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A role for Gcn5 in cardiomyocyte differentiation of rat mesenchymal stem cells

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Abstract MSCs possess the capacity of self-renewal and potential of differentiation into various kinds of specialized tissue cells including myocardiocytes. From self-renewing to oriented differentiation, chromatin is remodeled into heritable states that allow activation or maintain the repression of regulatory genes, which means specific genes in self-renewing switched off and specific genes in oriented differentiation activated (Bernstein et al. Cell 125:315–326, 2006). These epigenetic states are established and controlled largely by specific patterns of histone posttranslational modifications, in particular, histone acetylation (Li Nat Rev Genet 3:662–673, 2002). In cardiomyocyte differentiation of rat MSCs, we focused on Gcn5, which linked a known transcriptional coactivator with catalytic histone acetyltransferase activity (Brownell et al. Cell 84:843–851, 1996). To clarify participatory in vivo role of Gcn5, using an RNA interference (RNAi) strategy employing shRNA to specifically knockdown Gcn5 expression in MSCs, we found that HAT activity altered dynamically depended on the inhibition of Gcn5 during MSCs differentiation. Chromatin immunoprecipitation (ChIP) assay showed the increased binding of acetyl histone H3 to the early cardiomyocyte-specific genes GATA4 and NKx2.5 promoters in cardiomyocyte differentiation of MSCs by 5-azacytidine inducing, whereas the decreased binding with lower Gcn5 expression. Cell ultrastructure analysis revealed that MSCs induced by 5-azacytidine possess morphological characteristics of cardiomyocyte cells. The shape of MSCs transfected by Gcn5 RNAi was similar to normal MSCs, but the chromatin showed heavy electron-density and a hard-packed structure. This intermediate state of chromatin may be an inactive part of MSCs differentiation. These results demonstrate that Gcn5, possessing acetyltransferase activity, is involved in regulating chromatin configuration around GATA4 and NKx2.5 in cardiomyocyte differentiation of rat MSCs by changing the level of histone acetylation. HAT activity depending on Gcn5 is important in differentiation of MSCs into cardiomyocytes as a consequence of the remodeling of the chromatin configuration caused by modification of histone H3.

Keywords Mesenchymal stem cells · Differentiation · Cardiomyocyte · Gcn5 · Histone acetylation · HAT activity

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Introduction

MSCs are characterized by extensive self-renewal and the ability to differentiate into various cell-type specific progeny. They have characteristic shapes and specialized functions under certain conditions, or if they receive the correct signals (e.g., 5-azacytidine) [1]. Given the special properties, stem cells are tightly regulated by multiple genes and gene networks. Progression from stem cells

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into different differentiated progeny requires long-lasting changes in gene expression. Emerging evidences suggest that differentiation progress involves the action of a unique epigenetic program upon gene expression. Unique properties of stem cells defined as "stemness" may be determined by acetylation and methylation of histones near gene promoters that regulate gene transcription. Specific epigenetic modifications mark the degree of activity of the transcribing genes. Therefore, it is quite plausible that regulation of epigenetic modification patterns may lead to an altered potential of stem cell self-renewal and differentiation.

Histone deacetylation is one of the main components of an epigenetic program [2]. Histone acetyltransferases (HATs) are involved in the acetylation of core histones, which is an important event for transcription regulation through alterations in the chromatin structure in eukaryotes. In most cases, histone acetylation enhances transcription while histone deacetylation represses transcription. In agreement with this idea, the acetylation of lysine residues in histone tails is associated with active gene transcription [3]. Disturbances of HAT activity may shift the balance between an active and silent chromatin conformation. Molecular events provoking changes in the pattern of histone acetylation appears to govern transition from pluripotent to committed state, resulting in an altered differential state of MSCs.

A member of HATs, general control non-derepressible (Gcn5), is a transcriptional coactivator with histone acetyltransferase activity, which is conserved with regard to structure as well as its histone substrates throughout the eukaryotes. Gcn5, acts as a histone acetyltransferase within the Ada and SAGA adaptor complexes, is important in transcription activation mediated by interactions with transcription activators and general transcription factors [4]. Histone acetylation by Gcn5 has been implicated in displacement of promoter nucleosomes during transcriptional activation [5, 6] and in aiding recruitment of TBP, RNA Polymerase II (Pol II) [7], and coactivators [8, 9] to promoter regions. Meanwhile, the SAGA complex characterized as a histone acetyltransferase, because of the Gcn5 subunit, mediates histone acetylation of gene promoters to enhance transcriptional activation and facilitates elongation. The level of compaction of chromatin dictates accessibility to genomic DNA and, therefore, has a key role in establishing and maintaining distinct gene expression patterns and consequently pluripotent state and differentiation fates of stem cells. The HAT domain is the most highly conserved part of Gcn5 and it has been shown to be inter-changeable [10]. And some data indicate that Gcn5 is a major H3 HAT and/or H2B HAT in mammalian development. Thus, Gcn5 is structurally conserved throughout evolution and appears to function in a conserved fashion by acetylating a conserved set of lysine residues in target proteins. Therefore, Gcn5 is expected to play a distinct role for differential expression of regulatory genes during the differentiation of MSCs in this research. We hypothesized that Gcn5 is a distinct role for differential expression of regulatory genes, and pivotal regulatory site in chromatin remodeling caused by histone acetylation in MSCs differentiation [11].

In the present study, MSCs were induced into cardiomyocytes with the costimulatory signal of 5-azacytidine. Gcn5-shRNA was chemically synthesized and transferred into MSCs by Lipofectamine 2000. It was found that Gcn5 expression in Gcn5-RNAi-treated MSCs was significantly inhibited. MSCs with lower Gcn5 expression showed low level of HAT activity despite the costimulatory signal of 5-azacytidine. Furthermore, in cardiomyocyte differentiation of MSCs, MSCs obviously represented decreased binding of acetyl histone H3 to the early cardiomyocyte-specific genes GATA4 and NKx2.5 promoters when MSCs were co-treated with Gcn5 RNAi and 5-azacytidine, whereas the increased binding just with 5-azacytidine. Cell ultrastructure analysis revealed that MSCs possess morphological characteristics of cardiomyocyte cells with inducing signal of 5-azacytidine, but possess similar cell morphology to normal MSCs with Gcn5 RNAi despite of 5-azacytidine. Meanwhile, the chromatin showed heavy electron-density and a hard-packed structure when MSCs were co-treated with Gcn5 RNAi and 5-azacytidine.

Taken together, Gcn5-silenced MSCs by RNAi will be a useful model for clarifying the powerful impacts of Gcn5 on HAT activity in cardiomyocyte differentiation of MSCs. Gcn5, relating to transcriptional activation, is involved in regulating chromatin configuration around GATA4 and NKx2.5 by changing the level of histone acetylation. HAT activity depending on Gcn5 is important to differentiation of MSCs into cardiomyocytes as a consequence of the remodeling of the chromatin configuration caused by modification of histone H3.

Materials and methods

Isolation and culture of MSCs

Samples of bone marrow (BM) were collected from 2 month-old male wistar rats by flushing femurs and tibias with complete medium containing Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, USA), 10% fetal calf serum (FBS) (PAA, CA) and penicillin/streptomycin (Invitrogen, USA). The BM solution was concentrated and washed twice in DMEM, and then cultured at 37°C in an atmosphere of 5% CO2. Cells were retrieved by trypsinization and passaged when they reached a confluence of 90–95%. Cells were collected at passage 4.

Preparation of short-hairpin RNAs (shRNAs) and plasmid construction

pGenesil-1 plasmid containing human/mouse U6 promoter was used for the RNA interference (RNAi) vector. We constructed one recombinant plasmid for rat Gcn5 mRNA RNAi (Gcn5 RNAi) and one recombinant plasmid for negative control. The target sequence for rat Gcn5 mRNA RNAi was 5′-tgattcatcaggtgggtgc in rat K (lysine) acetyltransferase 2A (Kat2a) RNA (NM_001107050), and the negative control target sequence was 5′-gacttcataaggcgc atgc not found in rat cDNA database (HK control). We also purchased a pGenesil-1 recombinant plasmid containing enhanced green fluorescence protein (eGFP) from Genesil Biotech (Wuhan, China). Our previous experiment proved that the Gcn5 RNAi plasmid possessed significant inhibitory effect on the expression of Gcn5 mRNA [12].

Induction and transfection

1.0×10^5 MSCs were incubated in a well of a 6-well plate to 80% confluence, and then transfected with 4.8 μg Gcn5 RNAi plasmid (or negative control plasmid) by 10 μl lipofectamine2000 in 2 ml opti-MEM® I reduced serum medium without antibiotics. Six hours after the transfection, the medium was replaced by complete medium, and the cells were induced by 10 μmol/l 5-azacytidine (Sigma-Aldrich, USA) with complete medium for 24 h. Every experiment was repeated for eight dishes of cells.

Suppression of Gcn5 mRNA in MSCs by shRNA

MSCs were randomly divided into four groups: empty control group (parental MSCs), HK group (MSCs transfected by negative control plasmid (named HK) with lipofectamine2000), Gcn5 RNAi group (MSCs transfected by Gcn5 RNAi plasmid with lipofectamine2000) and Liposome control group (MSCs treated by lipofectamine2000). After the transfection for 24 h, total RNA was extracted with TRI zol® (Invitrogen, Inc.) and cDNA was synthesized using oligo d $(T)_{15}$ primer and M-MLV (Promega, USA) reverse transcriptase. GAPDH was used as internal control. The primers used were as follows: Gcn5 forward primer: ctggccttgatcaaggat; Gcn5 reverse primer: accttgatgtccttggag. GAPDH forward primer: acatcaaa tggggtgatgct; GAPDH reverse primer: gtggttcacacccatc acaa. RT reaction was performed at 25°C for 10 min, 37°C for 60 min. PCR was performed as follows: 94°C for 30 s, 40 cycles, 94°C for 30 s, 60°C (Gcn5) or 57°C (GAPDH) for 30 s, 72°C for 30 s, and an extension at 94°C for 7 min. PCR products were separated by agarose gel electrophoresis. The expression intensity of Gcn5 was denoted with the ratio of the photodensity of the RT-PCR products of Gcn5 to GAPDH.

Measurement of Gcn5 expressions by western blotting

Cultured cells (3×10^6) were harvested and lysed in a lysis buffer (0.5 mol/l EDTA, 1 mol/l Tris–HCl, pH 7.4, 0.3 mol/l sucrose, and protease inhibitor cocktail). Protein extract was separated by SDS-PAGE and transferred onto a nitrocellulose membrane. The primary antibody used was 1:400 anti-rat Gcn5 antibody (Santa Cruz, CA, USA) or 1:1000 anti-GAPDH antibody (Santa Cruz, CA, USA). HRP-conjugated secondary antibody (Santa Cruz, CA, USA) was used at 1:2000–5000. Blotted antibodies were visualized by ECL and X-ray film exposure (Kodak Scientific Imaging film, X-Omat Blue XB-1, USA). The density of positive bands was determined by an Imaging System (Alpha Innotech).

Nuclear extract preparation and HAT activity measurement

Cultured MSCs were harvested by scraping, and nuclear extract was isolated using a nuclear extract kit (Active Motif, USA). Protein concentrations were determined by the bicinchoninic acid (BCA) method using a commercial kit (Pierce, USA). The aliquots were stored at 80°C until use. The adjusted weight of nucleic extracts from MSCs (60 ng per sample) was detected according to the manufacturer's protocol. HAT assay was performed using the HAT Activity Colorimetric Assay kit (Upstate Biotech, USA). Samples were read in a microplate reader at 440 nm ($\varepsilon 440\ \text{nm} = 37000\ \text{M}^{-1}\text{cm}^{-1}$). HAT activity was measured three times per nuclear extract sample, and the mean value from three independent samples was calculated.

Chromatin immunoprecipitation (ChIP) assay

ChIP assay was preformed using a kit (EZ ChIPTM, Upstate). MSCs were treated in 1% formaldehyde for 10 min to cross-link the DNA binding protein in situ. The chromatin was sheared by sonication to 200 to 1000 bp (Fig. 4a), and then mixed with mouse anti-rat acetylhistone H3 antibody, non-specific mouse IgG (negative control), anti-RNA polymerase II antibody (positive control), or nothing added (input control) at 4°C overnight. Antibody/acetyl histone H3/genomic DNA fragment complexes were then collected using protein G agarose. Protein–DNA complexes were eluted from protein G-agarose, and their cross-links were reversed, and the proteins were digested with proteinase K. Input DNA, obtained from chromatin that was crosslink reversed in a similar way to the sample, served as a positive control for PCR

effectiveness. DNA fragments were purified with a QIAquick Spin Kit (Qiagen, CA). The primer pairs used were 5′-gcctggaatagggaggtagg and 5′-gggtcagttggtcagagagg for GATA4 promoter, 5′-ccacgtgaatgcttccttct and 5′-gtctgccc cagagtagcttg for NKx2.5 promoter. PCR products were separated by agarose gel electrophoresis. For PCR, 1 μl from a 50 μl DNA extraction and 25–30 cycles of amplification were used.

Ultrastructure of MSCs by transmission electron microscopy (TEM)

Fresh MSCs were immersed in 2.5% glutaraldehyde at 4°C. After washing with phosphate-buffered saline (PBS), they were fixed with 1% osmium tetroxide. They were then dehydrated in a graded series of ethanol solutions, embedded in araldite, and polymerized for 24 h at 37°C. Ultra-thin sections (50 nm) were cut with an ultramicrotome (LKB-V, Bromma, Sweden), contrasted with uranyl acetate and lead citrate, and observed under TEM (H-600, Hitachi, Tokyo, Japan).

Statistical analyses

Data are as means ± standard deviation (SD). Comparisons among groups were evaluated by one-way ANOVA followed by the least square difference test. The two sample *t*-test was used for comparison between two groups. $P < 0.05$ was considered significant.

Results

Gcn5 expression was down-regulated in MSCs by RNA interference

MSCs were transfected with the Gcn5 RNAi plasmid. MSCs transfected with the HK negative control plasmid, without transfection and only treated with lipofectamine 2000 were used as the three negative controls. After transfection for 24 h, Gcn5 mRNA in MSCs was reduced by 33.2% (Fig. 1), and Gcn5 protein in MSCs was reduced by 35.7% (Fig. 2), compared with those in MSCs without transfection. Gcn5 expression remained unchanged in MSCs transfection with the negative control plasmid or treated with lipofectamine 2000 only. We then used MSCs transfected with Gcn5 RNAi plasmid to study their biological behaviors, and those transfected with the HK negative control plasmid as the control.

HAT activity was increased in MSCs induced with 5-azacytidine, and was inhibited in MSCs with lower Gcn5 expression and induced with 5-azacytidine

5-Azacytidine induces the differentiation of MSCs into cardiomyocytes. In the induced MSCs, epigenetic changes are expected to take place in a set of genes leading to the changes of their transcription level shortly after the induction. Gcn5 is a catalytic subunit in multiple HAT

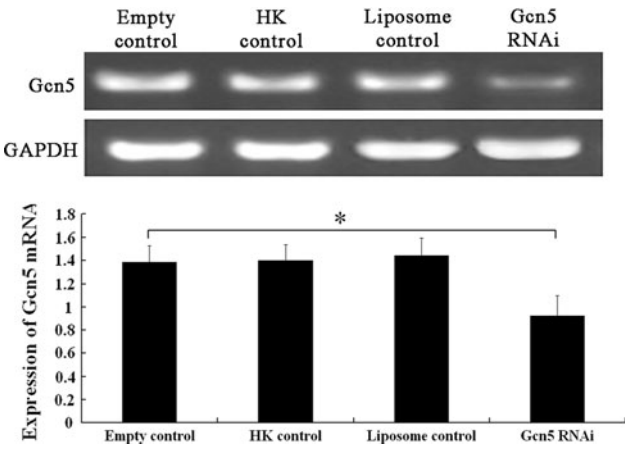

Fig. 1 Gcn5 mRNA in MSCs after transfection with Gcn5 RNAi plasmid. MSCs were transfected with Gcn5 RNAi plasmid (Gcn5 RNAi), or negative control plasmid (HK control). MSCs without treatment (empty control) or treated with lipofectamine 2000 only (liposome control) were also used as negative controls. After the transfection for 24 h, MSCs were harvested for RT-PCR. Gcn5 mRNA in MSCs was quantified by RT-PCR, and the result was normalized by GAPDH cDNA level. In MSCs transfected with Gcn5 RNAi plasmid, Gcn5 mRNA was reduce by 33.2% with respect to empty control group (* $P < 0.05$)

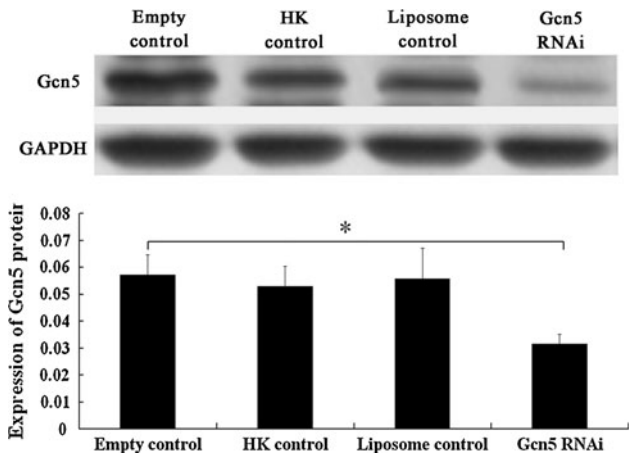

Fig. 2 Gcn5 protein in MSCs after transfection with Gcn5 RNAi plasmid. Protein samples for western blot were extracted from the four groups of MSCs as described in the legend to Fig. 1. The primary antibodies used were anti-rat Gcn5 antibody and anti-GAPDH antibody. Positive bands were measured by a densitometer and the result was normalized by the density of respective GAPDH band. In MSCs transfected with Gcn5 RNAi plasmid, Gcn5 was reduce by 35.7% compared with the amount of empty control group (* $P < 0.05$)

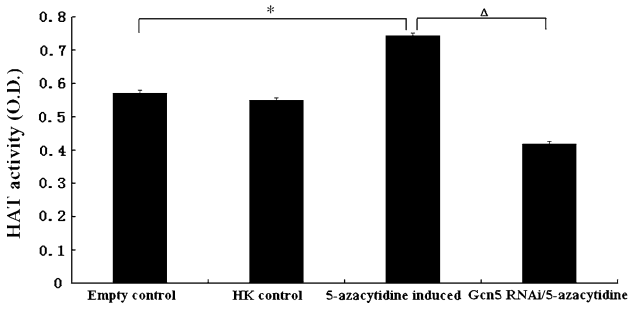

Fig. 3 Histone acetyltransferase (HAT) activity in MSCs after transfected with Gcn5 RNAi plasmid and induced with 5-azacytidine. After the transfection with Gcn5 RNAi plasmid for 6 h, MSCs were then treated with 10 μM 5-azacytidine for 24 h (Gcn5 RNAi/5-azacytidine). MSCs treated with 5-azacytidine for 24 h were used as the positive control (5-azacytidine inducd). MSCs transfected with the negative control plasmid (HK control), and those without any treatment (empty control) were used as the 2 negative controls. In the positive control MSCs, HAT activity increased significantly by 30.2% (* $P < 0.05$). Compared with empty control group. In MSCs transfected with Gcn5 RNAi plasmid and then induced with 5-azacytidine, HAT activity decreased by 43.8% compared with that in the positive control ($^{\Delta}P < 0.05$)

complexes. Therefore, Gcn5 is considered to be involved in this process. MSCs were transfected with the Gcn5 RNAi plasmid. MSCs were transfected with Gcn5 RNAi plasmid, and then induced by 5-azacytidine (Gcn5 RNAi/5-azacytidine group). MSCs were treated with 5-azacytidine used as positive control. MSCs were transfected with HK negative control plasmid (HK control group), or without treatment (empty control) were used as negative controls. In MSCs induced with 5-azacytidine as the positive control, HAT activity increased by 30.2% compared with empty control. In MSCs with lower Gcn5 expression and induced with 5-azacytidine, HAT activity decreased by 43.8% compared with that of the positive control (Fig. 3).

Acetyl histone H3 on GATA4 and NKx2.5 promoter regions was increased in MSCs induced with 5-azacytidine, and decreased in MSCs with lower Gcn5 expression and induced with 5-azacytidine

To observe Gcn5 in the acetylation of histone H3 on GATA4 and NKx2.5 promoters, 5-azacytidine was used to induce the differentiation of MSCs with normal Gcn5 expression and those with lower Gcn5 expression by RNAi. Samples were extracted from the four groups of MSCs as described in the results of HAT activity measurement. In MSCs with normal Gcn5 expression and induced with 5-azacytidine, acetyl histone H3 on GATA4 and NKx2.5 promoters increased significantly compared with those of HK negative control. In MSCs with lower Gcn5 expression and induced with 5-azacytidine, acetyl histone H3 on GATA4 promoter region decreased by 21.1% compared with the result from empty control, and by 48% compared with that from MSCs of normal Gcn5 expression induced with 5-azacytidine; acetyl histone H3 on NKx2.5 promoter region decreased by 51.4% compared with the result from empty control, and by 66.7% compared with that from MSCs of normal Gcn5 expression induced with 5-azacytidine (Fig. 4). Therefore, acetylation of histone H3 catalyzed by Gcn5 may alter the status of histone acetylation at the promoter region of GATA4 and NKx2.5 genes, and then, regulate the transcription of GATA4 and NKx2.5 genes during cardiomyocyte differentiation of MSCs.

Ultrastructural changes of MSCs induced with 5-azacytidine

Parental MSCs contained a large elliptical nucleus, scattered chromatin, and a few organelles (Fig. 5a). MSCs induced with 5-azacytidine showed a long shape, intracellular parallel filaments resembling sarcomeres with intercalated large mitochondria, and more organelles in the cytoplasm including several secretory vesicles, rough endoplasmic reticulum, and ribosomes, resembling the ultrastructure of a cardiomyocyte (Fig. 5b, c). The appearance of MSCs with lower Gcn5 expression and induced with 5-azacytidine was similar to that of normal MSCs (Fig. 5d). In normal MSCs and 5-azacytidine-treated MSCs, chromatin showed light electron density and had a loose structure (Fig. 5e–f). The appearance of MSCs treated with Gcn5 siRNA before 5-azacytidine was similar to that of normal MSCs except that chromatin condensation was present (Fig. 5g–h).

Discussion

MSCs possess the proliferation and multi-lineage differentiation potentials. Given the special properties, MSCs require long-lasting changes in genes expression and gene networks. In the recent few years, it has been established that epigenetic mechanisms could be critical for determining the fate of MSCs. Unique properties of stem cells defined as "stemness" may be determined by acetylation and methylation of histones near gene promoters that regulate gene transcription. Specific epigenetic modifications mark the degree of activity of the transcribing genes. And it is believed that acetylation of lysine residues by HAT enzymes is associated with active gene transcription. However, the functions of individual HAT in specific differentiation programs are not well defined.

The most well-characterized HAT enzymes are those in the Gcn5 family. Gcn5 was the first nuclear HAT protein to be identified [13], and previous studies of Gcn5 as a transcription cofactor [14] set the stage for genetic,

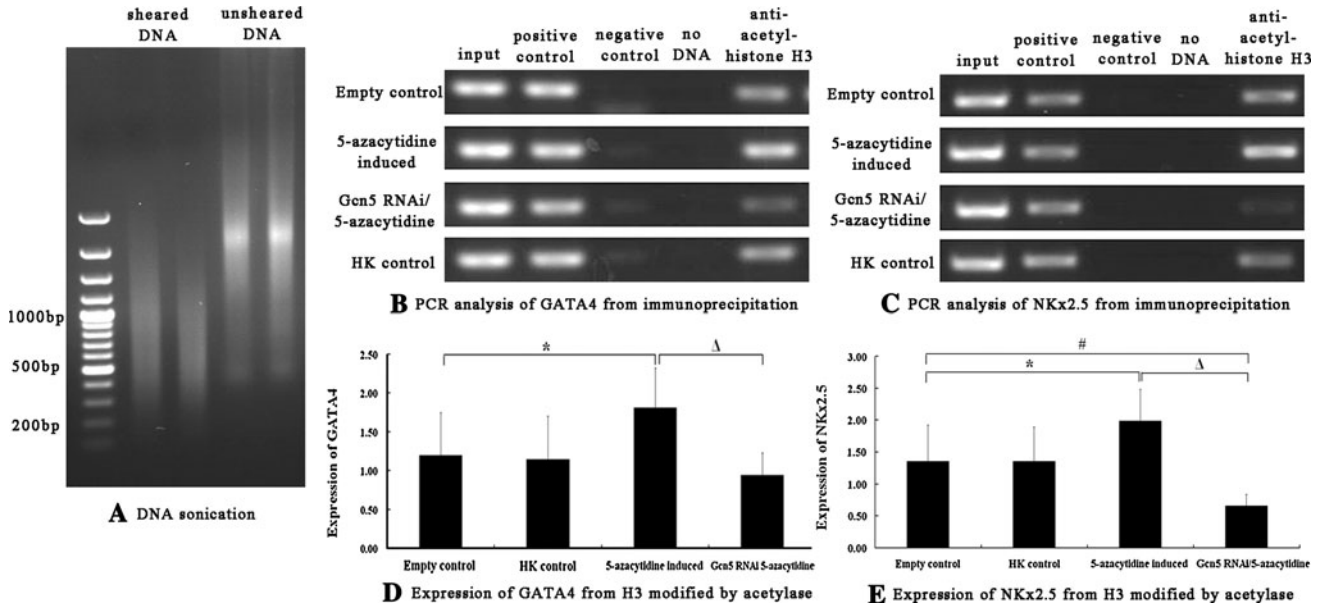

Fig. 4 The increased binding of acetyl histone H3 to GATA4 and NKx2.5 promoter regions in MSCs differentiating into cardiomyocyte induced by 5-azacytidine, whereas the decreased binding with lower Gcn5 expression. **a** Genomic DNA samples were sheared by sonication to 200 to 1000 bp before treated with antibodies for ChIP assay. **b**, **c** PCR product bands of GATA4 promoter region and NKx2.5 promoter region were amplified from the genomic DNA obtained in ChIP. Nuclear extracts were prepared and immunoprecipitated by acetyl-histone H3, or normal rabbit IgG (as negative control) or RNA polymerase II (as positive control). Input and a no DNA tube act as controls for DNA contamination. **d**, **e** The amount of GATA4 promoter region and NKx2.5 promoter region were calculated from the density of the PCR product bands normalized by the respective densities of the PCR product bands from input controls. In MSCs induced with 5-azacytidine, acetyl histone H3 on GATA4 and NKx2.5 promoter regions increased significantly (* $P < 0.05$, versus empty control). However, in MSCs with lower Gcn5 expression and induced with 5-azacytidine, the binding of acetyl histone H3 at NKx2.5 promoter was decreased significantly ($^{\#}P < 0.05$, vs. Gcn5-inducd group). When Gcn5 was inhibited, the binding of acetyl histone H3 at GATA4 promoter could not be increased anymore ($^{\Delta}P < 0.05$ vs. empty control). Data are means of independent experiments ± standard deviation. The pixel intensity for each band was normalized to the input DNA in the immune precipitates

biochemical, and structural studies of the enzymatic activity. In the present study, to define the functions of Gcn5 during cardiomyocyte differentiation of MSCs, Gcn5-shRNA was chemically synthesized and transferred into MSCs. And then, we determined whether or not the GCN5 deficiency influences on the expression of HAT activity. Data showed that Gcn5 inhibition could lead to a dramatic reduction in HAT activity. Past studies have shown that HAT activity of Gcn5 was required for Gcn5's function in transcriptional activation [15], which correlated with acetylation of histones at Gcn5-dependent promoters [13] and chromatin remodeling [16].

The HAT members play much diverse and broader roles in cell differentiation [17, 18]. In our study, to clarify the participation of HAT activity depending on Gcn5 in cardiomyocyte differentiation of MSCs, ChIP was used to detect the biding of acetyl histone H3 across the early cardiomyocyte-specific gene GATA4 and NKx2.5 promoter regions in MSCs provided Gcn5 RNAi or not. Data showed decreased binding of acetyl histone H3 in the promoter regions of GATA4 and NKx2.5 because of inhibition of Gcn5 during the cardiomyocyte differentiation of MSCs. GATA4 and NKx2.5 are the initial expression of a cascade of cardiac-associated transcription factors [19]. Some studies [20] found that stem-cell expression of GATA4 and NKx2.5 may be important for the stem cell to acquire a cardiac phenotype in culture. Meanwhile, acetylation of the lysine residues of H3 has a crucial role in chromatin packaging and in the regulation of gene transcription [21]. Therefore, we have reason to believe the decreased binding of acetyl histone H3 caused by the inhibition of Gcn5 could repress the transcription of NKx2.5 and GATA4 during the cardiomyocyte differentiation of MSCs.

Genome of MSCs undergoes global changes in gene expression during the transition from a pluripotent to a committed state. The level of compaction of chromatin dictates accessibility to genomic DNA and, therefore, has a key role in establishing and maintaining distinct gene expression patterns and consequently pluripotent state and differentiation fates of MSCs. In this work, electron microscopy was used to observe the ultrastructure of the MSCs co-cultured with 5-azacytidine and Gcn5 RNAi, or 5-azacytidine alone. Ultrastructural investigation revealed that MSCs possess morphological characteristics of cardiomyocyte cells with inducing signal of 5-azacytidine, but possess similar cell morphology to normal MSCs with

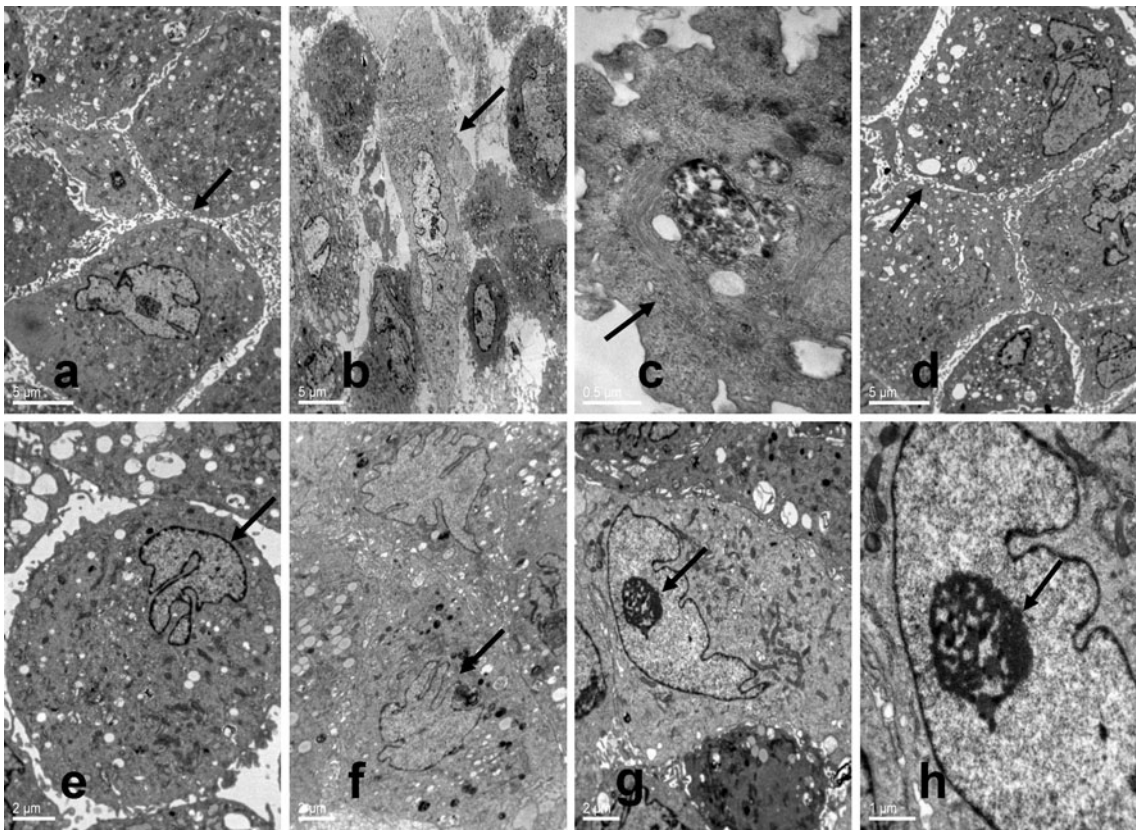

Fig. 5 Ultrastructural appearance of MSCs under different processing conditions. **a** Mature MSC contained a large elliptical nucleus, scattered chromatin, and a few organelles. Bar = 5 μm. **b** and **c** 5-azacytidine-treated MSC showed a long shape and parallel filaments resembling sarcomeres with intercalated large mitochondria, and more organelles in cytoplasm, including several secretory vesicles, rough endoplasmic reticulum, and ribosomes. Bar = 5 μm. **d** The appearance of a MSC transfected with Gcn5 RNAi plasmid and induced with 5-azacytidine was similar to that of normal MSCs. Bar = 5 μm. **e**, **f** In normal MSCs and 5-azacytidine-treated MSCs, chromatin showed light electron-density and a loose structure. Bar = 2 μm. **g** (Bar = 2 μm), **h** (Bar = 5 μm). In MSCs treated with Gcn5 RNAi berore 5-azacytidine, chromatin showed a heavy electron-density and a hard-packed structure

Gcn5-RNAi-treated despite 5-azacytidine. Meanwhile, the chromatin showed heavy electron-density and a hard-packed structure when MSCs were co-cultured with Gcn5-RNAi and 5-azacytidine. Histone acetylation, depending on Gcn5, may facilitate chromatin-based processes by unwinding chromatin structure and ensuring DNA accessibility during the cardiomyocyte differentiation of MSCs.

Conclusion

As a transcription-related HAT, Gcn5 is required for HAT activity in the cardiomyocyte differentiation of MSCs. The loose balance of H3 modification caused by Gcn5 inhibition could interfere the biological characteristics of MSCs, and take part in the regulation of MSCs differentiation by changing acetylation level of H3 in regulatory gene promoters. Gcn5 biology and its participation in HAT activity are important "switches" for formulating strategies for cardiomyocyte differentiation of MSCs.

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