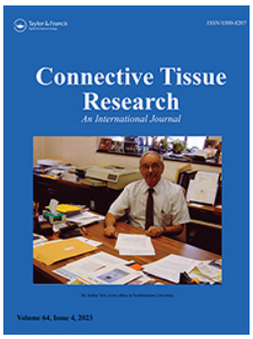

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MetaLnc9 facilitates osteogenesis of human bone marrow mesenchymal stem cells by activating the AKT pathway

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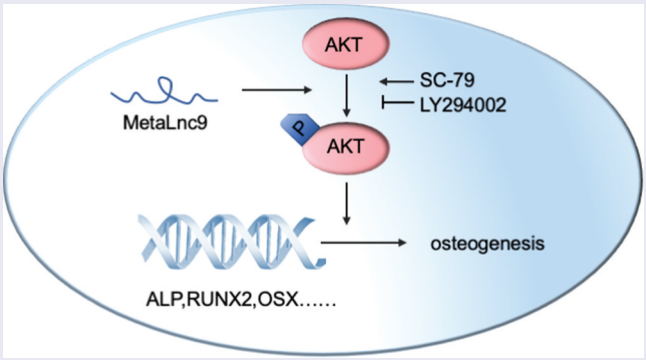
ABSTRACT

Aim of the study: To investigate the role of MetaLnc9 in the osteogenesis of human bone marrow mesenchymal stem cells (hBMSCs).

Materials and Methods: We used lentiviruses to knockdown or overexpress MetaLnc9 in hBMSCs. qRT-PCR was employed to determine the mRNA levels of osteogenic-related genes in transfected cells. ALP staining and activity assay, ARS staining and quantification were used to identify the degree of osteogenic differentiation. Ectopic bone formation was conducted to examine the osteogenesis of transfected cells in vivo. AKT pathway activator SC-79 and inhibitor LY294002 were used to validate the relationship between MetaLnc9 and AKT signaling pathway.

Results: The expression of MetaLnc9 was significantly upregulated in the osteogenic differentiation of hBMSCs. MetaLnc9 knockdown inhibited the osteogenesis of hBMSCs, whereas overexpression of it promoted the osteogenic differentiation both in vitro and in vivo. Taking a deeper insight, we found that MetaLnc9 enhanced the osteogenic differentiation by activating AKT signaling. The inhibitor of AKT signaling LY294002 could reverse the positive effect on osteogenesis brought by MetaLnc9 overexpression, whereas the activator of AKT signaling SC-79 could reverse the negative effect caused by MetaLnc9 knockdown.

Conclusion: Our works uncovered a vital role of MetaLnc9 in osteogenesis via regulating the AKT signaling pathway.



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AKT signaling; BMSCs; lncRNAs; MetaLnc9; osteogenesis

Introduction

Bone defect is worldwide popular and has always been a difficult problem in the field of orthopedic treatment. With its great regeneration and reconstruction ability, bone has attracted much attention in tissue engineering and regenerative medicine[1, 2]. Stem cell therapy takes an emerging role in bone tissue regeneration. Human bone marrow mesenchymal stem cells (hBMSCs) are the most promising candidates. Reasons for this are easy access, their anti-inflammatory character, ability

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of multilineage differentiation, and so on [3, 4]. In many studies, stem cells were genetically engineered to express specific genes or differentiate into specific cell lines before implantation[5, 6].

Long non-coding RNAs (lncRNAs) are RNA molecules that are not able to code protein, and are initially considered as the "noise" of transcription[7]. Abundant studies have displayed that lncRNAs take part in many important life activities, such as cell proliferation, differentiation, and apoptosis, as important regulatory factors[8–10]. In recent years, the molecular mechanisms of lncRNAs involved in bone-related diseases have been extensively studied[11]. More and more studies have exhibited that lncRNAs are essential regulators of bone homeostasis and play key roles in stem cell differentiation[12]. Since lncRNAs play important roles in bone formation, tissue engineering techniques that use stem cells modified by lncRNAs combined with appropriate scaffolds are a promising bone regeneration strategy. MetaLnc9 (Linc00963) belongs to the lncRNAs family and has been identified to regulate biological functions of multiple cancer cell lines[13–16]. Effects of MetaLnc9 on the differentiation of stem cells are rarely researched and still need more in-depth studies.

In our study, MetaLnc9 was found to increase during osteogenesis of hBMSCs. Overexpressing MetaLnc9 facilitated osteogenesis of hBMSCs while silencing MetaLnc9 inhibited the osteogenic differentiation. MetaLnc9 knockdown caused a decreased level of phosphorylated AKT (p-AKT). AKT pathway activator SC-79 could reverse the inhibition triggered by MetaLnc9 knockdown on osteogenesis of hBMSCs. AKT pathway inhibitor LY294002 could reverse the positive effect MetaLnc9 overexpression has on osteogenesis (graphical abstract).

Materials and methods

Cell culture and treatment

Primary hBMSCs were purchased from ScienCell company (Carlsbad, CA, USA). Cells were cultivated in proliferation medium (PM) composed of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 U/mL streptomycin. When the cell fusion degree arrived 70%-80%, medium was changed into osteogenic medium (OM) to induce the osteogenic differentiation. OM was consisted of standard PM supplemented with 100 nM dexamethasone, 0.2 mM ascorbic acid, and 10 mM β-glycerophosphate. Medium was changed every two or 3 days.

Transfection

Recombinant lentiviruses containing full-length MetaLnc9 (LINC OVER) and the scramble control (NC) were purchased from Cyagen Biosciences (Guangzhou, China). Recombinant lentiviruses targeting MetaLnc9 (LINC sh-1 and LINC sh-2) and the scramble control (shNC) were obtained from GenePharma Co. (Shanghai, China). Transfection was carried out by exposing hBMSCs to the dilutions of the viral supernatant for 72 h.

Alkaline Phosphatase (ALP) staining and activity

ALP staining and activity assay were completed as described previously[17, 18]. After 1 week of osteogenic induction, cells were rinsed with PBS for three times, fixed in 4% paraformaldehyde and incubated with the ALP substrate solution (CoWin Biotech, Beijing, China). To detect ALP activity more precisely, an ALP activity Kit was used (Biovision, Milpitas, CA). Results are normalized to the total protein contents.

Alizarin red S staining and quantification

After 14 days of osteogenic induction, Alizarin red S staining was used to detect calcium deposits. Cells were washed with PBS for three times and then fixed with 4% paraformaldehyde for 15 min, and stained with 1% Alizarin red S dye (Sigma-Aldrich; Merck KGaA) for 20 min. Deionized water was used to wash the cells after staining for three times. Cetylpyridinium chloride was used to solubilize the staining. Quantification was carried out to measure the degree of bone nodule formation by using a spectrophotometer at 570 nm. Images of ALP and ARS staining were recorded by a scanner (Image Scanner III, GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA).

RNA isolation and quantitative reverse transcription-polymerase chain reaction (Qrt-PCR) analysis

Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Then, a PrimeScript RT Reagent Kit (Takara, Tokyo, Japan) was applied to synthesize cDNA from RNA. qRT-PCR was performed with SYBR Green Master Mix (Roche Applied Science, Mannheim, Germany) on a 7500 real-time PCR System (Applied Biosystems, Foster City, CA, USA) using GAPDH for normalization. Relative quantification of gene expression was calculated by using the

comparative CT (ΔΔCT) method. Sequences of primers used are listed in Supplemental Table 1.

Ectopic bone formation in vivo

Before in vivo experiments, hBMSCs were transfected with lentiviruses and induced in OM for 1 week. Then, 5×10^6 cells were resuspended and incubated with 6 mm × 6 mm × 3 mm Bio-Oss Collagen (Geistlich, GEWO GmbH, Baden-Baden, Germany) scaffolds for 1 h at 37 °C. Afterward, the mixture of cells and scaffolds were implanted subcutaneously into the dorsal surface of 8-week-old BALB/c homozygous nude (nu/nu) male mice, as described previously[18, 19]. The mice were bought from Charles River Laboratories (Beijing, China) and maintained under specific pathogen-free condition. The mice were randomized into five groups (six mice per group): shNC-hBMSCs/Collagen group, LINC sh-1-hBMSCs/Collagen group, LINC sh-2-hBMSCs/Collagen group, NC-hBMSCs/Collagen group, and LINC OVER-hBMSCs/Collagen group. The mice were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). After 8 weeks, all mice were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and killed by sodium pentobarbitone overdose (100 mg/kg, i.p.). Implants were harvested after 2 months, fixed with 4% paraformaldehyde, decalcified, embedded in paraffin wax, and cut into 5 µm sections. HE and Masson's trichrome staining were performed to identify bone formation. Researchers who conducted the experiments and analyzed the outcomes are different people. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Peking University Health Science Center (LA2021078) and were performed according to the Institutional Animal Guidelines.

Western blot analysis

Proteins were extracted by means of radioimmunoprecipitation assay (RIPA) lysis buffer. Samples were first separated by sodium dodecyl sulfate polyacrylamide-gel electrophoresis and then transferred to PVDF membranes (Millipore, Billerica, MA, USA). After being blocked for 1 h with skimmed milk, membranes were incubated with primary antibodies against AKT (Cell Signaling Technology, Beverly, MA, USA), phosphorylated-AKT (Ser473) (Cell Signaling Technology) and GAPDH (HuaxingBio Science, Beijing, China) at 4 °C overnight. After being washed with TBS containing Tween-20, the membranes were incubated with secondary antibodies for 1 h at room temperature. Signals of protein bands were visualized using the ECL Kit (CoWin Biotech).

Subcellular fractionation

A nuclei isolation Kit (Invent-biotech, Minnesota, USA) was used to isolate the nuclear and cytoplasmic fractions of hBMSCs. RNA from both fractions was extracted using Trizol and then reverse transcribed into cDNA. qRT-PCR was carried out to quantify the RNA expression. MALAT1 and GAPDH were detected as the fractionation indicators. Primers used in the qRT-PCR are shown in Supplementary Table 1.

Statistics

All statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Independent sample t-test was applied to evaluate statistical differences between two groups, and one-way analysis of variance (ANOVA) was used to analyze the statistical significance when there were more than two groups. The results were expressed as mean ± standard deviation from at least three biologically repeated experiments with three technical replicates per sample. We considered p values less than 0.05 as statistically significant.

Results

MetaLnc9 was upregulated during the osteogenic differentiation of hBmscs and was mainly located in the nucleus

To investigate the effect of MetaLnc9 on osteogenesis, we first detected the expression level of MetaLnc9 during osteogenic differentiation. The mRNA levels of osteogenesis-related genes Runt-related transcription factor 2 (RUNX2), ALP, and osterix (OSX) were also detected to figure out whether we successfully induce the osteogenesis of hBMSCs. qRT-PCR results showed a great rise of osteogenic marker genes as expected (Figure 1(a–c)). Moreover, Metalnc9 was significantly upregulated during the osteogenesis of BMSCs (Figure 1d), which indicated a role it might play in the osteogenic differentiation. Subcellular location of lncRNAs is important because it is concerned with the function of most lncRNAs. We performed subcellular fractionation assay to examine the distribution of MetaLnc9 of hBMSCs. As presented in Supplementary Fig.2, MetaLnc9 was primarily located in the nucleus of hBMSCs, meanwhile the marker RNAs (MALAT1 and GAPDH) were enriched in their expected fractions.

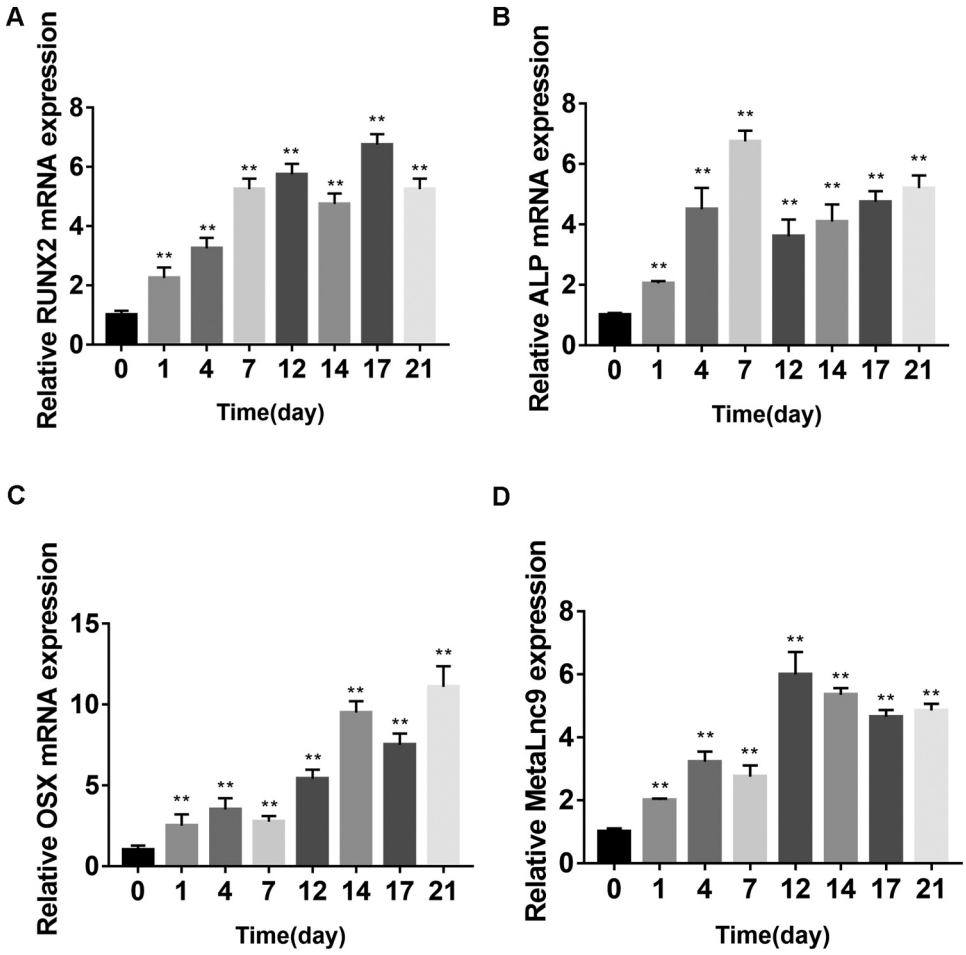

Figure 1. The expression pattern of osteo-related genes and MetaLnc9 during the osteogenesis of hBMSCs. A-C. Relative mRNA expression of osteogenic markers RUNX2, ALP and OSX were measured during day 0, 1, 4, 7, 12, 14, 17, 21. D. Relative mRNA expression of MetaLnc9 was measured during day 0, 1, 4, 7, 12, 14, 17, 21. Results are presented as the mean ± SD, $^{**}P < 0.01$, compared with day 0.

Gain- and loss-of-function of MetaLnc9 in vitro affect osteogenic differentiation

To explore whether MetaLnc9 regulates osteogenic differentiation, we next used lentivirus to knockdown and overexpress MetaLnc9 in hBMSCs (Supplementary Fig.1A-C). Transfected hBMSCs were cultured in PM or OM for 1 week. ALP activity was significantly increased in the MetaLnc9 overexpression group and decreased in the MetaLnc9 knockdown group (Figure 2(a,b)). Consistently, the mRNA expression levels of ALP, RUNX2, and OSX were decreased in the MetaLnc9 knockdown group and upregulated in MetaLnc9 overexpression group (Figure 2(e–g)). After 14 days of culture in PM or OM, ARS staining and quantification results uncovered that the mineralized nodules were improved in the MetaLnc9 overexpression group and diminished in the MetaLnc9 knockdown group (Figure 2(c,d)).

Gain- and loss-of-function of MetaLnc9 in vivo affect bone formation

Subsequently, ectopic bone formation was conducted to further uncover the effect MetaLnc9 had on osteogenesis in vivo. HE staining and Masson's trichrome staining indicated MetaLnc9 overexpression group (LINC over) promoted new bone formation compared with its control group (NC), whereas MetaLnc9 knockdown (LINC sh-1, LINC sh-2) formed less bone-like tissues compared with the control group (shNC) (Figure 3). Furthermore, we also examined osteogenic maker genes of ectopic samples. As shown in Supplemental Figure 3, MetaLnc9 overexpression upregulated osteogenic marker genes RUNX2 and ALP in vivo, while MetaLnc9 knockdown had an opposite effect.

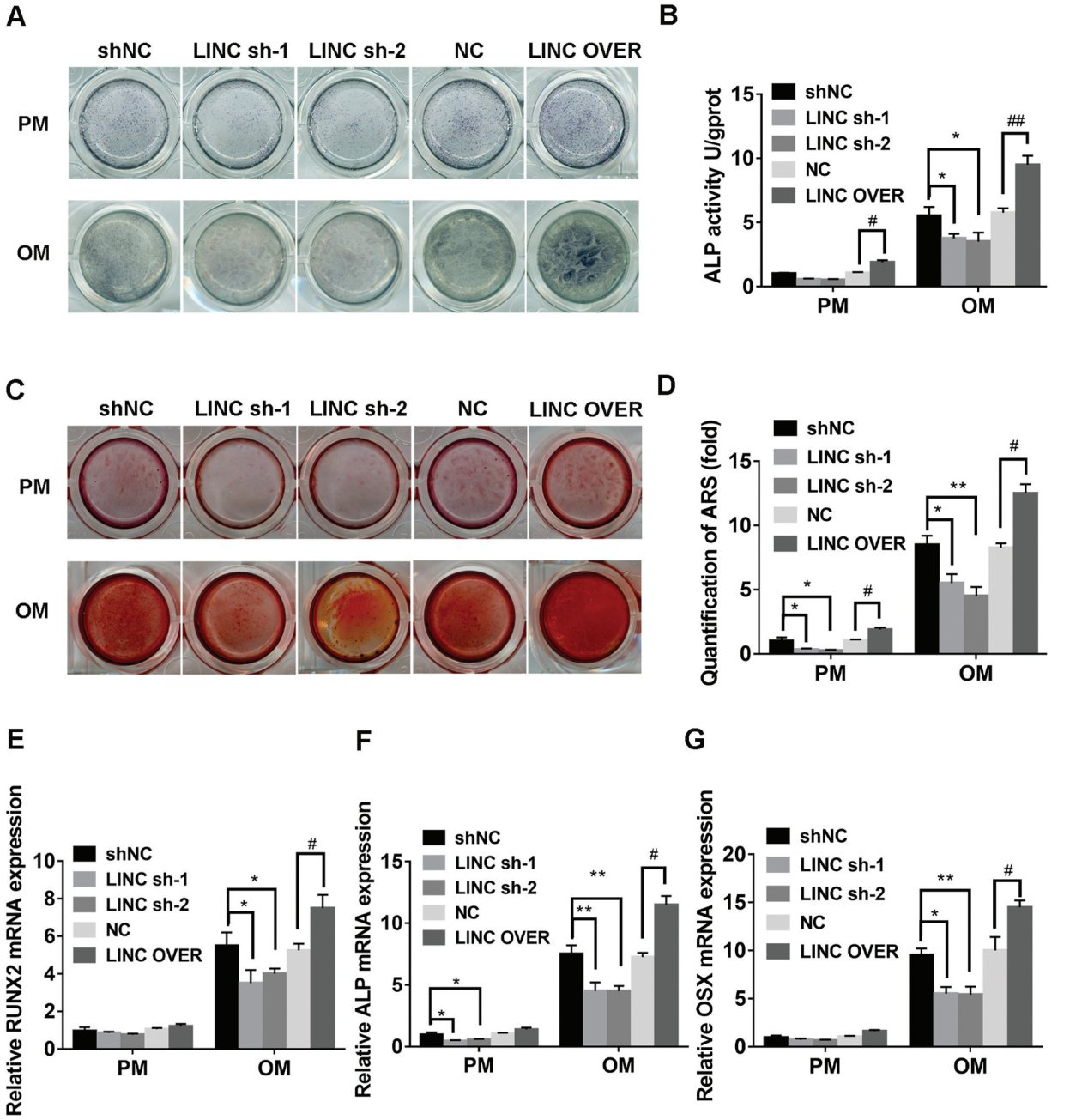

Figure 2. Gain- and loss-of-function of MetaLnc9 in the osteogenic differentiation of hBMSCs in vitro. A-B. ALP staining and activity were detected in shNC, LINC sh-1, LINC sh-2, NC, LINC over groups. C-D. ARS staining and quantification were measured in shNC, LINC sh-1, LINC sh-2, NC, LINC over groups. E-G. mRNA levels of ALP, RUNX2, and OSX were measured in shNC, LINC sh-1, LINC sh-2, NC, LINC over groups. Results are presented as the mean ± SD, **/**$P < 0.01$, */##$P < 0.05$, *compared with shNC, # compared with NC.

Silencing MetaLnc9 suppressed osteogenic differentiation via inhibition of the AKT signaling pathway

We next screened several osteogenesis-related signaling pathways to explore the mechanism by which MetaLnc9 regulates osteogenesis and found MetaLnc9 knockdown significantly decreased the protein level of p-AKT (Figure 4(a,b)). We inspected the effect of MetaLnc9 on osteogenic differentiation in the presence of AKT signaling activator SC-79 (10 μM) to validate whether MetaLnc9 regulated osteogenesis via AKT pathway. The decreased ALP activity induced by MetaLnc9 knockdown was successfully abrogated by treatment of SC-79 (Figure 4(c,d)). Moreover, the negative effect MetaLnc9 knockdown had on extracellular

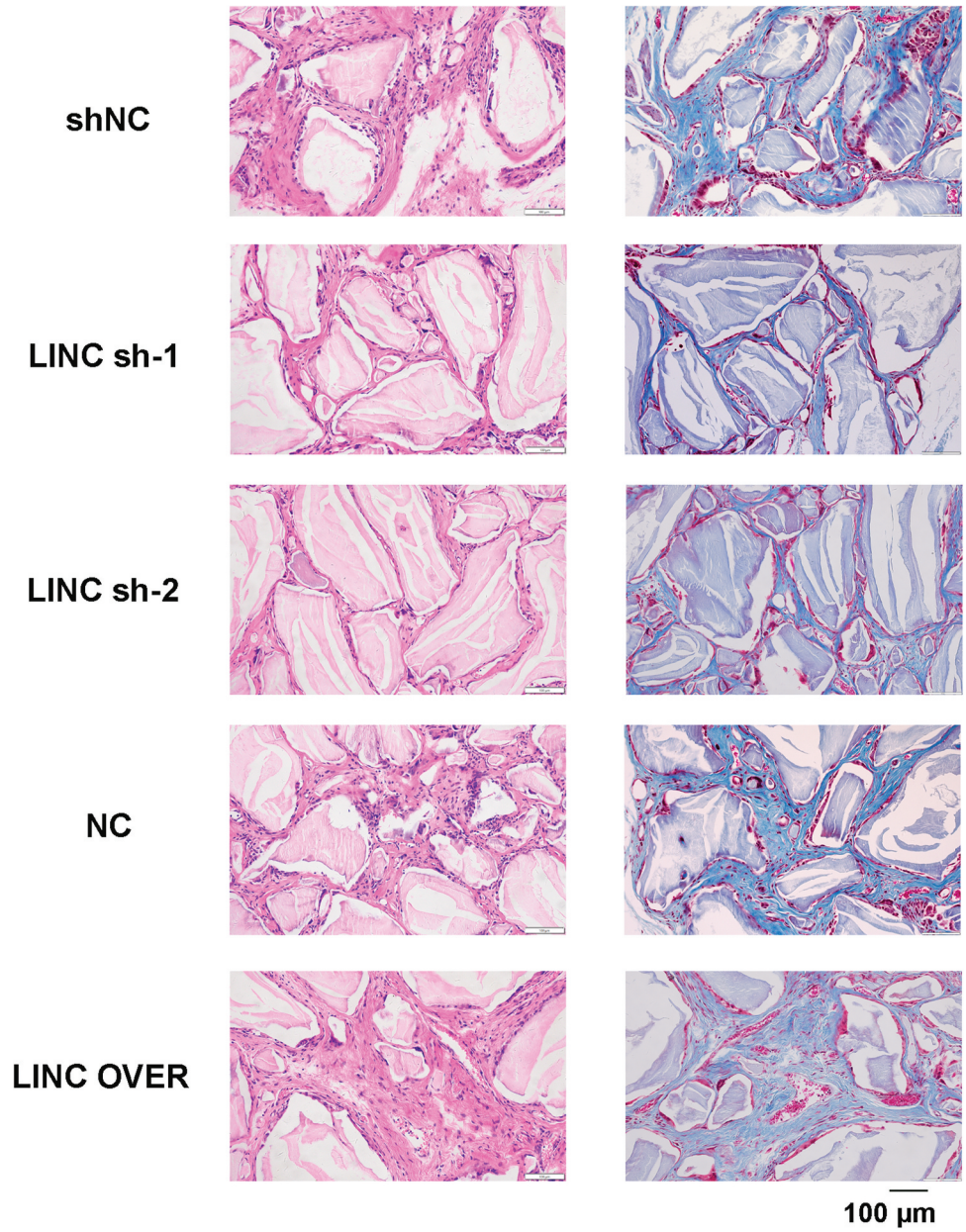

Figure 3. MetaLnc9 promoted bone formation of hBmscs in vivo. Mice were transplanted with Bio-oss collagen scaffold loaded hBmscs overexpressing or knocking down MetaLnc9 in the dorsal surface. H&E staining and Masson's trichrome staining in shNC, LINC sh-1, LINC sh-2, NC, LINC over groups. Scale bar = 100 μm.

matrix calcium deposits was also reversed by SC-79 (Figure 4(e,f)). Besides, adding SC-79 moderated the reduction of mRNA expression levels of osteogenic markers RUNX2, ALP, and OSX caused by MetaLnc9 silencing (Figure 4(g–i)).

Overexpression of MetaLnc9 enhanced osteogenic differentiation via activation of the AKT signaling pathway

Inhibitor of AKT signaling pathway LY294002 (20 μM) was applied to confirm that overexpression of MetaLnc9 enhanced osteogenic differentiation via activating the AKT pathway. Consistent with knockdown experiments, overexpression of MetaLnc9 increased protein level of p-AKT compared to NC group (Figure 5(a,b)). The enhanced ALP activity caused by MetaLnc9 overexpression was significantly overturned (Figure 5(c,d)) after using LY294002. Moreover, the reinforcement resulted from MetaLnc9 overexpression on calcium deposits was also diminished by LY294002 (Figure 5(e,f)). Treatment of LY294002 reduced the upregulation of mRNA expression levels of osteogenic markers RUNX2, ALP, and

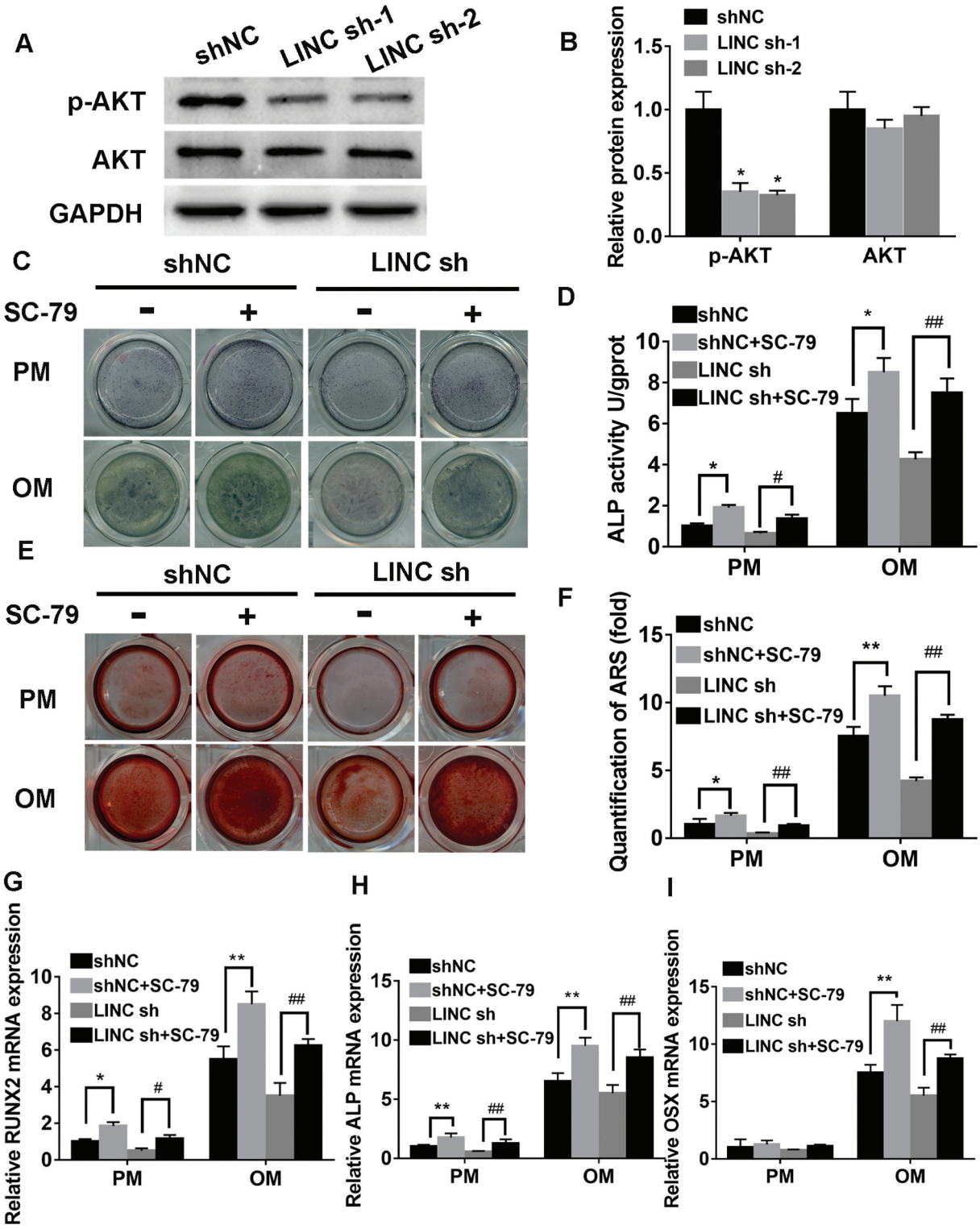

Figure 4. Silencing MetaLnc9 promoted the osteogenesis of hBMSCs via inhibiting AKT signaling pathway. A-B. The protein levels of p-AKT and AKT were measured and quantified in shNC, LINC sh-1, LINC sh-2 groups, normalized to GADPH. C-D. ALP staining and activity were detected in shNC, shNc+SC-79, LINC sh, LINC sh+SC-79 groups. E-F. ARS staining and quantification were detected in shNC, shNc+SC-79, LINC sh, LINC sh+SC-79 groups. G-I. mRNA levels of ALP, RUNX2, and OSX were measured in shNC, shNc+SC-79, LINC sh, LINC sh+SC-79 groups. Results are presented as the mean ± SD, **/**$P < 0.01$, */##$P < 0.05$, *compared with shNC, # compared with LINC sh.

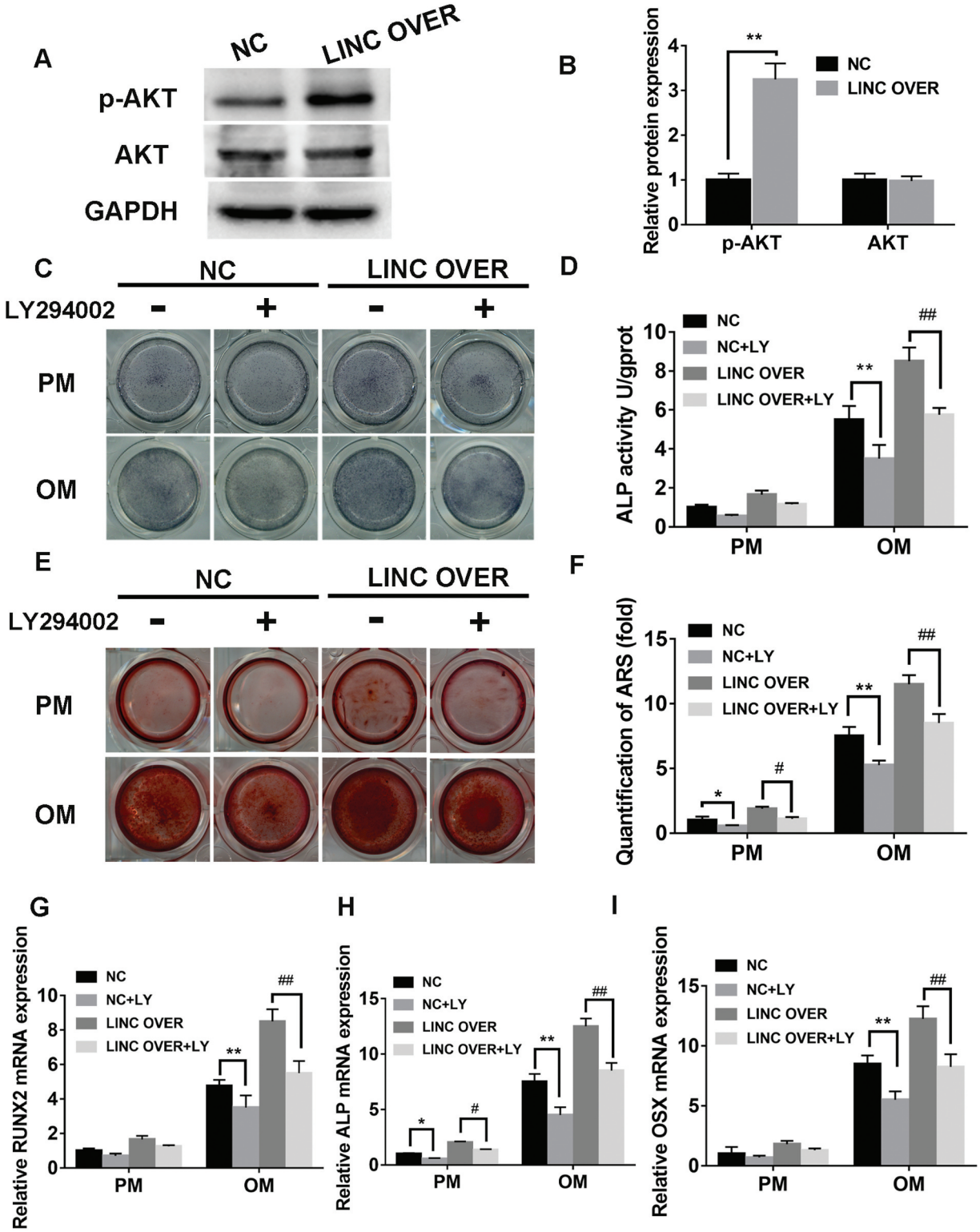

Figure 5. Overexpressing MetaLnc9 promoted the osteogenesis of hBMSCs via activating AKT signaling pathway. A-B. P-AKT and AKT were measured and quantified in NC and LINC over groups, normalized to GADPH. C-D. ALP staining and activity were detected in NC, NC+LY294002, LINC over, LINC over+LY294002 groups. E-F. ARS staining and quantification were detected in NC, NC+ LY294002, LINC over, LINC over+LY294002 groups. G-I. mRNA levels of ALP, RUNX2, and OSX were detected in NC, NC+ LY294002, LINC over, LINC over+LY294002 groups. Results are presented as the mean ± SD, **/**$P < 0.01$, */##P < 0.05, *compared with NC, # compared with LINC over.

OSX triggered by MetaLnc9 overexpression (Figure 5 (g–i)).

Discussion

Numerous lncRNAs exert their effects via AKT signaling, which is widely considered to affect stem cells' ability of osteogenic differentiation[20–22]. Phosphorylated AKT played a crucial role in the osteogenesis of hBMSCs through many downstream genes[23, 24]. For example, it could inhibit the ability of GSK3β to form a complex with β-catenin, contributing to the accumulation of β-catenin and enhancing osteogenic gene expression[25]. Notwithstanding, direct phosphorylation by AKT prevent GSK3β from attenuating RUNX2 activity[26].

In present study, we discovered that MetaLnc9 promoted the osteogenesis of hBMSCs through activating AKT signaling pathway. Subcellular fractionation assay disclosed that Metalnc9 was largely distributed in the nuclear area of hBMSCs. However, inactivated AKT existed mostly in the cytoplasm. Our further study would focus on whether the majority of MetaLnc9 located in the nucleus activated AKT or the minority cytoplasmic MetaLnc9 exerted the effects and the detailed mechanisms. Similar to our study, MetaLnc9 was found to be located in both cytoplasm and nucleus in non-small cell lung cancer (NSCLC) cells. It activated AKT signaling through interacting with the glycolytic kinase PGK1 and prevented its ubiquitination in NSCLC cells. PGK1 is located mainly in cytoplasm and researchers speculated that PGK1 might be a downstream target of MetaLnc9 in the cytosol[15]. We examined the expression of PGK1 in transfected BMSCs via western blot. Similarly, we also found out overexpressing MetaLnc9 enhanced PGK1 and silencing MetaLnc9 reduced PGK1. Our results suggested that Metalnc9 might activate AKT signaling via promoting the expression of PGK1 in BMSCs (Supplemental Figure 4). Moreover, Metalnc9 fostered hepatocellular carcinoma progression via stimulating PI3K/AKT pathway with the detailed mechanisms remaining to be explored[27]. In contrast to our findings, a previous research uncovered that MetaLnc9 was primarily situated in cytoplasm in bladder cancer cells[28]. These findings suggested MetaLnc9 played diverse roles in different cell lines.

Researchers have worked hard on new approaches to repair bone defect. Tissue engineering involved stem cells, especially BMSCs, is a potential strategy and has received much attention[29, 30]. However, as seed cells, BMSCs also have some deficiencies, such as inefficiency of osteogenic differentiation, difficulty in quality control, and decline in their proliferative ability. Therefore, several studies used gene-modified BMSCs in the defect area and received promising repair effects[31–33]. Our research implied that MetaLnc9 might serve as a gene target to modify BMSCs to improve its osteogenic differentiation ability. Moreover, there are some clinical medicines targeting AKT signaling pathway to treat diseases such as breast cancer[34]、cervical cancer[35] and renal cell carcinoma[36]. It is reasonable to hypothesize that MetaLnc9 might be used to treat bone defect as a nucleotide drug targeting AKT signaling in the future.

Our study discovered that MetaLnc9 was upregulated during the osteogenesis of hBMSCs. Overexpression of MetaLnc9 facilitated the osteogenic differentiation of hBMSCs by activating AKT signaling pathway. In future, we will try to figure out the detailed mechanisms of how MetaLnc9 regulated phosphorylation of AKT and the downstream gene reactions, especially those involved in the osteogenesis.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

1. Khare D, Basu B, Dubey AK. Electrical stimulation and piezoelectric biomaterials for bone tissue engineering applications. Biomater. 2020;258:120280. doi:10.1016/j.biomaterials.2020.120280.
2. Riester O, Borgolte M, Csuk R, Deigner HP. Challenges in bone tissue regeneration: stem cell therapy, biofunctionality and antimicrobial properties of novel materials and its evolution. Int J Mol Sci. 2020;22(1):192. doi:10.3390/ijms22010192.
3. Fan XL, Zhang Y, Li X, Fu QL. Mechanisms underlying the protective effects of mesenchymal stem cell-based therapy. Cell Mol Life Sci. 2020;77(14):2771–2794. doi:10.1007/s00018-020-03454-6.
4. Jones E, Yang X. Mesenchymal stem cells and bone regeneration: current status. Injury. 2011;42(6):562–568. doi: 10.1016/j.injury.2011.03.030.
5. Hu B, Li Y, Wang M, Zhu Y, Zhou Y, Sui B, Tan Y, Ning Y, Wang J, He J, et al. Functional reconstruction of critical-sized load-bearing bone defects using a sclerostin-targeting mir-210-3p-based construct to enhance osteogenic activity. Acta Biomater. 2018;76:275–282.

6. Li KC, Chang YH, Yeh CL, Hu YC. Healing of osteoporotic bone defects by baculovirus-engineered bone marrow-derived MSCs expressing microRNA sponges. Biomater. 2016;74:155–166. doi:10.1016/j.biomaterials.2015.09.046.
7. Ponjavic J, Ponting CP, Lunter G. Functionality or transcriptional noise? Evidence for selection within long noncoding rnas. Genome Res. 2007;17(5):556–565. doi: 10.1101/gr.6036807.
8. Zhu Y, Gu L, Lin X, Cui K, Liu C, Lu B, Zhou F, Zhao Q, Shen H, Li Y. Linc00265 promotes colorectal tumorigenesis via zmiz2 and usp7-mediated stabilization of beta-catenin. Cell Death Differ. 2020;27(4):1316–1327. doi: 10.1038/s41418-019-0417-3.
9. Hudson WH, Prokhnevska N, Gensheimer J, Akondy R, McGuire DJ, Ahmed R, Kissick HT. Expression of novel long noncoding RNAs defines virus-specific effector and memory cd8(+) t cells. Nat Commun. 2019;10(1):196. doi: 10.1038/s41467-018-07956-7.
10. Shen SN, Li K, Liu Y, Yang CL, He CY, Wang HR. Down-regulation of long noncoding RNA pvt1 inhibits esophageal carcinoma cell migration and invasion and promotes cell apoptosis via microRNA-145-mediated inhibition of fscn1. Mol Oncol. 2019;13(12):2554–2573. doi: 10.1002/1878-0261.12555.
11. Yang Q, Jia L, Li X, Guo R, Huang Y, Zheng Y, Li W. Long noncoding RNAs: new players in the osteogenic differentiation of bone marrow- and adipose-derived mesenchymal stem cells. Stem Cell Rev And Rep. 2018;14(3):297–308. doi: 10.1007/s12015-018-9801-5.
12. Aurilia C, Donati S, Palmini G, Miglietta F, Iantomasi T, Brandi ML. The involvement of long non-coding RNAs in bone. Int J Mol Sci. 2021;22(8):3909. doi: 10.3390/ijms22083909.
13. Wang L, Han S, Jin G, Zhou X, Li M, Ying X, Wang L, Wu H, Zhu Q. Linc00963: a novel, long non-coding RNA involved in the transition of prostate cancer from androgen-dependence to androgen-independence. Int J Oncol. 2014;44(6):2041–2049. doi: 10.3892/ijo.2014.2363.
14. Ye J, Liu J, Tang T, Xin L, Bao X, Yan Y. Linc00963 affects the development of colorectal cancer via mir-532-3p/hmga2 axis. Cancer Cell Int. 2021;21(1):87. doi: 10.1186/s12935-020-01706-w.
15. Yu T, Zhao Y, Hu Z, Li J, Chu D, Zhang J, Li Z, Chen B, Zhang X, Pan H, et al. Metalnc9 facilitates lung cancer metastasis via a pgk1-activated akt/mtor pathway. Cancer Res. 2017;77(21):5782–5794. doi:10.1158/0008-5472.CAN-17-0671.
16. Zuo W, Zhou K, Deng M, Lin Q, Yin Q, Zhang C, Zhou J, Song Y. Linc00963 facilitates acute myeloid leukemia development by modulating mir-608/mmp-15. Aging (Albany NY). 2020;12(19):18970–18981. doi: 10.18632/aging.103252.
17. Jin C, Shuai T, Tang Z. Hspb7 regulates osteogenic differentiation of human adipose derived stem cells via erk signaling pathway. Stem Cell Res Ther. 2020;11(1):450. doi: 10.1186/s13287-020-01965-4.
18. Jin C, Jia L, Tang Z, Zheng Y. Long non-coding RNA mir22hg promotes osteogenic differentiation of bone marrow mesenchymal stem cells via PTEN/AKT pathway. Cell Death Dis. 2020;11(7):601. doi: 10.1038/s41419-020-02813-2.
19. Jin C, Jia L, Huang Y, Zheng Y, Du N, Liu Y, Zhou Y. Inhibition of lncrna mir31hg promotes osteogenic differentiation of human adipose-derived stem cells. Stem Cells. 2016;34(11):2707–2720. doi: 10.1002/stem.2439.
20. Meng YB, Li X, Li ZY, Zhao J, Yuan XB, Ren Y, Cui ZD, Liu YD, Yang XJ. microRNA-21 promotes osteogenic differentiation of mesenchymal stem cells by the pi3k/beta-catenin pathway. J Orthop Res. 2015;33(7):957–964. doi: 10.1002/jor.22884.
21. Zhang Y, Cao X, Li P, Fan Y, Zhang L, Ma X, Sun R, Liu Y, Li W. Lncrna nkila integrates rxfp1/akt and nf-kappab signalling to regulate osteogenesis of mesenchymal stem cells. J Cell Mol Med. 2020;24(1):521–529. doi: 10.1111/jcmm.14759.
22. Guntur AR, Rosen CJ, Naski MC. N-cadherin adherens junctions mediate osteogenesis through pi3k signaling. Bone. 2012;50(1):54–62. doi: 10.1016/j.bone.2011.09.036.
23. Baker N, Sohn J, Tuan RS. Promotion of human mesenchymal stem cell osteogenesis by pi3-kinase/akt signaling, and the influence of caveolin-1/cholesterol homeostasis. Stem Cell Res Ther. 2015;6(1):238. doi: 10.1186/s13287-015-0225-8.
24. McGonnell IM, Grigoriadis AE, Lam EW, Price JS, Sunters A. A specific role for phosphoinositide 3-kinase and akt in osteoblasts? Front Endocrinol (Lausanne). 2012;3:88. doi:10.3389/fendo.2012.00088.
25. Sunters A, Armstrong VJ, Zaman G, Kypta RM, Kawano Y, Lanyon LE, Price JS. Mechano-transduction in osteoblastic cells involves strain-regulated estrogen receptor alpha-mediated control of insulin-like growth factor (igf) i receptor sensitivity to ambient igf, leading to phosphatidylinositol 3-kinase/akt-dependent wnt/lrp5 receptor-independent activation of beta-catenin signaling. J Biol Chem. 2010;285(12):8743–8758. doi: 10.1074/jbc.M109.027086.
26. Kugimiya F, Kawaguchi H, Ohba S, Kawamura N, Hirata M, Chikuda H, Azuma Y, Woodgett JR, Nakamura K, Chung UI, Heisenberg C-P. GSK-3β controls osteogenesis through regulating Runx2 activity. PLoS One. 2007;2(9):e837. doi: 10.1371/journal.pone.0000837.
27. Wu JH, Tian XY, An QM, Guan XY, Hao CY. Linc00963 promotes hepatocellular carcinoma progression by activating pi3k/akt pathway. Eur Rev Med Pharmacol Sci. 2018;22(6):1645–1652. doi: 10.26355/eurrev_201803_14574.
28. Zhou N, Zhu X, Man L. Linc00963 Functions as an Oncogene in Bladder Cancer by Regulating the miR-766-3p/MTA1 axis. Cancer Manag Res. 12:3353–3361. 10.2147/CMAR.S249979. 2020.
29. Kim SH, Kim KH, Seo BM, Koo KT, Kim TI, Seol YJ, Ku Y, Rhyu IC, Chung CP, Lee YM. Alveolar bone regeneration by transplantation of periodontal ligament stem cells and bone marrow stem cells in a canine peri-implant defect model: a pilot study. J Periodontol. 2009;80(11):1815–1823. doi: 10.1902/jop.2009.090249.

30. Li Q, Yang G, Li J, Ding M, Zhou N, Dong H, Mou Y. Stem cell therapies for periodontal tissue regeneration: a network meta-analysis of preclinical studies. Stem Cell Res Ther. 2020;11(1):427. doi: 10.1186/s13287-020-01938-7.
31. Zhang Y, Cao X, Li P, Fan Y, Zhang L, Ma X, Sun R, Liu Y, Li W. microRNA-935-modified bone marrow mesenchymal stem cells-derived exosomes enhance osteoblast proliferation and differentiation in osteoporotic rats. Life Sci. 2021;272:119204. doi:10.1016/j.lfs.2021.119204.
32. Zhu C, Chang Q, Zou D, Zhang W, Wang S, Zhao J, Yu W, Zhang X, Zhang Z, Jiang X. Lvbmp-2 gene-modified bmscs combined with calcium phosphate cement scaffolds for the repair of calvarial defects in rats. J Mater Sci Mater Med. 2011;22(8):1965–1973. doi: 10.1007/s10856-011-4376-6.
33. Zhu L, Chuanchang D, Wei L, Yilin C, Jiasheng D. Enhanced healing of goat femur-defect using bmp7 gene-modified bmscs and load-bearing tissue-engineered bone. J Orthop Res. 2010;28(3):412–418. doi: 10.1002/jor.20973.
34. Kim SB, Dent R, Im SA, Espie M, Blau S, Tan AR, Isakoff SJ, Oliveira M, Saura C, Wongchenko MJ, et al. Ipatasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer (lotus): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Oncol. 2017;18(10):1360–1372. doi:10.1016/S1470-2045(17)30450-3.
35. Bell MC, Crowley-Nowick P, Bradlow HL, Sepkovic DW, Schmidt-Grimminger D, Howell P, Mayeaux EJ, Tucker A, Turbat-Herrera EA, Mathis JM. Placebo-controlled trial of indole-3-carbinol in the treatment of cin. Gynecol Oncol. 2000;78(2):123–129. doi: 10.1006/gyno.2000.5847.
36. Yuan R, Kay A, Berg WJ, Lebwohl D. Targeting tumorigenesis: development and use of mTOR inhibitors in cancer therapy. J Hematol Oncol. 2009;2(1):45. doi: 10.1186/1756-8722-2-45.