Original Paper

Acetylation of GATA4 on Lysine Residue K313 Promotes Osteoblastic Cells Growth

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Key Words

Gata4 • Osteoporosis (OS) • hFOB1.19 cells • Acetylation • P300/CBP • Cell growth

Abstract

Background/Aims: GATA4, a protein related to cardiac differentiation and mineralization, whose acetylation is essential for cardiac development. Here we aimed to explore the functional impacts of GATA4 acetylation on osteoporosis. **Methods:** GATA4 acetylation in hFOB1.19 and 293T cells was detected after exposure to histone deacetylase inhibitors (TSA and SAHA). Co-immunoprecipitation was conducted to determine which HATs and HDACs was involved in the modulation of GATA4 acetylation. **Results:** GATA4 acetylation was identified as a pivotal event in hFOB1.19 cells. GATA4 can be acetylated by P300/CBP, and the acetylation site was on lysine residue K313. Besides, the acetylation of GATA4 can be impaired by HDAC1, rather than by HDAC2-5. GATA4 acetylation contributed to the stability and transcription of GATA4. Moreover, GATA4 acetylation activated CCND2 transcription, and mutation of GATA4 on K-313 reduced cell growth and increased a mitochondria-dependent apoptosis in hFOB1.19 cells. **Conclusion:** Our data suggest that GATA4 exists as an acetylated protein in hFOB1.19 cells. Acetylation promotes the stability and transcription of GATA4, and activates CCND2 transcription, which may explain the growth-promoting functions of GATA4 in hFOB1.19 cells.

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Published by S. Karger AG, Basel

Introduction

Osteoporosis (OS) is defined as low bone mineral density caused by altered bone microstructure leading to skeletal fragility and fracture [1]. OS is one of these serious health problems, affecting millions of people worldwide [2, 3]. Under normal physiological conditions, osteoblasts are responsible for the formation of new bone in the developing skeleton and during the process of bone remodeling [4]. Recently, therapeutic strategies for OS attempt to promote bone formation by increasing the number or activity of mature

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osteoblasts or by preventing their apoptosis [5]. There is a need for therapy that target osteoblastic cells to increase their survival.

GATA4 is the founding member of the GATA family of zinc finger transcription factors, which consists of 6 members, GATA1 to GATA6 [6]. GATA4 is well known for its central role in embryogenesis [7], and in myocardial differentiation and function [8]. Besides, this protein is thought to be necessary for testicular development [9]. Mutations in this gene have been associated with various diseases. For the selected examples, GATA4 mutations/deletions are causes of neonatal or childhood-onset diabetes with or without exocrine insufficiency [10]. GATA4 genetic mutations are significantly associated with congenital heart disease risk [11, 12]. In terms of OS, GATA4 has been previously identified as a pioneer factor for estrogen receptor in osteoblasts [13]. Further investigation demonstrated that knock-out of GATA4 specifically in osteoblasts leads to embryonic and/or perinatal lethality, providing *in vivo* evidence that GATA4 is essential for bone mineralization [14]. Besides, GATA4 has been reported to be a negative regulator in osteoblast differentiation possibly via down-regulating Run2 [15], and bone sialoprotein (Bsp) [16].

Chromatin structure is determined by histone proteins, which undergo a multitude of covalent modifications that influence chromatin architecture and gene expression [17]. Acetylation is certainly the best characterized post-translational modification of both chromatin accessibility by modulating histone function, and for functional regulation of non-histone proteins. Accumulating studies indicated that histone acetylation and deacetylation have many links to congenital cardiac abnormalities [18], immune response [19], epilepsy [20, 21], and multiple kind of cancers, like ovarian cancer [22], gastric cancer [23], colorectal cancer [24], and lung cancer [25]. The balance between histone acetylation and deacetylation is maintained by the equilibrium of two kinds of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs), that HATs are responsible for histone acetylation while the duty of HDACs is deacetylation [26].

A previous study has reported that HAT is able to acetylate GATA4, resulting in enhanced DNA binding and transcription activity [27]. In transgenic mice, HAT in the heart induces GATA4 acetylation and cardiac hypertrophy [28, 29]. These authors demonstrated an essential role of GATA4 acetylation in cardiac defects; however, the functional impacts of GATA4 acetylation on OS, to our best knowledge, have not been previously revealed. Here, we explored how GATA4 acetylation affects human osteoblastic hFOB1.19 cells, and examined the functions of GATA4 acetylation on the growth of hFOB1.19 cells.

Materials and Methods

Cell Culture

The human osteoblastic cell line hFOB1.19 (CRL-11372™) and the human embryonic kidney cell line HEK293T (CRL-3216™) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). hFOB1.19 cells were cultured in DMEM: Ham's F-12 (1:1) medium (Sigma-Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) and 0.3 mg/mL G418 (Sigma-Aldrich). HEK293T cells were cultured in DMEM medium (Gibco) supplemented with 10% FBS. hFOB1.19 and HEK293T cells were maintained in a humidified atmosphere containing 5% CO₂ at 34°C and 37°C respectively.

HPLC grade of trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA) and cycloheximide (CHX) were obtained from Sigma-Aldrich. For HDAC inhibition, cells were treated with 20, 40, and 80 nM TSA or SAHA for 24 h. For blocking protein synthesis, cells were treated with 5 mg/kg CHX for the indicated time [31].

Cell transfection

Full-length GATA4 was generated by PCR and cloned into pc-DNA3.1-Flag vectors (Invitrogen, Carlsbad, CA, USA) to construct Flag-GATA4 vector. CBP, PCAF, P300, and GCN5 were sub-cloned into pcDNA3.1-Myc

(Invitrogen). HDAC1 to HDAC5 were sub-cloned into pcDNA3.1-HA vectors, which were constructed by inserting a copy of the HA epitope tag (YPYDVPDYA) into the pcDNA3.1 vector (Invitrogen).

The position of lysines (K) 247, 257, 301, 313, 320, 322, 324, 328, 330, 366, 405, and 424 in GATA4 were substituted to arginines (R). The mutated GATA4 sequence was cloned into pc-DNA3.1-Flag vectors. Point mutations in GATA4 were introduced by generating two overlapping fragments by PCR and were verified by sequencing. For GATA4 silence, cells were transfected with siRNA against GATA4 (RiboBio, Guangzhou, China). Control cells were transfected with empty vectors or non-targeting scrambled controls. Transfection was performed by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Transfected cells were subjected to 1.0 µg/mL neomycin for stable cell selection.

Co-immunoprecipitation (Co-IP) and western blot

The protein used for co-immunoprecipitation and immunoblotting were extracted from cells using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China). The whole-cell lysates were centrifuged at 14,000 *g* for 10 min at 4°C, and the supernatant was incubated with anti-GATA4 (Cell Signaling Technology, Danvers, MA, USA), anti-IgG (Santa Cruz Biotechnology, Santa Cruz, CA), anti-Flag (Santa Cruz Biotechnology) and protein-A sepharise (Zymed Laboratories, South San Francisco, CA, USA), and immunoblotted with anti-acetylated-Lys (New England Biolabs, Beverly, MA, USA), anti-GATA4 (Cell Signaling Technology), anti-Myc (Abcam, Cambridge, MA), and anti-HA (BioVision, Mountain View, CA, USA). After rinsing, the protein bound were separate by SDS-PAGE and transferred into PVDF membranes. The membranes were incubated with the secondary antibody and the bands were visualized using chemiluminescence (PerkinElmer, Wellesley, MA, USA).

For western blotting, the following antibodies were used: anti-GATA4 (Cell Signaling Technology), anti-HDAC1 (Abcam), anti-cleaved caspase-3 (Abcam), anti-Bcl-2 (Abcam), anti-Bax (Abcam), anti-Bcl-2 (Abcam), and anti-GAPDH (Abcam).

Promoter reporter and dual luciferase assay

293T cells were seeded in 24-well plates (1 × 10⁵ cells /well) for adherence, and transfected with Lipofectamine 2000 (Invitrogen) in antibiotic free medium for 48 h. The biological activity of luciferase reporter construct was assessed by Dual-Luciferase® Reporter Assay System (Promega, Madison, WI, USA). The relative luciferase activity was normalized to that of firefly luciferase.

Cell viability

After correspondingly transfection, hFOB1.19 cells were planted in 96-well plates with a density of 5000 cells/well for adherence. After 3 and 4 days of culturing, the culture medium was removed, cells were washed twice with PBS, and 20 µL MTT (Sigma-Aldrich) solution was added into each well. The plates were incubated at 37°C for 4 h, after which 10 µL DMSO was added to dissolve crystal. The absorbance was measured by microplate reader (Bio Rad Laboratories, Hercules, CA, USA) at a wavelength of 570 nm.

Apoptosis assay

The Annexin V-FITC Apoptosis Staining/Detection Kit from Abcam (Cambridge, MA, USA) was utilized in the present work, according to the manufacturer's protocols. hFOB1.19 cells were seeded in 6-well plates at a density of 5 × 10⁵ cells/well for adherence. After the indicated transfection, cells in each sample were collected by trypsin digestion and centrifugation, and were resuspended in 200 µL of binding buffer containing 5 µL Annexin V-FITC solution and 5 µL PI. The samples were incubated in the dark for 30 min at 4°C, after which cells were analyzed by the FACS can (Beckman Coulter, Fullerton, CA). The early apoptotic cells were calculated, which characterized by FITC-positive and PI-negative.

Statistical analysis

All experiments were repeated three times. Data were expressed as mean ± SD. Differences between groups were analyzed by one-way analysis of variance (ANOVA) with Duncan procedure in SPSS version 19.0 program (SPSS Inc., Chicago, IL, USA). A P-value of <0.05 was considered to indicate a statistically significant result.

Results

GATA4 acetylation was observed in hFOB1.19 cells, and acetylation contributed to GATA4 stability

hFOB1.19 cells were subjected to various dose of HDAC inhibitors (TSA and SAHA) for 24 h. As a result, we found that protein level expressions of GATA4 were significantly increased by TSA and SAHA in a dose-dependent fashion ($P < 0.01$ or $P < 0.001$, Fig. 1A), indicating GATA4 acetylation promoted GATA4 expression. This observation was confirmed in 293T cells, that protein expressions of GATA4 in 293T cells pre-transfected with Flag-GATA4 were also increased by TSA or SAHA treatment ($P < 0.01$ or $P < 0.001$, Fig. 1B). Then, acetylation of GATA4 in 293T cells were detected by immunoblot with anti-Acetylated-Lys post-transfection with Flag-GATA4 vector or Flag vector. As results shown in Fig. 1C, visible acetylation signal was found in cells transfected with Flag-GATA4, rather than in cells transfected with the Flag vector. Co-IP was conducted in hFOB1.19 cells to determine the regulation of endogenous GATA4 in hFOB1.19 cells upon TSA treatment. Fig. 1D showed that acetylation signal was observed in hFOB1.19 cells treated with TSA and acetylation further promoted the signal.

GATA4 was acetylated by P300/CBP

Then, we asked which NAT isoforms was involved in mediating GATA4 acetylation in hFOB1.19 cells. To do this, 293T cells were co-transfected with Flag-GATA4 and expression vectors encoding CBP, PCAF, P300 or GCN5. Co-IP results revealed that co-transfection of Flag-GATA4 and Myc-CBP,

Fig. 1. GATA4 is acetylated in hFOB1.19 cells. (A) hFOB1.19 cells were subjected to various dose of HDAC inhibitors (TSA and SAHA). Extracts of cells were subjected to Western blot analysis using anti-GATA4 antibody. (B) 293T cells were transfected with Flag-GATA4 and exposed to TSA and SAHA. Western blot analysis was performed by using anti-Flag antibody. (C) 293T cells were transfected with Flag-GATA4 or Flag vector. The cell extract was subjected to immunoblot with anti-Acetylated-Lys antibody. (D) hFOB1.19 cells were treated with TSA (40 nM). The cellular extract was immunoprecipitated with anti-GATA4 or anti-IgG antibody followed by immunoblot with anti-Acetylated-Lys antibody.

Fig. 2. Acetylation of GATA4 by P300/CBP. (A) 293T cells were transfected with Flag-GATA4 and expression vectors encoding CBP, PCAF, P300 or GCN5. The cellular extract was immunoprecipitated with anti-Flag or anti-IgG antibody followed by immunoblot with anti-Acetylated-Lys antibody. (B) 293T cells were transfected with Flag-GATA4 and expression vectors encoding CBP or P300. The extract was immunoprecipitated with anti-Flag or anti-IgG antibody followed by immunoblot with anti-CBP and anti-P300 antibody. (C) The extract from hFOB1.19 cells was subjected with immunoprecipitation using anti-Flag or anti-IgG antibody followed by immunoblot with anti-CBP and anti-P300.

as well as co-transfection of Flag-GATA4 and Myc-P300 resulted in visible acetylation signals (Fig. 2A), indicating GATA4 acetylation was mediated by CBP and P300, rather than by PCAF and GCN5. The interaction between GATA4 and P300/CBP was determined by Co-IP in 293T cells. Results in Fig. 2B showed that GATA4 specifically interacted with CBP and P300, but not with the IgG control. The phenomenon was also observed in hFOB1.19 cells, that GATA4 could interact with CBP and P300 rather than IgG at an endogenous level (Fig. 2C).

The site of acetylation occurred on lys-313 in GATA4

To determine which region in GATA4 was acetylated by P300/CBP, different K-point mutation was designed (K mutated to R), and then the mutant GATA4 together with Myc-CBP was transfected into 293T cells. Fig. 3A showed that, except the group co-transfected with Myc-CBP and Flag-GATA4-K313R, all other groups post-transfection exhibited visible signals of acetylation. Interestingly, the region in GATA4 acetylated by P300 was the same as CBP, that no acetylation signal was observed in cells co-transfected with Myc-P300 and Flag-GATA4-K313R (Fig. 3B). There data strongly evidenced that CBP and P300 directly interact with the region of lys-313 in GATA4. Fig. 3C showed that TSA could not induce the acetylation of GATA4 when Flag-GATA4-K313R was transfected into 293T cells, further implying the site of acetylation occurred on lys-313 in GATA4.

Inhibition of GATA4 by HDAC1

To determine which HDAC is specially involved in the acetylation of GATA4, 293T cells were co-transfected with Flag-GATA4, Myc-CBP and expression vectors encoding HDAC1 and HDAC5. Co-IP results revealed that acetylation signal induced by GATA4-CBP interaction was reduced by transfection with HA-HDAC1, rather than other HA-HDACs (Fig. 4A). The interaction between GATA4 and HDAC1 was then confirmed in 293T (Fig. 4B) and hFOB1.19 cells (Fig. 4C).

Acetylation promoted the transcriptional activity of GATA4

CHX is widely used as an inhibitor of protein synthesis. Herein, 293T cells were transfected with Flag-GATA4 or transfected with Flag-GATA4 plus Myc-CBP, and then subjected with CHX for 24 h. Down-regulated expression of GATA4 was observed in Flag-GATA4 transfected cells prolonged exposure to CHX (Fig. 5A). However, the down-regulations of GATA4

Fig. 3. Identification of GATA4 acetylation site. (A) 293T cells were co-transfected with Myc-CBP and expression vectors of GATA4, in which the indicated position of lysines were substituted to arginines (R). Cell extract was subjected to immunoblot with anti-Acetylated-Lys antibody. (B) 293T cells were co-transfected with Myc-P300 and expression vectors encoding mutated GATA4, after which immunoblot was performed. (C) 293T cells were transfected with Flag-GATA4-wt (wild type) and Flag-GATA4-K313R (mutated at lys-313). The transfected cells were cultured with or without 40 nM TSA for 24 h, after which the extract was collected for immunoprecipitation using anti-Flag antibody followed by immunoblot using anti-acetylated-Lys antibody.

induced by CHX exposure were not that remarkable when cells were co-transfected with Flag-GATA4 and Myc-CBP. Considering that the synthesis of GATA4 protein was sharply declined at 8 h of CHX exposure, 3, 6, and 9 h were selected for the CHX-treating conditions for use in the following experiments. Fig. 5B showed that, the down-regulations of GATA4 induced by CHX exposure were accelerated by co-transfection with Flag-GATA4 and HA-HDAC1 relative to transfection with Flag-GATA4 alone. Fig. 5C indicated that, the protein level expressions of GATA4 in 293T cells transfected with Flag-GATA4-wt or Flag-GATA4-K313R were reduced by prolonged CHX exposure.

GATA4 is known to activate CCND2 promoter [32]. Herein, we overexpressed wt or mutated GATA4 and CBP or HDAC1 in 293T cells, and measured CCND2 promoter activity, to detect whether GATA4 acetylation will impact the targeting effects between GATA4 and CCND2. As results shown in Fig. 5D and 5E, GATA4 and CBP together synergistically increased the CCND2 promoter activity, while HDAC1 antagonized GATA4-induced

Fig. 4. Deacetylation of GATA4 by HDAC1. (A) 293T cells were transfected with Flag-GATA4-wt, Myc-CBP and expression vectors encoding HDAC1-5. The cellular extract was immunoprecipitated with anti-Acetylate-Lys antibody. (B) 293T cells were transfected with Flag-GATA4-wt and HA-HDAC1. Cellular extract was collected for immunoprecipitation using anti-Flag or anti-IgG antibody followed by immunoblot using anti-HDAC1 antibody. (C) Extract from 293T cells were immunoprecipitated by anti-GATA4 antibody followed by immunoblotting by anti-HDAC1 antibody.

Fig. 5. Enhanced transcriptional activity of GATA4 by acetylation. (A) 293T cells were transfected with Flag-GATA4 or transfected with Flag-GATA4 plus Myc-CBP, and then subjected to CHX for 24 h. Protein expressions of GATA4 were probed by anti-Flag antibody. (B) 293T cells were transfected with Flag-GATA4 and/or HA-HDAC1 and subjected with CHX for 9 h. Protein expressions of GATA4 were probed by anti-Flag antibody. (C) 293T cells transfected with Flag-GATA4-wt or Flag-GATA4-K313R were subjected with CHX for 9 h. Protein expressions of GATA4 were probed by anti-Flag antibody. (D) 293T cells were transfected with Flag-GATA4-wt/Flag-GATA4-K313R and Myc-CBP. The CCND2 promoter activity was then detected. n = 3. (E) 293T cells were transfected with Flag-GATA4-wt/Flag-GATA4-K313R and Myc-HDAC1, after which CCND2 promoter activity was detected. n = 3. * P<0.05, ** P<0.01, and *** P<0.001 compared to control group. Ns, no significance, # P<0.05, and ## P<0.01 compared to the indicated group.

Fig. 6. Acetylation-defective mutant of GATA4 reduces hFOB1.19 cells growth. (A) hFOB1.19 cells were transfected with siGATA4, oeGATA4-wt or oeGATA4-K313R. Western blot was performed to detect GATA4 expression to ensure the transfection efficiency. (B) Viability of hFOB1.19 cells after siGATA4 transfection. $n = 3$. (C) Viability of hFOB1.19 cells after oeGATA4-wt or oeGATA4-K313R transfection. $n = 3$. (D) Apoptotic rate of hFOB1.19 cells after siGATA4, oeGATA4-wt or oeGATA4-K313R transfection. $n = 3$. (E) Protein levels of cleaved caspase-3, Bid, Bax, and Bcl-2 in hFOB1.19 cells after the indicated transfection. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared to control group. # $P < 0.05$ comparison between oeGATA4-wt and oeGATA4-K313R groups.

increase of CCND2 promoter activity. However, CBP and HDAC1 could not affect CCND2 promoter activity when lys-313 in GATA4 was not mutated ($P > 0.05$).

GATA4 mutation at K313 reduced the growth-promoting activities of GATA4 on hFOB1.19 cells

In order to investigate the role of acetylation-defective mutant of GATA4 on the growth of hFOB1.19 cells, siGATA4, oeGATA4-wt, oeGATA4-K313R were respectively transfected into cells. Western blot was performed to detect the transfection efficiency, and results showed that the protein expression of GATA4 was notably reduced by siGATA4 transfection, while was increased by oeGATA4-wt and oeGATA4-K313R transfection (Fig. 6A). MTT assay result indicated the viability of hFOB1.19 cells were significantly decreased by siGATA4 ($P < 0.01$), increased by oeGATA4 ($P < 0.01$ or $P < 0.001$), while oeGATA4-induced increase of cell viability was neutralized by GATA4 mutation at lys-313 (Fig. 6B and 6C). Flow cytometry detection results showed that the apoptotic cell rate was significantly increased by siGATA4 ($P < 0.01$), decreased by oeGATA4 ($P < 0.01$); unsurprisingly oeGATA4-induced decrease of cell apoptosis was neutralized by GATA4 mutation at lys-313 (Fig. 6D). Western blot analytical results further confirmed the alleviating effect of GATA4 point mutation on apoptosis. As shown in Fig. 6E, up-regulations of cleaved caspase-3, Bid and Bax, and down-regulation of Bcl-2 were observed in siGATA4 transfected cells; oeGATA4 affected these protein expressions resulted in a completely opposite impacts, and these alterations induced by oeGATA4 were alleviated by GATA4 mutation at lys-313.

Discussion

Bone remodeling is controlled by osteoblasts and osteoclasts, the imbalance between osteoblasts and osteoclasts leads to osteopetrosis or OS by changing bone mass. Recently, studies have demonstrated the necessity of GATA4 in osteoblasts, that GATA4 regulates osteoblast differentiation [15, 16], which contributes in estrogen-independent mineralization [14]. Here we identified GATA4 acetylation as a pivotal event in human osteoblastic hFOB1.19 cells. Specifically, we observed that GATA4 in hFOB1.19 cells was acetylated by P300/CBP, and the acetylation site was on lys-313; besides, the acetylation of

GATA4 could be impaired by HDAC1, rather than by HDAC2-5. GATA4 acetylation contributed to the transcriptional activity of GATA4. What's more, mutation of GATA4 on lys-313 reduced the growth-promoting activities of GATA4 on hFOB1.19 cells, suggesting the acetylation of GATA4 on lys-313 promoted osteoblastic cells growth *in vitro*.

Human HATs can be divided into several large families: MYST (TIP60, MOZ/MYST1, MORF/MYST4, HBO1/MYST2, MOF/MYST1), P300/CBP (P300, CBP), and GNAT (GCN5, PCAF, ELP3) [33]. These HATs have different lysine specificities, and remarkably, P300/CBP acetylates all four core histones in nucleosomes [34]. A previous study has revealed that P300 is able to acetylate lys-311, -318, -320 and -322 of GATA4 in myocardial cells [27]. In addition to P300, CBP possesses HAT activity and P300/CBP is able to acetylate GATA4 on two highly conserved, lysine-rich motifs near the zinc finger domains [35, 36]. Zeng *et al.* [27] for the first time observed that GATA4 was acetylated by P300/CBP on lysine residue K313, rather than by PCAF or GCN5.

In contrast to HAT, HDACs mediate histone deacetylation. According to the homology with the yeast counterparts, HDACs are grouped into five sub-families: class I (HDAC1, 2, 3, and 8), class IIa (HDAC4, 5, 7, and 9), class IIb (HDAC6 and HDAC10), class III (sirtuins) and class IV (HDAC11) [37]. In the current study, GATA4 co-immunoprecipitated with CBP was almost abolished by HDAC1 except by HDAC2-5, suggesting the acetylation of GATA4 mediated by CBP can be impaired by HDAC1.

Acetylation has been mentioned to regulate the function of GATAs at multiple levels such as DNA-binding activity, protein interaction, nuclear localization, protein stability, and transactivation activity [38]. In the current study, two inhibitors of HDAC activity (TSA and SAHA), which cause accumulation of acetylated histone species in a variety of mammalian cell lines [39, 40], were used to promote the acetylation of GATA4. As a result, the protein expressions of GATA4 were increased by TSA and SAHA, suggesting GATA4 protein has in some manner maintained its stability via histone acetylation. The accumulated GATA4 due to its stability promotes GATA4's functions, for example senescence, a terminal arrest of proliferation triggered by various cellular stresses including dysfunctional telomeres, DNA damage, oxidative stress, and genomic mutations [41]. On the other hand, our data from Co-IP suggested that GATA4 accumulation can be enhanced by histone acetylation via triggering GATA4 transcription. This observation was confirmed with the widely accepted viewpoint that histone acetylation is associated with an "open" chromatin configuration and active transcription [42].

It is well-known that CCND2, a protein response for cell cycle G1/S transition, is encoded by the CCND2 gene in humans. A previous study has pointed out that GATA4 could activate CCND2 promoter [43]. In the current study, GATA4 acetylation was observed to increase CCND2 promoter activity, while GATA4 mutated at lys-313 showed low transactivation potential. Considering that GATA4 acetylation is implicated in the transcription of CCND2, we speculate that it will also participate in hFOB1.19 cells growth. To test this hypothesis, the viability and apoptosis of hFOB1.19 cells were detected. Our data evidenced that GATA4 acetylation at lys-313 may be a pathway for increasing cell viability, and repressing apoptosis in a mitochondria-dependent pathway. Mutation at lys-313 decreased the ratio of Bcl-2 to Bax, increased Bid expression and sequentially activated caspase-3. This finding is consistent with the studies of Yanazume *et al.* [28], and Trivedi *et al.* [43], of which GATA4 acetylation was reported to be implicated in cardiac myocyte growth. Although, the mechanisms have not been fully resolved, one of the putative mechanisms of GATA4 acetylation for growth may be a functional modulation of CCND2 or other transcription factors related to cell growth.

Conclusion

In summary, our data suggest that GATA4 exists as an acetylated protein in osteoblastic hFOB1.19 cells. GATA4 was acetylated by P300/CBP on lysine residue K313. Acetylation regulates the stability and transcription of GATA4, and activates CCND2 transcription, may be via which histone posttranslational modification of GATA4 promoted hFOB1.19 cells

growth. The findings in this study provide a possibility for use GATA4 as an efficient targeted therapy in the control of OS. However, more effort is required to evaluate the potential usage of GATA4-based target in OS.

Disclosure Statement

No conflict of interests exists.

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