Biochemical and Biophysical Research Communications 446 (2014) 235–240

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Dux4 induces cell cycle arrest at G1 phase through upregulation of p21 expression

Hongliang Xu [a], Zhaoxia Wang [a], Suqin Jin [a], Hongjun Hao [a], Lemin Zheng [b], Boda Zhou [c], Wei Zhang [a], He Lv [a], Yun Yuan [a,*]

[a] Department of Neurology, Peking University First Hospital, Beijing 100034, China
[b] The Institute of Cardiovascular Sciences, Peking University Health Science Center, Key Laboratory of Molecular Cardiovascular Sciences of Education Ministry, Key Laboratory of Cardiovascular Molecular Biology and Regulatory Peptides of Health Ministry, Beijing 100191, China
[c] The Department of Cardiology, Peking University Third Hospital, Beijing 100191, China

ARTICLE INFO

Article history:
Received 10 February 2014
Available online 28 February 2014

Keywords:
FSHD
Dux4
p21
Cell cycle

ABSTRACT

It has been implicated that Dux4 plays crucial roles in development of facioscapulohumeral dystrophy. But the underlying myopathic mechanisms and related down-stream events of this retrogene were far from clear. Here, we reported that overexpression of Dux4 in a cell model TE671 reduced cell proliferation rate, and increased G1 phase accumulation. We also determined the impact of Dux4 on p53/p21 signal pathway, which controls the checkpoint in cell cycle progression. Overexpression of Dux4 increased p21 mRNA and protein level, while expression of p53, phospho-p53 remained unchanged. Silencing p21 rescued Dux4 mediated proliferation defect and cell cycle arrest. Furthermore, we demonstrated that enhanced Dux4 expression increased p21 promoter activity and elevated expression of Sp1 transcription factor. Mutation of Sp1 binding site decreased dux4 induced p21 promoter activation. Chromatin immunoprecipitation (ChIP) assays confirmed the Dux4-induced binding of Sp1 to p21 promoter *in vivo*. These results suggest that Dux4 might induce proliferation inhibition and G1 phase arrest through upregulation of p21.

1. Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is a repeat disease, characterized by progressive skeletal muscle weakness and decrease of muscle cells and tissue. Contraction of D4Z4 macrosatellite repeat in chromosome 4q, results in the inefficient repression of a retrogene Dux4, which was recently correlated with the development of FSHD disease [1]. Dux4, first identified through sequencing the 3.3-kb repeat elements remaining in an FSHD patient in 1999, encodes a protein containing 2 homodomains [2]. Expression of Dux4 mRNAs and protein can be detected in primary myoblasts of patients with FSHD but not in control lines [3,4]. As a transcriptional factor, DUX4 activates germline genes, retroelements, and immune mediators, which supports that the gene is a casual factor for FSHD [5]. Overexpression of this gene promoted cell apoptosis, with elevated caspase 3/7 activity [6], interferes with myogenic regulators and abolishes myoblast differentiation [5,7].

It has been implicated that coordinate regulation of cell cycle plays important roles during myogenesis. The cell cycle regulation of muscle stem cells was crucial for proliferation and differentiation of myoblast during development or repair of muscle tissue [8,9]. Development of skeletal muscle from myoblast to multinucleate fibers is a multistep process, in which cell cycle arrest was required for the myoblast to terminal differentiation [10,11]. However, whether Dux4 affects the cell cycle progression remains unclear.

Myopathic effects of Dux4 were p53 dependent [7], because p53 inhibition mitigated Dux4 toxicity in vitro. As we knew, p53 was also a cell cycle regulator, which induced cell cycle arrest through transcriptional activation of p21 [12]. P21 is a p53 target gene, whose functions were dependent on activated p53 [13]. P21 is also a potent cyclin-dependent kinase inhibitor, which induce cell cycle arrest through directly binding to cyclins and CDKs [14]. To date, plenty of investigation demonstrated that p21 can induce G1/S arrest and cell death [12]. But whether p21 was a downstream effector of Dux4 remains unclear.

* Corresponding author. Address: Department of Neurology, Peking University First Hospital, No.8 Xishiku Street, Xicheng District, Beijing 100034, China. Fax: +86 1066176450.
E-mail address: yuanyun2002@sohu.com (Y. Yuan).

http://dx.doi.org/10.1016/j.bbrc.2014.02.105
0006-291X/

In this investigation, we studied how Dux4 inhibits cell proliferation, and influence cell cycle progression in a rhabdomyosarcoma cell model. We found that dux4 induced G1 phase arrest through upregulation of P21 protein without p53 and phospho-p53 elevation. We also revealed a transcriptional factor Sp1, was responded in Dux4 induced p21 upregulation.

2. Materials and methods

2.1. Cell culture and reagents

Rhabdomyosarcoma cell line TE671 was obtained from the cell culture center of School of Basic Medicine, Peking Union Medical College. DMEM medium was obtained from Hyclone (USA), and fetal bovine serum (FBS) was purchased from Gibco (USA). Cocktail was purchased from Amresco (USA).

2.2. Plasmid construction and transient transfection

Open reading frame of Dux4 gene (NM_003178) was synthesized (Genescript) and subcloned to pcDNA3.1 expression vector (Invitrogen). Using Lipofectamine®2000, about 80–90% transfection efficiency can be achieved for TE671 cells according to manufactures' protocol. The p21-shRNA (targeting sequence: 5′-GTCACTGTCTTGTACCCTTGT-3′) and unspecific scrambled shRNA plasmids (control) were purchased from Genechem Company (Shanghai).

2.3. Proliferation assays

TE671 cells were transfected with the pcDNA 3.1 empty vector or Dux4-pcDNA3.1 Vector. At the time point of 0, 24, 48, 72, 96, 120 h, 10 μl of CCK-8 solution was added to each well of the plate containing 100 μl of culture media, and incubated for 1 h in the cell incubator. The absorbance at 450, which were proportional to cell number in each well, was measured by BioTek Synergy H1 Hybrid Multi-Mode Microplate Readers (USA).

2.4. Western-blot analysis

Equivalent amounts of protein were electrophoresed on denaturing polyacrylamide gradient gel and transferred to PVDF membrane (Pall). The membranes were blocked, incubated with a specific primary antibody against Dux4, p21 (Santa Cruz), P53, phospho-p53 and Sp1 (Cell Signal Company), washed, and exposed to an appropriate horseradish peroxidase-conjugated secondary antibody. Immunoreactive bands were visualized by enhanced chemiluminescence (Perice, USA) and subsequent luminesence signal was obtained by KODAK Image Station 4000.

2.5. Reverse transcription and real-time PCR

Total mRNA was extracted from the cultured cells using Trizol regent (Invitrogen). 2 μg total mRNA was used in a 20 μl reaction system following the High Capacity cDNA Reverse Transcription Kits (ABI) protocol. The cDNA product was subject to PCR using the Power SYBR® Green PCR Master Mix (Invitrogen) on a Real-Time PCR System (Applied Biosystems 7500). The following primers were used: GAPDH: 5′-TCTTTTGCGTCGCCAGCCGAG-3′ (sense), 5′-CAGAGTTAAAAGCAGCCCTGGTGAC-3′ (antisense); p21: 5′-TCTTGTACCCTTGTGCCTCg-3′ (sense), 5′-CGGCGTTTGGAGTGGTAgAA-3′ (antisense). Sp1: 5′-TCCAGGTGCACCCAATTCAA-3′ (sense), 5′-CTTCCTCTCCACCTGCTGTG-3′ (antisense). A comparative Ct method (2^{-ddCt}) was used to calculate the relative expression and GAPDH was used as an internal control. All values are the means of 3 independent experiments.

2.6. Flow cytometry (FCM) analysis of cell cycle

Measurement of cellular DNA content by flow cytometry was performed for the analysis of cell cycle. Briefly, adherent cells (less than 70% confluence) were collected with trypsin and centrifuged at 300×*g* and fixed in cold 70% ethanol for at least 3 h. Fixed cells were pelleted, washed once in phosphate-buffered saline (PBS), and then resuspended in propidium iodide (PI) stain buffer (0.1% (v/v) Triton X-100, 10 μg/mL PI, and 100 μg/mL DNase-free RNaseA) in PBS for 30 min. After staining, samples were analyzed on a flow cytometry (BD Biosciences FACS Calibur) and data were analyzed with Modifit LT software.

2.7. Measurement of p21 promoter activity

The 934 bp (from −717 to +217) upstream region of the p21 promoter was subcloned into pGL3-Basic luciferase plasmid (Promega). Site-directed mutagenisis of Sp1 binding site was conducted using the QuikChange site-directed mutagenesis kit (Stratagene). Cells were seeded in 96-well plates (1×10^4 cells/well) prior to cotransfection with 0.2 μg of p21 propmoter-pGL3 basic chimeric plasmid construct, which expresses firefly luciferase, and 0.02 μg of the pRL-CMV plasmid (Promega), which expresses renilla luciferase, using Lipofectamin2000 (Invitrogen). Transfected cells were cultured for 24 h, the activities of firefly and renilla luciferases were measured following the protocol of Dual-Luciferase ® Reporter Assay System (Promega, USA) on the BioTek Synergy H1 Hybrid Multi-Mode Microplate Readers (USA). Renilla luminescence of each well was measured as an internal control. Firefly luciferase values were standardized to renilla values.

2.8. Chromatin immunoprecipitation assay (ChIP)

ChIP experiments were performed following the instructions of Pierce Agarose ChIP Kit (Thermo Scientific). TE671 cells were treated with formaldehyde solution, lysed, and digested with micrococcal nuclease. The digested chromatin was isolated and immunoprecipitated with Sp1 antibody, followed by incubation with the protein A/G Plus agarose resin. Immunoprecipitation with a normal rabbit IgG (Thermo Scientific) was used as a negative control. The captured chromatin was eluted and then uncross-linked, and the DNA was recovered. ChIP DNA was subjected to PCR using specific primers flanking the DNA-binding site for Sp1. The amplified promoter region located at nucleotides from −150 to −4, and the primer sequences were: 5′-GCTGGGCAGCCAGGAGCCTG-3′ (forward); 5′-CTGCTCACACCTCAGCTGGC-3′ (reverse).

3. Results

3.1. Overexpression of Dux4 inhibited cell proliferation and induced cell cycle arrest at G1 phase

One of main symptoms of FSHD is atrophy of muscle, which might result form cell proliferation inhibition. In this assay, we determined the effect of Dux4 on cell proliferation in TE671 cells. TE671 cells were transiently transfected with pcDNA3.1 empty vector (control), or Dux4-pcDNA 3.1, which enforced Dux4 expression. As shown in Fig. 1A, expression of Dux4 cannot be detected in TE671 cells transfected with empty pcDNA3.1 vector. Delivery of Dux4-pcDNA3.1 vector to TE671 cells induced overexpression of Dux4 protein within 8 h. As shown in Fig. 1B, cells transfected with

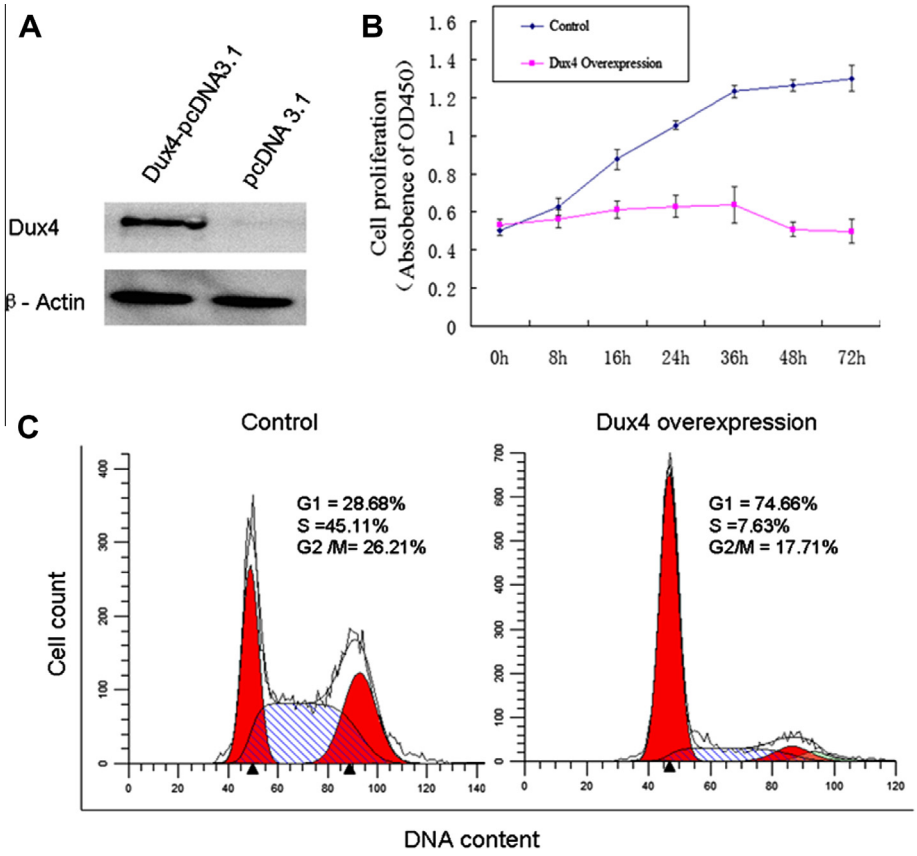

Fig. 1. Overexpression of Dux4 inhibited proliferation of TE671 cells and induced cell cycle arrest at G1 phase. TE671 cells were transfected with pcDNA3.1 empty vector (control) or Dux4-pcDNA3.1 vector and cultured in DMEM with 10% FBS. (A) Western-blot demonstrated overexpression of Dux4 in TE671 cells 8 h after transfection of Dux4-pcDNA3.1. (B) Proliferation rate was determined at indicated time point in following 3 days. Data from 4 paralleled experiments are expressed as mean ± SD of absorbance of OD450 (CCK-8 method) which was proportional to the cell number in the wells. (C) At the indicated time point, DNA content of the cells were determined to analyze the distribution of cell cycle phase. The mathematical model MODFIT was used to calculate the proportions of cells at each cell cycle phase.

pcDNA3.1 grew in a linear manner (control), while overexpression of Dux4 inhibited cells proliferation obviously as compared with control. Cell cycle progression plays important roles in cell proliferation, but the impact of dux4 on the cell cycle progression has not been studied. As shown in Fig. 1C, Dux4-pcDNA3.1 transfection resulted in accumulation of cells in G1 phase (74.66%) as compared with control (28.68%).

3.2. Dux4 overexpression upregulated p21 expression without activating p53

Recent study has implicated that P53 was a down stream target of Dux4, and FSHD myopathy was p53 dependent [7]. P53 played a central role in deciding the cell fate in response to stress [15–17], and p53 can induce cell cycle arrest in both p21 dependent and independent ways. So we detected whether the Dux4 induced cell cycle arrest was depend on the activity of p53/p21 pathway. As shown in Fig. 2A, Dux4 protein cannot be detected in the cell transfected with control vector, while delivery of Dux4-pcDNA3.1 vector results in overexpression of Dux4 in TE671 cells following cultured for 12 h, 24 h, and 36 h. P53 and its phosphorylated form seem not affected by overexpression of Dux4, while the p21 elevated significantly as compared with control at either time point we detected. To determine whether upregulation of p21 protein was resulted from the elevation of transcriptional activity of p21, we determined the expression of p21 mRNA after overexpression of Dux4. As shown in the Fig. 2B, real time PCR demonstrated that p21 mRNA was elevated about 3-fold 24 h after Dux4-pcDNA3.1 transfection as compared with control.

3.3. Silencing p21 rescued Dux4 mediated proliferation defect and cell cycle arrest

We next assessed whether p21 depletion could affect the inhibitory effect of Dux4 overexpression in TE671 cells. TE671 cells were transfected with pcDNA3.1 + control-shRNA vectors, or Dux4-pcDNA3.1 + control-shRNA vectors, or Dux4-pcDNA3.1 + p21-shRNA vectors, respectively. 24 h after transfection, elevated levels of Dux4 and p21 protein were readily detected as compared with control in the presence of control-shRNA vector, and p21-shRNA vector inhibited Dux4-pcDNA3.1 induced p21 expression efficiently (Fig. 3A). Fig. 3B showed the anti-proliferative effect induced by Dux4 overexpression was significantly impaired by silencing of p21 expression by p21-shRNA. Fig. 3C showed that Dux4-induced G1 phase arrest was also significantly impaired through silencing p21 expression. Taken together, these experiments strongly supported an anti-proliferative role for Dux4 and indicate that p21 was an important effector during these processes. The rescue effect was partial, which probably due to the fact that p21 knock-down in TE671 cells was not complete (Fig. 3A).

3.4. Sp1 binding site was required for Dux4-induced activation of p21 promoter

To study the mechanism of the Dux4-dependent increase in p21 expression, we sought to identify Dux4-responsible elements in p21 promoter region. Fig. 4A showed that Dux4 overexpression increased transcriptional activity of p21 promoter about 4.87-fold as compared with empty pcDNA3.1 vector transfection. The p21

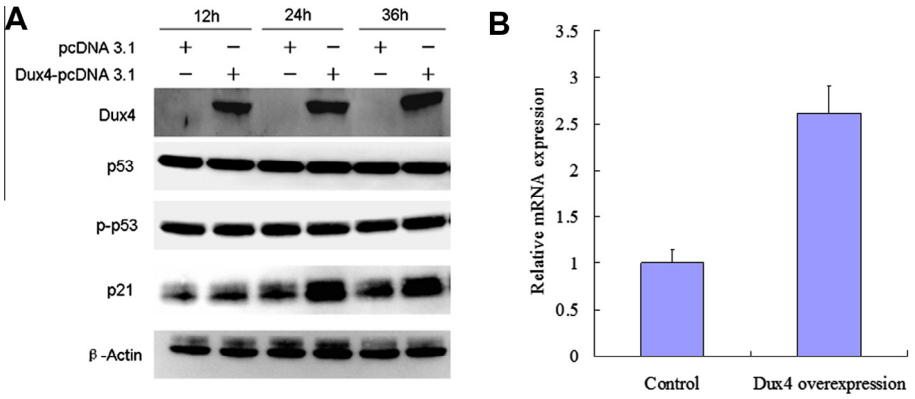

Fig. 2. Dux4 overexpression increased p21 expression but not p53 and phospho-p53. TE671 cells were transfected with pcDNA3.1 empty vector or Dux4-pcDNA3.1 vector and cultured in DMEM with 10% FBS. (A) At the indicate time point, Western-blot were performed to quantify the expression of key regulator of p53/p21 signal pathway. Whole cell lysates were separated on SDS–PAGE and immunoblotted for Dux4, p53, p-p53, p21. Similar results were obtained in three separate experiments and a representative blot was shown. (B) 24 h after transfection, total RNA was isolated and analyzed for the expression of p21 and GAPDH. Data shown were expression of p21 normalized by the expression of GAPDH (internal control).

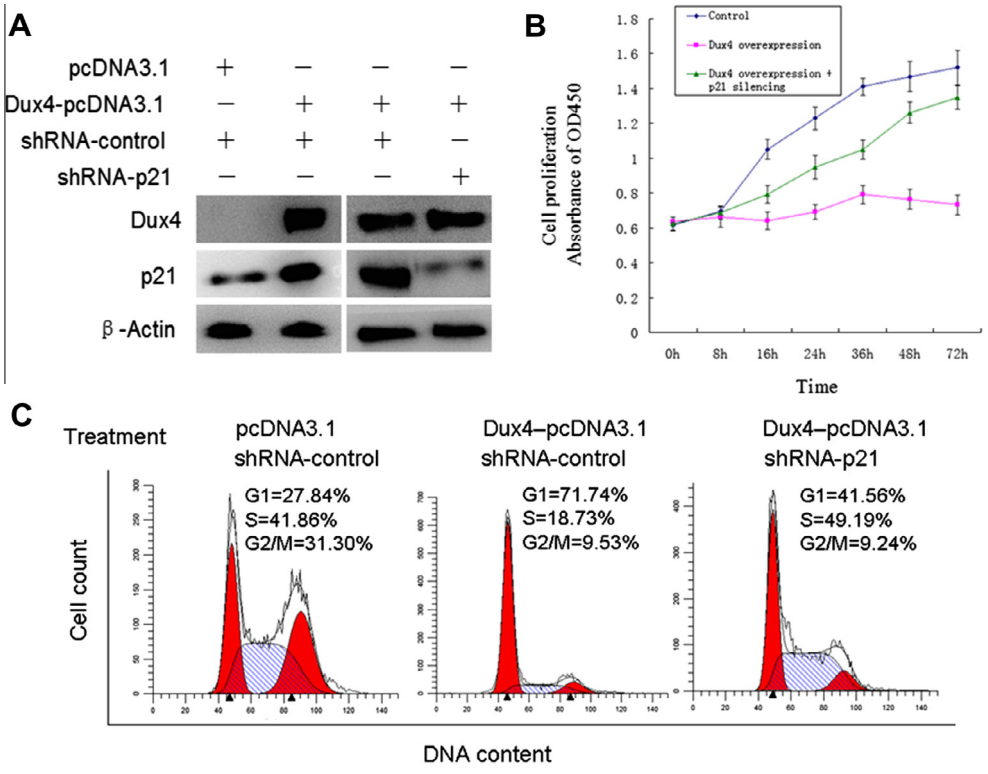

Fig. 3. Silencing p21 rescued Dux4 induced proliferation defect and cell cycle arrest TE671 cells were transfected with pcDNA3.1 + control-pSilencer4.1, Dux4-pcDNA3.1 + control-pSilencer4.1, Dux4-pcDNA3.1 + p21-pSilencer4.1, respectively. (A) Western blot analysis was performed to examine the knockdown efficiency of the p21-shRNA. (B) Proliferation assay was performed to determine effect of p21 silencing on the Dux4 induced proliferation defect. (C) Effect of p21 silencing on Dux4 induced G1 phase accumulation. Similar results were obtained in three separate experiments and a representative result was shown.

promoter encodes six GC-rich sites, clustered in the proximal promoter region, which were reported to bind Sp1 transcription factors [18,19]. By now, the Sp1 bind site from nucleoside −82 to −77 was the most reported element that mediate p21 promoter activation by various factors, and mutation or deletion of this site resulted in obviously decrease of p21 promoter activity [18,20,21]. So we detected whether this site was responsible to Dux4 overexpression. Fig. 4B showed that Sp1 mRNA (upper graph) and protein (lower graph) was elevated accompanied with p21 upregulation after Dux4 overexpression. Fig. 4C left graph showed the 6 Sp1 binding sites and the sequence from −82 to −77 in wild-type p21 promoter and the substituted nucleotide in the mutated construct, which were used in the following dual-luciferase assay. Fig. 4C right graph showed the site-directed mutations of Sp1 binding site reduced Dux4-induced promoter activation by about 67.3% (Fig. 4C). ChIP assay confirmed binding of Sp1 on the p21 promoter after overexpression of Dux4; while this binding cannot be detected in the control cells transfected with pcDNA3.1 empty vector (Fig. 4D). Morphogenetic protein2 (Bmp2) mRNA was elevated after enforced Dux4 expression (Supplementary Fig. A). Activation of Bmp2-smad signal pathway was accompanied with Sp1 and p21 elevation; moreover, LDN193189, which inhibited phosphorylation

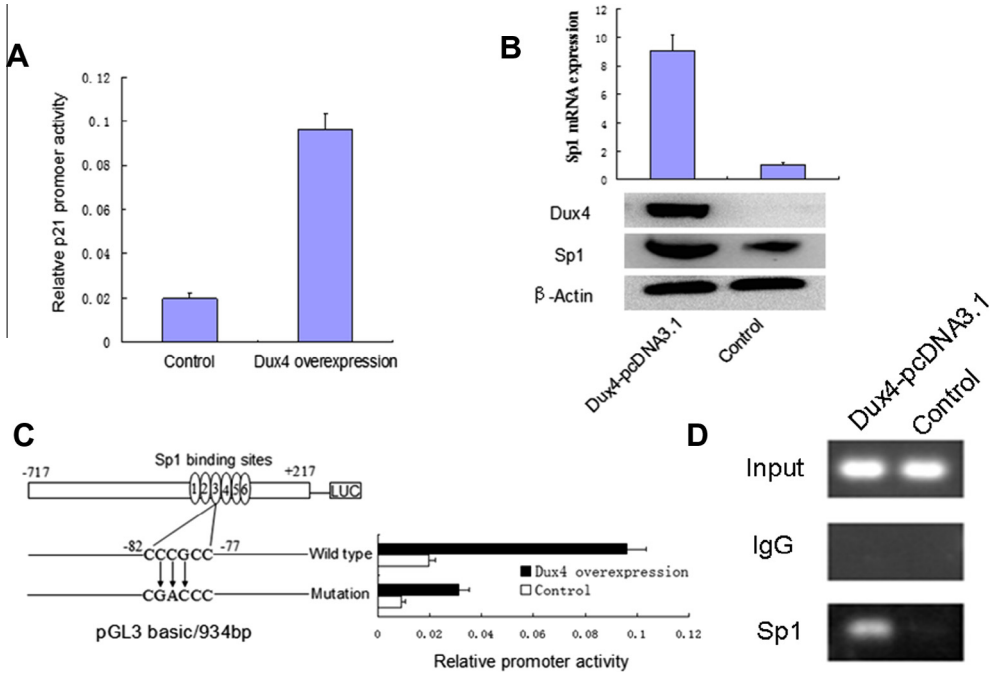

Fig. 4. Sp1 binding site was required for Dux4 induced p21 promoter activation. (A) TE671 cells were transfected with p21 promoter luciferase reporter plasmid in the presence of Dux4-pcDNA3.1 vector or empty pcDNA3.1 vector (control). After 24 h, cells were harvested, and extracts were assayed for luciferase activity. (B) TE671 cells were transfected with Dux4-pcDNA3.1 vector or empty vector (control). After 24 h, realtime PCR and Western blot were performed to determine Sp1 mRNA and protein expression. (C) Schematic of 934 bp upstream region of human p21 promoter showed the 6 Sp1 binding sites in the proximal region of p21 promoter. The positions were numbered from the transcription start site. The Sp1 binding sequence (−82/−77) were showed and substituted nucleotide in mutation analysis were indicated by vertical arrows. Luciferase assays were performed with wild type reporter plasmids or mutated type in the presence of Dux4-pcDNA3.1 or empty vector (control) and the relative luciferase activities were showed on the right graph. (D) ChIP assays were conducted using the chromatins prepared from TE671 cells transfected with Dux4-pcDNA3.1 or empty vector. The antibodies used in immunoprecipitation are indicated on the left. IgG served as a negative control. The region flanking Sp1 binding site (spanning from −150 to −4) was amplified by PCR with the primer sets described in Section 2.

of smad1/5/8, decreased Sp1 and p21 protein (Supplementary Fig. B).

4. Discussion

FSHD is the third most common muscular dystrophy. Most FSHD patients harbor a large deletion in the polymorphic D4Z4 macrosatellite repeat array at 4q35, which was 1–10 repeats in FSHD patients and 11–150 repeats in non-affected individuals [22]. The contraction of the D4Z4 repeats resulted in the chromatin remodelling of the 4qter region, activated the expression of the Dux4 gene, a protein that has been regarded as the candidate pathogenic factor of FSHD [4]. The Dux4 gene encoded a protein of 55kd, and can be immunodetected on FSHD myoblast [3] or human testis extracts [23]. Rhabdomyosarcoma cell, which possess a genetic background of human muscle, has been used usually for the investigation of Dux4 function in vitro [6,24,25]. Several researches have demonstrated that Dux4 is a pro-apoptotic protein [6]. Though cell cycle is an important biological process that link with proliferation, apoptosis, and differentiation to sustain tissue homeostasis [26,27], no report has been published for the impact of Dux4 on the progression of cell cycle. In this investigation, we demonstrate that Dux4 significantly inhibited cell proliferation and induced cell cycle arrest at G1 phase in a rhabdomyosarcoma cell model.

Like most other transcriptional factor, Dux4 modulates cell proliferation through activating or suppressing transcription of the target gene. Currently, only a limited number of genes have been confirmed as the down stream effector of this protein. It has been reported that p53 was required for Dux4 to exert its adverse effect, and p53/p21 signal pathway plays crucial roles in controlling the G1/S checkpoint in cell cycle progression [28]. However, our results showed that expression of p53 and phosphor-p53 were not affected after the overexpression of Dux4 in TE671 cells. P21, a negative regulator of cell cycle, exert its biological activities through binding to and inhibiting the activity of cyclin-dependent kinases CDK2 and CDK1, and promoting cell cycle arrest at specific stages in response to many stimuli [29]. P21 bind and inactivate cyclin D/CDK4/6 and cyclin E/CDK2 kinase complexes, and inhibits Rb phosphorylation and induces cell cycle arrest in G1 phase [12,30]. The interaction of p21 with PCNA blocks the ability of PCNA to activate polδ, and therefore inhibitied the DNA replication and arrest cell cycle at intra-S phase [31,32]. In this investigation, our data shows the Dux4 induced P21 elevation and accumulation of cells arrested at G1 phase, which was consistent with the cell cycle regulation function of p21. Silencing p21 partially abrogates Dux4-induced proliferation defect and G1 phase accumulation. All these data indicated that p21 was a mediator of Dux4-induced proliferation defect and cell cycle arrest.

Sp1 was a zinc finger transcription factor which binds to GC-rich motifs of many promoters, and by this way Sp1 enhance or repress the activity of target genes involved in many cellular processes including proliferation, cell cycle progression, apoptosis, cell differentiation, and ontogenesis [33]. Six Sp1 binding elements have been identified in the proximal region of p21 promoter, to which the sp1 bind and increased p21 expression to induce cell cycle arrest at G1 phase [34]. The binding site from nucleotide −82 to −77 was the most reported one previously to mediate p21 induction under many conditions [19,20,35]. In this investigation, mutation of this site (−82/−77) decreased Dux4 induced p21 promoter activity significantly, which suggested that this site was the responsible element under Dux4 overexpression. The *in vivo* binding of Sp1 to p21 promoter was confirmed by ChIP. Bmp2 can be an

autocrine factor and plays important roles in development of muscle tissue [36]. It has been reported that Bmp signaling pathway was misregulated by Dux4 [24] and activation of Bmp2-smad signal pathway can cause Sp1 [37] and p21 [38] elevation. In this investigation, we showed that Dux4 overexpression induced Bmp2-Smad signal pathway, which mediated sp1 and p21 elevation.

In summary, we conclude that Dux4 induces cell cycle arrest at G1 phase and inhibits cell proliferation. In this pathologic process, p21 was transcriptionally upregulated without P53 elevation, and Sp1 was involved in the p21 promoter activation. These findings shed new light on the molecular mechanisms underlying muscle cell waste during development of FSHD.

Conflict of interest statement

None declared.

Contribution

Xu Hongliang and Yuan Yun, designed the research project, performed the research, analyzed the data, and wrote the paper; Jin Suqin, Wang Zhaoxia, Hao Hongjun, Zheng Lemin, Zhou Boda, Zhang Wei, Lv He, performed the research, analyzed the data, and revised the paper.

Acknowledgment

This work was supported by the Twelfth Five-year Plan of China [No. 2011ZX09307-001-07].

Thanks Zhang Gaolei in department of dermatology, Peking University First Hospital for his help in p21 RNA interfere and promoter analysis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.02.105.

References

[1] J.C. de Greef, R.R. Frants, S.M. van der Maarel, Epigenetic mechanisms of facioscapulohumeral muscular dystrophy, Mutat. Res. 647 (2008) 94–102.
[2] J.E. Hewitt, R. Lyle, L.N. Clark, E.M. Valleley, T.J. Wright, C. Wijmenga, J.C. van Deutekom, F. Francis, P.T. Sharpe, M. Hofker, et al., Analysis of the tandem repeat locus D4Z4 associated with facioscapulohumeral muscular dystrophy, Hum. Mol. Genet. 3 (1994) 1287–1295.
[3] M. Dixit, E. Ansseau, A. Tassin, S. Winokur, R. Shi, H. Qian, S. Sauvage, C. Matteotti, A.M. van Acker, O. Leo, D. Figlewicz, M. Barro, D. Laoudj-Chenivesse, A. Belayew, F. Coppee, Y.W. Chen, DUX4, a candidate gene of facioscapulohumeral muscular dystrophy, encodes a transcriptional activator of PITX1, Proc. Natl. Acad. Sci. USA 104 (2007) 18157–18162.
[4] S.M. van der Maarel, R. Tawil, S.J. Tapscott, Facioscapulohumeral muscular dystrophy and DUX4: breaking the silence, Trends Mol. Med. 17 (2011) 252–258.
[5] L.N. Geng, Z. Yao, L. Snider, A.P. Fong, J.N. Cech, J.M. Young, S.M. van der Maarel, W.L. Ruzzo, R.C. Gentleman, R. Tawil, S.J. Tapscott, DUX4 activates germline genes, retroelements, and immune mediators: implications for facioscapulohumeral dystrophy, Dev. Cell 22 (2012) 38–51.
[6] V. Kowaljow, A. Marcowycz, E. Ansseau, C.B. Conde, S. Sauvage, C. Matteotti, C. Arias, E.D. Corona, N.G. Nunez, O. Leo, R. Wattiez, D. Figlewicz, D. Laoudj-Chenivesse, A. Belayew, F. Coppee, A.L. Rosa, The DUX4 gene at the FSHD1A locus encodes a pro-apoptotic protein, Neuromuscul. Disord. 17 (2007) 611–623.
[7] L.M. Wallace, S.E. Garwick, W. Mei, A. Belayew, F. Coppee, K.J. Ladner, D. Guttridge, J. Yang, S.Q. Harper, DUX4, a candidate gene for facioscapulohumeral muscular dystrophy, causes p53-dependent myopathy in vivo, Ann. Neurol. 69 (2011) 540–552.
[8] K. Walsh, Coordinate regulation of cell cycle and apoptosis during myogenesis, Prog. Cell Cycle Res. 3 (1997) 53–58.
[9] M.A. Ciemerych, K. Archacka, I. Grabowska, M. Przewozniak, Cell cycle regulation during proliferation and differentiation of mammalian muscle precursor cells, Results Probl. Cell Differ. 53 (2011) 473–527.
[10] F.E. Stockdale, Myogenic cell lineages, Dev. Biol. 154 (1992) 284–298.
[11] J.B. Miller, L. Schaefer, J.A. Dominov, Seeking muscle stem cells, Curr. Top. Dev. Biol. 43 (1999) 191–219.
[12] Y.S. Jung, Y. Qian, X. Chen, Examination of the expanding pathways for the regulation of p21 expression and activity, Cell. Signal. 22 (2010) 1003–1012.
[13] K.H. Vousden, C. Prives, Blinded by the light: the growing complexity of p53, Cell 137 (2009) 413–431.
[14] A.L. Gartel, M.S. Serfas, A.L. Tyner, P21–negative regulator of the cell cycle, Proc. Soc. Exp. Biol. Med. 213 (1996) 138–149.
[15] Z.A. Stewart, J.A. Pietenpol, P53 signaling and cell cycle checkpoints, Chem. Res. Toxicol. 14 (2001) 243–263.
[16] N.R. Farid, P53 and other cell cycle regulators, Cancer Treat. Res. 122 (2004) 149–164.
[17] E. Senturk, J.J. Manfredi, P53 and cell cycle effects after DNA damage, Methods Mol. Biol. 962 (2013) 49–61.
[18] A.L. Gartel, A.L. Tyner, Transcriptional regulation of the p21((WAF1/CIP1)) gene, Exp. Cell Res. 246 (1999) 280–289.
[19] K. Nakano, T. Mizuno, Y. Sowa, T. Orita, T. Yoshino, Y. Okuyama, T. Fujita, N. Ohtani-Fujita, Y. Matsukawa, T. Tokino, H. Yamagishi, T. Oka, H. Nomura, T. Sakai, Butyrate activates the WAF1/Cip1 gene promoter through Sp1 sites in a p53-negative human colon cancer cell line, J. Biol. Chem. 272 (1997) 22199–22206.
[20] Y.C. Chew, G. Adhikary, G.M. Wilson, W. Xu, R.L. Eckert, Sulforaphane induction of p21(Cip1) cyclin-dependent kinase inhibitor expression requires p53 and Sp1 transcription factors and is p53-dependent, J. Biol. Chem. 287 (2012) 16168–16178.
[21] G. Koutsodontis, I. Tentes, P. Papakosta, A. Moustakas, D. Kardassis, Sp1 plays a critical role in the transcriptional activation of the human cyclin-dependent kinase inhibitor p21(WAF1/Cip1) gene by the p53 tumor suppressor protein, J. Biol. Chem. 276 (2001) 29116–29125.
[22] M. Richards, F. Coppee, N. Thomas, A. Belayew, M. Upadhyaya, Facioscapulohumeral muscular dystrophy (FSHD): an enigma unravelled?, Hum Genet. 131 (2012) 325–340.
[23] L. Snider, L.N. Geng, R.J. Lemmers, M. Kyba, C.B. Ware, A.M. Nelson, R. Tawil, G.N. Filippova, S.M. van der Maarel, S.J. Tapscott, D.G. Miller, Facioscapulohumeral dystrophy: incomplete suppression of a retrotransposed gene, PLoS Genet. 6 (2010) e1001181.
[24] V. Sharma, N. Harafuji, A. Belayew, Y.W. Chen, DUX4 differentially regulates transcriptomes of human rhabdomyosarcoma and mouse C2C12 cells, PLoS One 8 (2013) e64691.
[25] J. Gabriels, M.C. Beckers, H. Ding, A. De Vriese, S. Plaisance, S.M. van der Maarel, G.W. Padberg, R.R. Frants, J.E. Hewitt, D. Collen, A. Belayew, Nucleotide sequence of the partially deleted D4Z4 locus in a patient with FSHD identifies a putative gene within each 3.3 kb element, Gene 236 (1999) 25–32.
[26] F.Q. Alenzi, Links between apoptosis, proliferation and the cell cycle, Br. J. Biomed. Sci. 61 (2004) 99–102.
[27] C.H. Golias, A. Charalabopoulos, K. Charalabopoulos, Cell proliferation and cell cycle control: a mini review, Int. J. Clin. Pract. 58 (2004) 1134–1141.
[28] G. Liu, G. Lozano, P21 stability: linking chaperones to a cell cycle checkpoint, Cancer Cell 7 (2005) 113–114.
[29] T. Abbas, A. Dutta, P21 in cancer: intricate networks and multiple activities, Nat. Rev. Cancer 9 (2009) 400–414.
[30] N. Abdel-Wahab, B.S. Weston, T. Roberts, R.M. Mason, Connective tissue growth factor and regulation of the mesangial cell cycle: role in cellular hypertrophy, J. Am. Soc. Nephrol. 13 (2002) 2437–2445.
[31] R. Li, S. Waga, G.J. Hannon, D. Beach, B. Stillman, Differential effects by the p21 CDK inhibitor on PCNA-dependent DNA replication and repair, Nature 371 (1994) 534–537.
[32] S. Waga, G.J. Hannon, D. Beach, B. Stillman, The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA, Nature 369 (1994) 574–578.
[33] L. Li, J.R. Davie, The role of Sp1 and Sp3 in normal and cancer cell biology, Ann. Anat. 192 (2010) 275–283.
[34] M. Ocker, R. Schneider-Stock, Histone deacetylase inhibitors: signalling towards p21cip1/waf1, Int. J. Biochem. Cell Biol. 39 (2007) 1367–1374.
[35] M.B. Datto, Y. Yu, X.F. Wang, Functional analysis of the transforming growth factor beta responsive elements in the WAF1/Cip1/p21 promoter, J. Biol. Chem. 270 (1995) 28623–28628.
[36] H. Amthor, B. Christ, M. Weil, K. Patel, The importance of timing differentiation during limb muscle development, Curr. Biol. 8 (1998) 642–652.
[37] J.J. Dore, J.C. DeWitt, N. Setty, M.D. Donald, E. Joo, M.A. Chesarone, S.J. Birren, Multiple signaling pathways converge to regulate bone-morphogenetic-protein-dependent glial gene expression, Dev. Neurosci. 31 (2009) 473–486.
[38] G.A. Wong, V. Tang, F. El-Sabeawy, R.H. Weiss, BMP-2 inhibits proliferation of human aortic smooth muscle cells via p21Cip1/Waf1, Am. J. Physiol. Endocrinol. Metab. 284 (2003) E972–E979.