Biochemical and Biophysical Research Communications 432 (2013) 612–617

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Downregulated LncRNA-ANCR promotes osteoblast differentiation by targeting EZH2 and regulating Runx2 expression

Lin Zhu, Pei-Cheng Xu *

Department of Oral Medicine, Dental Clinic of Xuhui District, 685 Zhaojiabang Road, Shanghai 200032, People's Republic of China

ARTICLE INFO

Article history:
Received 4 February 2013
Available online 21 February 2013

Keywords:
ANCR
EZH2
Runx2
Osteoblast differentiation

ABSTRACT

Long noncoding RNAs (lncRNAs) are key regulators of diverse biological processes such as transcriptional regulation, cell growth and differentiation. Previous studies have demonstrated that the lncRNA-ANCR (anti-differentiation ncRNA) is required to maintain the undifferentiated cell state within the epidermis. However, little is known about whether ANCR regulates osteoblast differentiation. In this study, we found that the ANCR expression level is significantly decreased during hFOB1.19 cell differentiation. ANCR-siRNA blocks the expression of endogenous ANCR, resulting in osteoblast differentiation, whereas ANCR overexpression is sufficient to inhibit osteoblast differentiation. We further demonstrated that ANCR is associated with enhancer of zeste homolog 2 (EZH2) and that this association results in the inhibition of both Runx2 expression and subsequent osteoblast differentiation. These data suggest that ANCR is an essential mediator of osteoblast differentiation, thus offering a new target for the development of therapeutic agents to treat bone diseases.

1. Introduction

Mesenchymal stem cells (MSCs) have the potential to differentiate into multiple connective tissue cell types, including osteoblasts, myoblasts, adipocytes, and chondrocytes [1,2]. The differentiation of MSCs into specific lineage depends on the extracellular cues received by these cells. Bone morphogenetic protein (BMP)-2, parathyroid hormone (PTH), and Runt-related transcription factor 2 (Runx2) plays an important roles in the regulation of osteoblast differentiation [2–4]. The alteration or malfunction of this regulation results in pathological consequences [5].

Long noncoding RNAs (lncRNAs) have been proposed to be a key regulators of diverse biological processes, such as transcriptional regulation, cell growth, and differentiation [6,7]. Ng et al., demonstrated that lncRNAs promote neuronal differentiation by associating with chromatin modifiers and transcription factors [6]. Some hESC-specific lncRNAs involved in pluripotency maintenance were shown to physically interact with SOX2 and SUZ12 [6]. Sunwoo et al., found that MEN ε/β lncRNAs are upregulated during muscle differentiation and are essential components of paraspeckles [8]. The knockdown of MEN ε/β results in the disruption of nuclear paraspeckles. In the epidermis, lncRNA-ANCR (anti-differentiation ncRNA) loss abolishes the normal blockade of differentiation in the progenitor-containing compartment. Thus, ANCR is required to maintain the undifferentiated cell state within the epidermis. However, little is known about the role of ANCR in the osteoblast differentiation of hMSCs.

EZH2 is highly expressed in embryonic stem cells (ESCs) and is involved in the differentiation of these cells into different cell lineages, including osteoblasts, hepatocytes and neurons [9,10]. EZH2 catalyzes histone H3 trimethylation at lysine 27 (H3K27me3) in target gene promoters to silence gene expression [11]. Wei et al., demonstrated that the CDK1-dependent phosphorylation of EZH2 disrupts methyltransferase activity of EZH2 and suppresses the methylation of H3K27, promoting the differentiation of MSCs into osteoblasts. Yu et al., found that EZH2 regulates the neuronal differentiation of MSCs through PIP5K1C-dependent calcium signaling [9]. EZH2 also regulates muscle gene expression and skeletal muscle differentiation [12]. Recent studies have demonstrated that some lncRNAs physically interact with EZH2 to regulate gene expression. lncRNA-HOTAIR targets Polycomb Repressive Complex 2 (which contains the H3K27 methylase EZH2) to silence the *HOXD* gene [13]. Therefore, we speculated that ANCR may regulate the osteoblast differentiation of ESCs by associating with EZH2.

Based on these findings, we investigated the molecular mechanism by which ANCR regulates osteoblast differentiation. We found that ANCR is an important regulator of osteoblast differentiation. ANCR associates with EZH2, and this association results in the inhibition of both Runx2 expression and subsequent osteoblast differentiation.

* Corresponding author. Fax: +86 21640343096.
E-mail address: xupeicheng11@yahoo.cn (P.-C. Xu).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.02.036

2. Materials and methods

2.1. Cell culture

The human fetal osteoblastic cell line hFOB1.19 was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and maintained at 33.5 °C in complete medium consisting of 1:1 Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 medium supplemented with 10% FBS (Atlas Biologicals, Fort Collins, CO) and 0.3 mg/mL G418/geneticin (Calbiochem, Gibbstown, NJ). For osteoblastic differentiation, confluent cultures of hFOB1.19 cells were maintained in complete medium with the addition of the following differentiation cocktail: 100 μg/μL ascorbic acid, 10^{-8} M menadione, 5 mM β-glycerophosphate, and 10^{-7} M 1–25(OH)2-vitamin D3 (Sigma, St. Louis, MO) [2].

2.2. Real-time polymerase chain reaction (PCR)

The total RNA was extracted from hFOB1.19 cells using the Trizol reagent (Invitrogen, Carlsbad, CA), and reverse transcription (RT) reactions were performed using random primers and an M-MLV Reverse Transcriptase kit (Invitrogen, Carlsbad, CA). Real-time PCR was performed using a standard protocol and the SYBR Green PCR kit (Toyobo, Osaka, Japan). β-Actin was used as a reference for the RNAs. The ΔCt values were normalized to β-actin level. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative gene expression levels. Each sample was analyzed in triplicate.

2.3. Measurement of hFOB1.19 cell differentiation

Alkaline phosphatase (ALP) and osteocalcin are phenotypic markers of early-stage differentiated osteoblasts and terminally differentiated osteoblasts, respectively [14]. In this study, hFOB1.19 cell differentiation was analyzed by assaying the ALP and osteocalcin mRNA levels as described above. The ANCR-siRNAs and EZH2-siRNAs were mixtures of three siRNAs and were purchased from Santa Cruz (the sequences are not disclosed). pcDNA-ANCR and pcDNA-EZH2 were constructed in our laboratory.

2.4. RNA pull-down and RNA immunoprecipitation (RIP)

ANCR RNA was *in vitro* transcribed from pcDNA-ANCR and was biotin-labeled with the T7 RNA polymerase (Roche) and the Biotin RNA Labeling Mix (Roche Diagnostic, Indianapolis, USA). The nuclear extract of hFOB1.19 cells (3 μg) was mixed with biotin-labeled RNAs (200 pmol), incubated with streptavidin agarose beads (Invitrogen) and washed. The retrieved proteins were detected using the standard Western blot technique.

RIP experiments were performed using a Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore, USA) according to the manufacturer's instructions. The antibody for EZH2 used in the RIP assays was ab3748 (Abcam, Cambridge, MA). The coprecipitated RNAs were detected by RT-PCR.

2.5. Western blot analysis

Western blot analysis to assess the EZH2 and β-actin expression levels was performed as previously described [15]. Primary antibodies for EZH2 and β-actin were purchased from Sigma (MO, USA).

2.6. Chromatin immunoprecipitation (ChIP)

ChIP was performed using the EZ ChIP™ Chromatin Immunoprecipitation Kit (Millipore, Bedford, MA) according to the manufacturer's protocol. Briefly, cross-linked chromatin was sonicated to yield 200–1000 bp fragments. The chromatin was immunoprecipitated using an anti-H3K27me3 antibody (Santa Cruz). Normal human IgG was used as a negative control. Quantitative PCR was performed according to the method described above.

2.7. Statistical analysis

All data are expressed as the mean ± standard deviation (SD) from at least three separate experiments. The differences between groups were analyzed using Student's *t* test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. The downregulation of ANCR promotes osteoblast differentiation

Previous studies have indicated that ANCR is required to maintain the undifferentiated cell state within the epidermis [16]. To investigate whether ANCR regulates osteoblast differentiation, we first assayed the ANCR levels during hFOB1.19 cell differentiation. We found that the ANCR levels were significantly decreased in hFOB1.19 cells cultured in osteoblastic differentiation medium (Fig. 1(A)). To investigate the biological role of ANCR in the regulation of hFOB1.19 cell differentiation, cells treated with ANCR-siRNA were analyzed. Fig. 1(B) shows that the ANCR-siRNA suppressed endogenous ANCR expression and resulted in increases in the mRNA levels of ALP and osteocalcin (osteoblast differentiation markers) (Fig. 1(C) and (D)). When ANCR was overexpressed in hFOB1.19 cells, the ALP and osteocalcin mRNA levels were reduced compared with the levels in the control cells (Fig. 1(E)). These observations suggest that the downregulation of ANCR contributes to osteoblast differentiation.

3.2. ANCR physically interacts with enhancer of zeste homolog 2 (EZH2)

lncRNAs typically function by binding to specific protein partners, serving important regulatory roles by influencing the activity and function of the proteins to which they bind [17]. To investigate whether ANCR functions by this mechanism, we performed an RNA pull-down assay to identify proteins that were associated with ANCR. Fig. 2(A) shows that ANCR was specifically associated with EZH2, whereas the negative control and the lncRNA-MEG3 control could not bind EZH2. To further validate the association between ANCR and EZH2, we performed a RIP assay with an antibody against EZH2 and hFOB1.19 cellular extracts. Consistent with the above results, we observed a significantly higher enrichment of ANCR with the EZH2 antibody than with the non-specific IgG antibody control (Fig. 2(B)). We next performed deletion–mapping experiments to determine whether EZH2 associates within a specific region of ANCR. We found that a 305-nt region at the 3′ end of ANCR is required for its association with EZH2 (Fig. 2(C)). Together, the RIP, RNA pull-down, and deletion-mapping experiments demonstrated that ANCR is specifically associated with EZH2.

3.3. ANCR regulates Runx2 expression by associating with EZH2

We then investigated the functional relevance of the association between ANCR and EZH2. EZH2 can silence gene expression by cat-

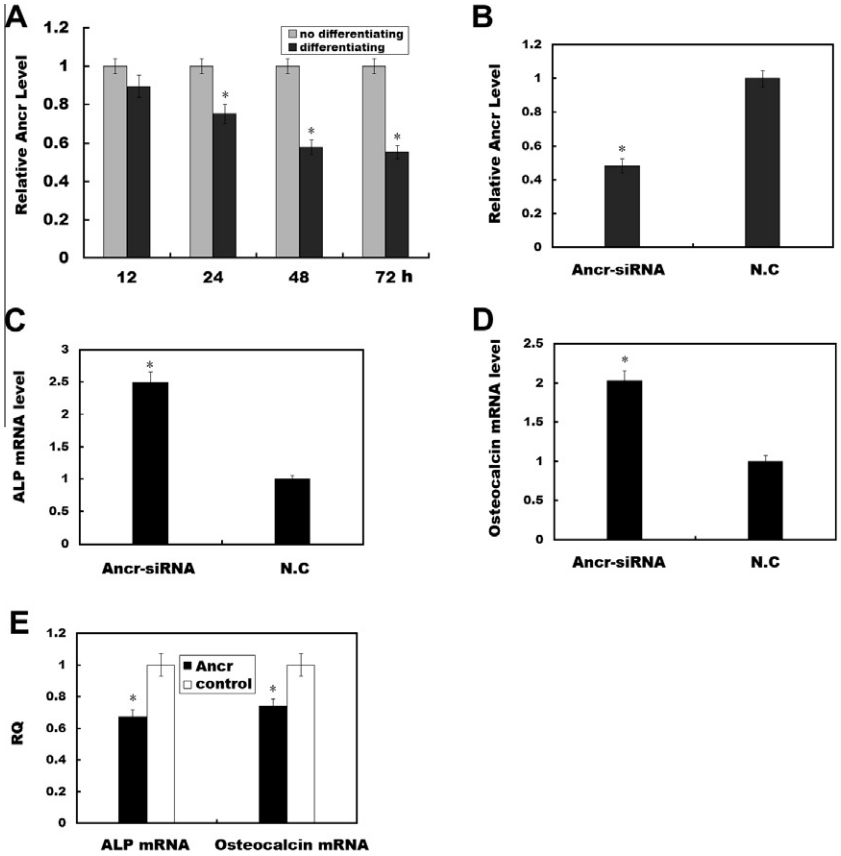

Fig. 1. ANCR downregulation promotes osteoblast differentiation. (A) Confluent cultures of hFOB1.19 cells were maintained in complete medium supplemented with a differentiation cocktail and were harvested after 12, 24, 48, and 72 h. The total RNA was extracted and subjected to real-time RT-PCR to analyze the ANCR level. β-Actin was used as an internal control. Each sample was analyzed in triplicate. $^{*}p < 0.05$. (B) hFOB1.19 cells were treated with ANCR-siRNA, and the ANCR expression levels were assayed by real-time PCR. (C) and (D) hFOB1.19 cells were treated with ANCR-siRNA and then cultured for 3 days under osteoblast differentiation conditions. The ALP mRNA and osteocalcin mRNA levels were assayed by real-time PCR. $^{*}p < 0.05$. (E) hFOB1.19 cells were transfected to overexpress ANCR and then cultured for 3 days under osteoblast differentiation conditions. The relative quantities (RQs) of the ALP and osteocalcin mRNAs were assayed by real-time PCR. $^{*}p < 0.05$.

alyzing histone H3 trimethylation at lysine 27 (H3K27me3) in target gene promoters [11]. Runx2 has been identified as a key transcription factor that regulates osteoblast differentiation [5]. We therefore speculated that ANCR might regulate Runx2 expression and subsequent osteoblast differentiation by associating with EZH2. To test this hypothesis, we determined whether the association of ANCR with EZH2 inhibits Runx2 expression in ANCR-overexpressing hFOB1.19 cells (Fig. 3(A)). ChIP arrays showed that ANCR increased the binding of H3K27me3 with the Runx2 promoter (Fig. 3(B) and (C)). Real-time PCR analysis further demonstrated that ANCR overexpression resulted in the decreased expression of Runx2, whereas EZH2 inhibition partially abrogated the ANCR-induced downregulation of Runx2 (Fig. 3(D)). These data suggests that the association of ANCR with EZH2 inhibits Runx2 expression by catalyzing H3K27me3 in the Runx2 gene promoter.

3.4. ANCR regulates osteoblast differentiation by associating with EZH2

We next investigated whether ANCR downregulation increases Runx2 expression and subsequent osteoblast differentiation by interacting with EZH2. Fig. 4(A) shows that ANCR inhibition increased the Runx2 expression level, whereas EZH2 overexpression inhibited Runx2 expression in ANCR-downregulated hFOB1.19 cells. As expected, ANCR inhibition also increased the ALP and osteocalcin mRNA levels, whereas EZH2 overexpression abrogated the ANCR-siRNA-induced downregulation of ALP and osteocalcin expression in hFOB1.19 cells (Fig. 4(B) and (C)). We further demonstrated that ANCR overexpression inhibited osteoblast differentiation, whereas EZH2 inhibition promoted osteoblast differentiation in ANCR-overexpressing hFOB1.19 cells (Fig. 4(D)). These results confirm that ANCR regulates Runx2 expression and subsequent osteoblast differentiation by interacting with EZH2.

4. Discussion

The human transcriptome has been shown to be more complex than simply a collection of protein-coding genes and their splice variants [18,19]. Using whole-transcriptome and whole-genome sequencing technologies, it has been demonstrated that approximately 90% of the genome is transcribed [19]. Although the transcription of non-coding RNAs was initially argued to be spurious transcriptional noise, new evidence suggests that this transcriptional activity of the genome may play a major biological role in cell differentiation and human diseases [20,21]. HOTAIR expression is elevated in primary breast tumors and metastases. Gupta et al., demonstrated that the enforced expression of HOTAIR in epithelial cancer cells induces the genome-wide retargeting of Polycomb repressive complex 2 (PRC2) to an occupancy pattern more closely resembling that of embryonic fibroblasts, leading to altered histone H3 lysine 27 methylation and gene expression. They also found that the enforced expression of HOTAIR increases cancer invasiveness and metastasis in a manner dependent on PRC2 [17]. Recently, the roles of lncRNAs in various developmental pro-

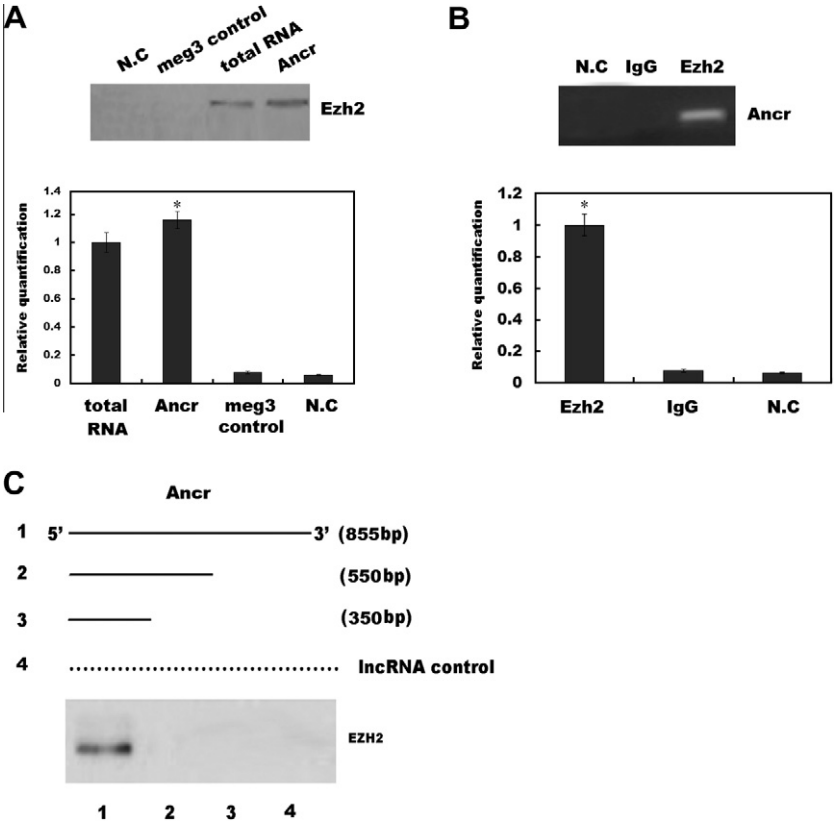

Fig. 2. ANCR physically interacts with enhancer of zeste homolog 2 (EZH2). (A) RNA pull-down was performed as described in the methods section. Biotinylated ANCR or the MEG3 control was incubated with nuclear extracts of hFOB1.19 cells, and the EZH2 protein was assayed by Western blot analysis. The relative quantifiation of the EZH2 protein level is presented (bottom). $^{*}p < 0.05$. (B) RIP was performed using an EZH2 antibody to immunoprecipitate ANCR, and a primer was used to detect ANCR. The relative quantification of the ANCR RNA levels is presented (bottom). $^{*}p < 0.05$ vs N.C. (C) RNAs corresponding to different fragments of ANCR or the lncRNA control sequence was incubated with nuclear extracts of hFOB1.19 cells, and the associated EZH2 was detected by Western blotting.

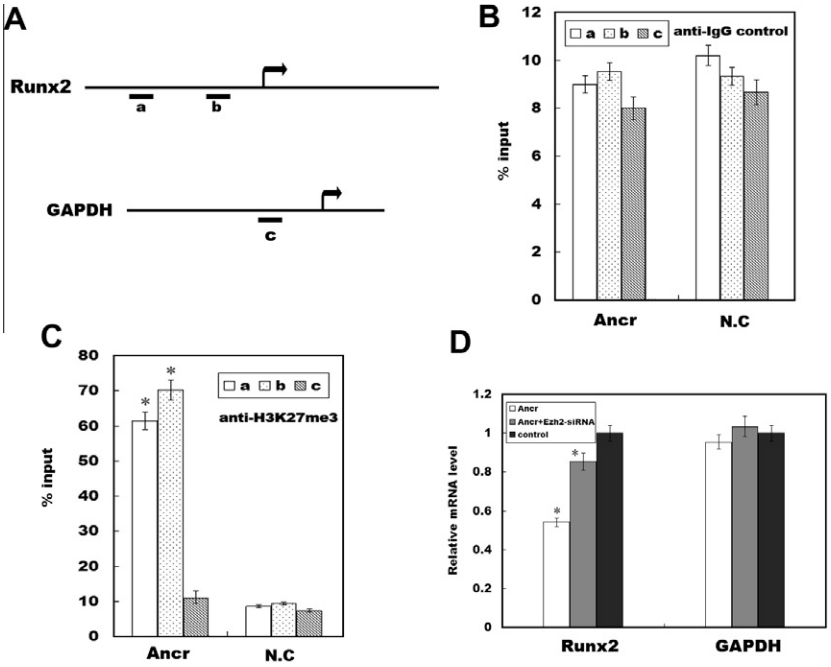

Fig. 3. ANCR regulates Runx2 expression by associating with EZH2 in Runx2 gene promoters. (A) Schematic representation of the Runx2 promoter region as mapped by quantitative PCR analysis and ChIP. (B) and (C) ChIP analysis was conducted for the Runx2 (primer set a–b) and GAPDH (primer c) promoter regions using the indicated antibodies. Enrichment was determined relative to the input controls. These results presented represents data from at least three independent experiments expressed as the mean ± SD. $^{*}p < 0.05$. (D) Analysis of the Runx2 mRNA levels was performed in hFOB1.19 cells transfected with pcDNA-ANCR or pcDNA-ANCR plus EZH2-siRNA. hFOB1.19 cells treated with pcDNA were used as control cells. $^{*}p < 0.05$.

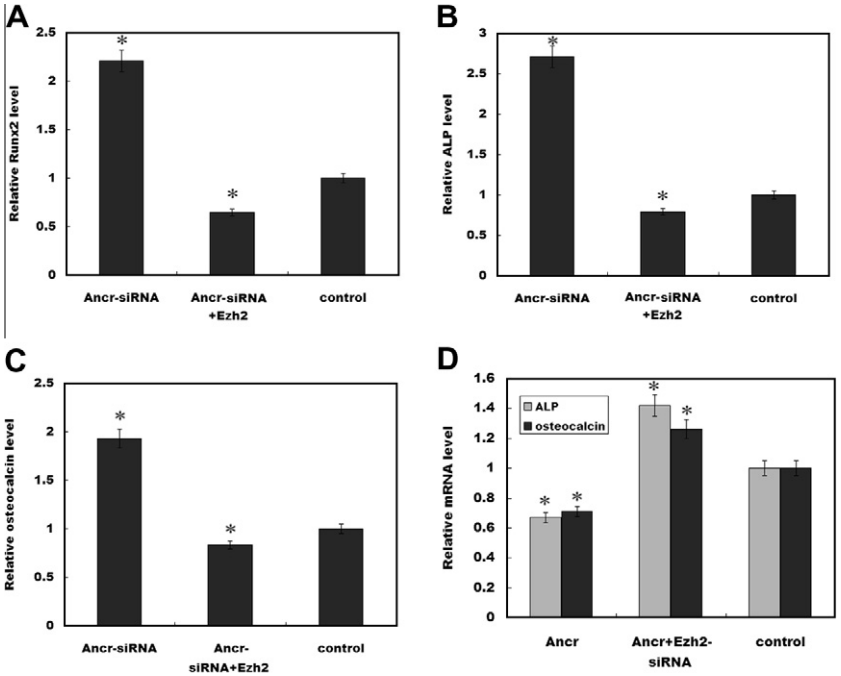

Fig. 4. ANCR regulates osteoblast differentiation by associating with EZH2. (A) Analysis of the Runx2 mRNA levels was performed in hFOB1.19 cells treated with ANCR-siRNA or ANCR-siRNA plus pcDNA-EZH2. $^{*}p < 0.05$. (B) and (C) hFOB1.19 cells were transiently transfected with ANCR-siRNA or ANCR-siRNA plus pcDNA-EZH2 and then cultured for 3 days under osteoblast differentiation conditions. The ALP mRNA and osteocalcin mRNA levels were assayed by real-time PCR. $^{*}p < 0.05$. (D) hFOB1.19 cells were treated with pcDNA-ANCR or pcDNA-ANCR plus EZH2-siRNA and then cultured for 3 days under osteoblast differentiation conditions. The relative quantities (RQs) of the ALP and osteocalcin mRNAs were assayed by real-time PCR. $^{*}p < 0.05$.

cesses, including muscle development and cell differentiation, were studied [22]. Sunwoo et al., found that MEN ε/β lncRNAs exhibit more than twofold upregulation upon the differentiation of C2C12 myoblasts into myotubes [8]. Kretz et al., identified ANCR as an 855-base-pair lncRNA that is downregulated during differentiation [16]. In the epidermis, ANCR loss abolishes the normal blockade of differentiation in the progenitor-containing compartment. The ANCR lncRNA is thus required to maintain the undifferentiated cell state within the epidermis [16].

In the present study, we demonstrated that the ANCR level significantly decreases during hFOB1.19 cell differentiation. ANCR-siRNA treatment inhibited the expression of endogenous ANCR, resulting in osteoblast differentiation, whereas the overexpression of ANCR suppressed osteoblast differentiation. We further demonstrated that ANCR physically interacts with EZH2 and that this association results in the inhibition of both Runx2 expression and subsequent osteoblast differentiation. These data confirm that ANCR is an essential mediator of the osteoblast differentiation of mesenchymal stem cells.

EZH2 is the catalytic subunit of PRC2 and catalyzes H3K27me3, which represses gene transcription. EZH2 has been shown to be a key regulator of hMSC differentiation processes, including osteogenesis and adipogenesis [23]. Chen et al., demonstrated that EZH2 binds to histone deacetylase 9c (HDAC9c) and represses its expression in adipocytes but not in osteoblasts [24]. The expression of HDAC9c accelerates hMSC osteogenesis and inhibits MSC adipogenesis through an interaction with PPARγ-2 in the nucleus of osteoblasts [24]. During osteogenesis, the phosphorylation of EZH2 at Thr487 by cyclin-dependent kinase 1 (CDK1) disrupts the binding of EZH2 with SUZ12 and EED, other PRC2 components [25]. As a result, the methyltransferase activity of EZH2 is inhibited, and hMSCs then undergo differentiation into osteoblasts. Runx2 is a transcription factor required for commitment to the osteoblast lineage. Runx2 directs MSCs to the osteoblastic lineage and prevents them from differentiating into cells in the adipocytic and chondrocytic lineages [26]. In this study, we found that ANCR recruits EZH2 and that the association of ANCR with EZH2 catalyzes H3K27me3 in Runx2 gene promoters, resulting in the inhibition of Runx2 and subsequent osteoblast differentiation.

5. Conclusion

Our data demonstrates that ANCR downregulation promotes osteoblast differentiation by regulating EZH2/Runx2. Thus, ANCR should be considered as an important candidate for a molecular target in osteoblastic differentiation and could be used in the development of therapeutic agents to treat osteogenic disorders.

References

[1] T. Wang, Z. Xu, miR-27 promotes osteoblast differentiation by modulating Wnt signaling, Biochem. Biophys. Res. Commun. 402 (2010) 186–189.
[2] K. Kapinas, C. Kessler, T. Ricks, G. Gronowicz, A.M. Delany, miR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop, J. Biol. Chem. 285 (2010) 25221–25231.
[3] R. Baron, G. Rawadi, Wnt signaling and the regulation of bone mass, Curr. Osteoporos. Rep. 5 (2007) 73–80.
[4] S. Yu, Q. Geng, F. Sun, Y. Yu, Q. Pan, A. Hong, Osteogenic differentiation of C2C12 myogenic progenitor cells requires the Fos-related antigen Fra-1 – a novel target of Runx2, Biochem. Biophys. Res. Commun. 430 (2013) 173–178.
[5] J. Huang, L. Zhao, L. Xing, D. Chen, MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell differentiation, Stem Cells 28 (2010) 357–364.
[6] S.Y. Ng, R. Johnson, L.W. Stanton, Human long non-coding RNAs promote pluripotency and neuronal differentiation by association with chromatin modifiers and transcription factors, EMBO J. 31 (2012) 522–533.
[7] L.L. Chen, G.G. Carmichael, Altered nuclear retention of mRNAs containing inverted repeats in human embryonic stem cells: functional role of a nuclear noncoding RNA, Mol. Cell 35 (2009) 467–478.
[8] H. Sunwoo, M.E. Dinger, J.E. Wilusz, P.P. Amaral, J.S. Mattick, D.L. Spector, MEN epsilon/beta nuclear-retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraspeckles, Genome Res. 19 (2009) 347–359.
[9] Y.L. Yu, R.H. Chou, L.T. Chen, W.C. Shyu, S.C. Hsieh, C.S. Wu, H.J. Zeng, S.P. Yeh, D.M. Yang, S.C. Hung, M.C. Hung, EZH2 regulates neuronal differentiation of mesenchymal stem cells through PIP5K1C-dependent calcium signaling, J. Biol. Chem. 286 (2011) 9657–9667.

[10] A.H. Juan, R.M. Kumar, J.G. Marx, R.A. Young, V. Sartorelli, Mir-214-dependent regulation of the polycomb protein Ezh2 in skeletal muscle and embryonic stem cells, Mol. Cell 36 (2009) 61–74.
[11] R. Cao, L. Wang, H. Wang, L. Xia, H. Erdjument-Bromage, P. Tempst, R.S. Jones, Y. Zhang, Role of histone H3 lysine 27 methylation in Polycomb-group silencing, Science 298 (2002) 1039–1043.
[12] G. Caretti, M. Di Padova, B. Micales, G.E. Lyons, V. Sartorelli, The Polycomb Ezh2 methyltransferase regulates muscle gene expression and skeletal muscle differentiation, Genes Dev. 18 (2004) 2627–2638.
[13] M.C. Tsai, O. Manor, Y. Wan, N. Mosammaparast, J.K. Wang, F. Lan, Y. Shi, E. Segal, H.Y. Chang, Long noncoding RNA as modular scaffold of histone modification complexes, Science 329 (2010) 689–693.
[14] Y.L. Hsu, J.K. Chang, C.H. Tsai, T.T. Chien, P.L. Kuo, Myricetin induces human osteoblast differentiation through bone morphogenetic protein-2/p38 mitogen-activated protein kinase pathway, Biochem. Pharmacol. 73 (2007) 504–514.
[15] N. Xu, C. Shen, Y. Luo, L. Xia, F. Xue, Q. Xia, J. Zhang, Upregulated miR-130a increases drug resistance by regulating RUNX3 and Wnt signaling in cisplatin-treated HCC cell, Biochem. Biophys. Res. Commun. 425 (2012) 468–472.
[16] M. Kretz, D.E. Webster, R.J. Flockhart, C.S. Lee, A. Zehnder, V. Lopez-Pajares, K. Qu, G.X. Zheng, J. Chow, G.E. Kim, J.L. Rinn, H.Y. Chang, Z. Siprashvili, P.A. Khavari, Suppression of progenitor differentiation requires the long noncoding RNA ANCR, Genes Dev. 26 (2012) 338–343.
[17] R.A. Gupta, N. Shah, K.C. Wang, J. Kim, H.M. Horlings, D.J. Wong, M.C. Tsai, T. Hung, P. Argani, J.L. Rinn, Y. Wang, P. Brzoska, B. Kong, R. Li, R.B. West, M.J. van de Vijver, S. Sukumar, H.Y. Chang, Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis, Nature 464 (2010) 1071–1076.
[18] S. Washietl, I.L. Hofacker, M. Lukasser, A. Huttenhofer, P.F. Stadler, Mapping of conserved RNA secondary structures predicts thousands of functional noncoding RNAs in the human genome, Nat. Biotechnol. 23 (2005) 1383–1390.
[19] E.A. Gibb, C.J. Brown, W.L. Lam, The functional role of long non-coding RNA in human carcinomas, Mol. Cancer 10 (2011) 38.
[20] T.R. Mercer, M.E. Dinger, J.S. Mattick, Long non-coding RNAs: insights into functions, Nat. Rev. Genet. 10 (2009) 155–159.
[21] J.E. Wilusz, H. Sunwoo, D.L. Spector, Long noncoding RNAs: functional surprises from the RNA world, Genes Dev. 23 (2009) 1494–1504.
[22] M.E. Dinger, P.P. Amaral, T.R. Mercer, K.C. Pang, S.J. Bruce, B.B. Gardiner, M.E. Askarian-Amiri, K. Ru, G. Solda, C. Simons, S.M. Sunkin, M.L. Crowe, S.M. Grimmond, A.C. Perkins, J.S. Mattick, Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation, Genome Res. 18 (2008) 1433–1445.
[23] Y.H. Chen, F.L. Yeh, S.P. Yeh, H.T. Ma, S.C. Hung, M.C. Hung, L.Y. Li, Myocyte enhancer factor-2 interacting transcriptional repressor (MITR) is a switch that promotes osteogenesis and inhibits adipogenesis of mesenchymal stem cells by inactivating peroxisome proliferator-activated receptor gamma-2, J. Biol. Chem. 286 (2011) 10671–10680.
[24] Y.H. Chen, M.C. Hung, L.Y. Li, EZH2: a pivotal regulator in controlling cell differentiation, Am. J. Transl. Res. 4 (2012) 364–375.
[25] Y. Wei, Y.H. Chen, L.Y. Li, J. Lang, S.P. Yeh, B. Shi, C.C. Yang, J.Y. Yang, C.Y. Lin, C.C. Lai, M.C. Hung, CDK1-dependent phosphorylation of EZH2 suppresses methylation of H3K27 and promotes osteogenic differentiation of human mesenchymal stem cells, Nat. Cell Biol. 13 (2011) 87–94.
[26] T. Komori, Regulation of osteoblast differentiation by transcription factors, J. Cell. Biochem. 99 (2006) 1233–1239.