Akt1 mediates the posterior Hoxc gene expression through epigenetic modifications in mouse embryonic fibroblasts

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The evolutionarily conserved Hox genes are organized in clusters and expressed colinearly to specify body patterning during embryonic development. Previously, Akt1 has been identified as a putative Hox gene regulator through in silico analysis. Substantial upregulation of consecutive 5’ Hoxc genes has been observed when Akt1 is absent in mouse embryonic fibroblast (MEF) cells. In this study, we provide evidence that Akt1 regulates the 5’ Hoxc gene expression by epigenetic modifications. Enrichment of histone H3K9 acetylation and a low level of the H3K27me3 mark were detected at the posterior 5’ Hoxc loci when Akt1 is absent. A histone deacetylase (HDAC) inhibitor de-repressed 5’ Hoxc gene expression when Akt1 is present, and a DNA demethylating reagent synergistically upregulated HDAC-induced 5’ Hoxc gene expression. A knockdown study revealed that Hdac6 is mediated in the Hoxc12 repression through direct binding to the transcription start site (TSS) in the presence of Akt1. Co-immunoprecipitation analysis revealed that endogenous Akt1 directly interacted with Hdac6. Furthermore, exogenous Akt1 was enriched at the promoter region of the posterior Hoxc genes such as Hoxc11 and Hoxc12, not the Akt1-independent Hoxc5 and Hoxd10 loci. The regulation of the H3K27me3 mark by Ezh2 and Kdm6b at the 5’ Hoxc gene promoter turned out to be Akt1 dependent. Taken together, these results suggest that Akt1 mediates the posterior 5’ Hoxc gene expression through epigenetic modification such as histone methylation and acetylation, and partly through a direct binding to the promoter region of the 5’ Hoxc genes and/or Hdac6 in mouse embryonic fibroblast cells.

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1. Introduction

The Hox transcription factor plays critical roles in pattern formation and differentiation. In mammals, there are 39 Hox genes clustered on four different chromosomal loci. The genomic location of the Hox genes correlates to their spatio-temporal expression, termed colinearity [1,2]. During development, the combination of expressed Hox genes decide the cellular and positional identity of specific cell types along the anteroposterior body axis, by orchestrating downstream target gene expression [3].

Retinoic acid (RA) is one of the most characterized factors regulating Hox gene expression. Retinoic acid response elements (RAREs) have been identified in several Hox genes and aberrant RA activity has led to distinct homeotic transformations through alterations in Hox expression [4,5]. The upstream transcription factors involved in Hox gene expression include Pbx, Meis, Prep cofactors, Krox20, Sox/Oct family members, Cdx, Jpk, and the Hox proteins themselves [6-9].

Recently, diverse mechanisms particularly, epigenetic regulations such as histone modification [10-17], DNA methylation [18-21], and chromatin architecture [20,22-28] have been suggested to regulate Hox gene expression. The progressive changes of chromatin marks and 3-dimensional conformation changes seemed to facilitate the temporal and spatial Hox gene regulation during early embryonic development [28-31]. Long noncoding RNAs (lncRNAs) have also been implicated in the control of vertebrate Hox gene expression recruiting trithorax or polycomb group members to Hox clusters [32-35]. In addition, Hox genes are not only expressed during development, but also in diverse organs in adult and play multiple roles in coordinating cellular processes such as cell division, proliferation, and differentiation [36].

Endocrine regulation of Hox genes and the aberrant Hox gene expression have been described in numerous human cancers [37-40]. In a recent study, we identified Akt1 as an additional Hox regulator. Several unexpressed consecutive 5’ Hoxc genes in wild-type (WT) cells were de-repressed in Akt1-null MEFs and the DNA methylation status showed reverse correlation with gene expression [21].
Akt, also known as protein kinase B (PKB), is a serine–threonine protein kinase. It is a downstream effector of the phosphatidylinositol 3-kinase (PI3K) pathway and plays a key role in multiple cellular processes such as glucose metabolism, cellular proliferation, apoptosis, transcription, cell migration, and development. In mammals, there are three closely related isoforms of protein kinase B (PKB)/Akt family members: PKBα/Akt1, PKBβ/Akt2, and PKBγ/Akt3. Among the three Akt isoforms, Akt1 is the predominantly expressed isoform in most tissues [41]. Recently, Akt was shown to modulate gene expression through epigenetic modifications. Akt1 enhances DNA methylation through the expression of DNA methyltransferase (DNMTs) and DNMT stability [42–44]. In several cancers, Akt1 has been reported to mediate epigenetic silencing by promoter methylation [45–47]. In addition to DNA methylation, Akt also has been shown to be involved in histone modifications; the activity of histone methyltransferase EZH2 and histone acetyltransferase CBP were shown to be regulated by Akt through phosphorylation [48,49].

To address the molecular mechanism underlying Hox gene regulations by Akt [21], we investigated epigenetic modifications such as histone modifications along the Hox loci. The loss of Akt1 in MEFs resulted in induced histone H3K9 acetylation and reduced histone H3K27me3 interaction on the 5′ Hox genes Hoxc11 and Hoxc12. In Akt1-null MEFs, the expression of several Hdac genes was reduced and an RNAi experiment suggested that Hdac6 regulates acetylation status at Hoxc12. Expression analyses and ChIP assays have shown that Ezh2 and Kdm6b regulate histone H3K27me3 levels at the Hoxc11 and Hoxc12 genes, which were dependent on the presence of Akt1. Our data demonstrate that Akt1 is important for the regulation of Hox gene expression through epigenetic modifications, probably through the direct binding to the promoter region of the 5′ Hox genes and/or Hdac6 in mouse embryonic fibroblasts.

2. Materials and methods

2.1. Cell culture, plasmid and transfection

Akt1+/+ (WT) and Akt1−/− MEFs (E13.5) were generated as previously described [50]. MEFs were maintained in Dulbecco’s modified Eagle’s medium (DMEM: WelGENE Inc., Daegu, Korea) supplemented with 10% fetal bovine serum (WelGENE Inc.), 100 units/ml penicillin, and 100 µg/ml streptomycin sulfate (WelGENE Inc.) at 37 °C in 5% CO2. Cells were transfected with siRNA against mouse Akt1 (s62215; Ambion Inc., Austin, TX, USA), HDAC6 (siRNA ID# s67425; Ambion), HDAC9 (siRNA ID# s95881; Ambion), or a universal negative control siRNA (ST Pharm. Co., Ltd. Seoul, Korea) using HiPerFect reagent (Qiagen, Hilden, Germany). For the long-term transfection, cells were transfected with 2 nM siRNA according to the manufacturer’s protocol. For the long-term transfection, cells were transfected with 2 nM siRNA according to the manufacturer’s protocol. For the long-term transfection, cells were transfected with 2 nM siRNA according to the manufacturer’s protocol.

2.2. Antibodies

The following antibodies were used for Western blot and ChIP analysis: anti-Akt1 (#2938, Cell Signaling Technology, Danvers, MA, USA), anti-Akt2 (#2964, Cell Signaling Technology), anti-Akt3 (#3788, Cell Signaling Technology), anti-histone H3 (ab1791, Abcam, Cambridge, UK), anti-histone H3 acetyl K9 (ab12179, Abcam), anti-histone H3 tri-methyl K27 (ab6002, Abcam), anti-Ezh2 (#5246, Cell Signaling Technology), anti-Suz12 (#3737, Cell Signaling Technology), anti-KDM6A (ab36938, Abcam), anti-KDM6B (ab85392, Abcam), anti-HA tag antibody (ab9110, Abcam), anti-Hdac6 antibody (ab12179, Abcam), anti-β-actin antibody (ab6276, Abcam), and normal mouse and rabbit IgG (sc-2025 and sc-2027, Santa Cruz Biotechnology, Inc., CA, USA).

2.3. Immunoprecipitation (IP)

Cells were harvested and resuspended in Nonidet-P40 (NP-40) lysis buffer (150 mM NaCl, 1% NP-40, 50 mM Tris–HCl [pH 8.0]) containing a protease inhibitor. After pre-clearing, the cell lysates were incubated with normal IgG (as a negative control) or primary antibodies overnight at 4 °C. The lysates were incubated with Protein A/G agarose beads and then washed with NP-40 lysis buffer. The precipitated proteins were released by boiling in loading buffer and resolved by SDS-PAGE.

2.4. RT- and real-time PCR analysis

Total RNA was isolated using the TRizol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer’s protocol. Two micrograms of total RNA was reverse-transcribed using the ImProm-II™ Reverse Transcription System (Promega, Madison, WI, USA) and cDNA was amplified with the specific primers listed in Table S1. PCR amplification conditions and primers for detecting Hox genes were the same as described previously [21]. For quantitative PCR analysis, the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster, CA, USA) and the Power SYBR Green PCR Master Mix (Applied Biosystems) kits were used.

2.5. Western blot

After cell lysis, protein content was determined using a Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). Ten micrograms of protein was loaded on 10% SDS poly-acrylamide gels, electrotransferred to PVDF membranes (Bio-Rad, Hercules, CA, USA), and probed with appropriate antibodies. Immunoreactive bands were detected with anti-rabbit (Abcam) or anti-mouse (Zymed, South San Francisco, CA, USA) HRP secondary antibody and visualized using an enhanced chemiluminescent substrate (SuperSignal West Pico Chemiluminescent Substrate kit; Thermo Scientific).

2.6. Chromatin immunoprecipitation (ChIP) and ChIP-PCR assay

ChIP analysis was performed following the protocol previously described by Min et al. [31] with minor modifications. Briefly, cells were cross-linked with 1% formaldehyde for 15 min at room temperature and the reaction was stopped by adding 125 mM glycine. After washing twice with cold PBS, cells were lysed for 10 min on ice in lysis buffer (1% SDS, 1% Triton X–100, 0.1% sodium deoxycholate, 10 mM EDTA, 50 mM Tris–HCl [pH 8.0]) containing a protease inhibitor. The chromatin was sonicated with 30 sets of 10-s pulses on ice to generate DNA fragments with an average length of 500–1000 bp. After pre-clearing, 1% of each sample was saved as the input fraction. Immunoprecipitation was performed using specific antibodies against the indicated proteins or IgG of a different species as the control. After cross-linking reversal, nucleic acids were prepared from the eluted complex and quantitative PCR analysis was performed using StepOnePlus™ Real-Time PCR System and Power SYBR Green PCR Master Mix. Primers for ChIP-qPCR are listed in Tables S2 and S3.

2.7. Statistical analysis

All quantitative data were from experiments repeated at least three times, and significance levels were calculated using one-way ANOVA or
downregulated Akt1 production, without affecting the production of Akt1 active 5′ regulator of Hoxc12 and blot analysis (Fig. 1A). As shown in Fig. 1, were transfected with siRNA against not a result of differences in WT regulation of the 5′ Akt1 versus correlation with gene expression. We of Hox (MEF) cells 3.1. Akt1 represses 5′ Hoxc gene expression in mouse embryonic fibroblast (MEF) cells

In search of regulatory genes responsible for the colinear expression of Hox genes during embryogenesis, we previously identified Akt1 as a regulator of Hox genes through in silico analysis [21]. Several consecutive 5′ Hoxc genes that were repressed in WT cells were de-repressed in Akt1-null MEFs. In addition, the DNA methylation status showed reverse correlation with gene expression. We first confirmed that down-regulation of the 5′ Hoxc gene expression was due to Akt1 itself and not a result of differences in WT vs. Akt1-null MEF status. WT MEFs were transfected with siRNA against Akt1 (siAkt1) for 21 days and the siRNA knockdown efficiency was confirmed by RT-PCR and Western blot analysis (Fig. 1A). As shown in Fig. 1, Akt1 siRNA specifically downregulated Akt1 production, without affecting the production of other Akt isoforms (Akt2 and Akt3). Similar to our previous findings in Akt1+/− MEFs [21], treatment with siAkt1 in WT MEFs significantly de-repressed 5′ Hoxc gene expression (Fig. 1B), confirming that Akt1 represses 5′ Hoxc gene expression in MEFs.

3.2. Histone modifications at the 5′ Hoxc gene loci

To address the molecular mechanism underlying Hox gene regulation by Akt1, epigenetic modifications such as DNA methylation and histone modifications were examined. Previously, profound DNA methylation differences at 5′ Hoxc gene promoter regions such as Hoxc11 (Fig. S1) and Hoxc12 [21] were detected through combined bisulfite restriction analysis (COBRA) and bisulfite sequencing. Results from these experiments indicated that Akt1 modulated DNA methylation patterns at the 5′ Hoxc loci.

Here, we examined whether histones were differentially modified in the presence or absence of Akt1. Hoxc11 and Hoxc12 genes, which are significantly upregulated by siAkt1 (Fig. 1B) and the control Hoxc5 and Hoxd10 genes, whose expression levels were not affected by Akt1, were chosen for analysis. As shown in Fig. 2, histone H3 lysine 9 acetylation (H3K9ac), a marker for transcription activation, was enhanced in Akt1+/− MEFs at the Hoxc11 and Hoxc12 loci. However, the acetylation levels at Hoxc5 were strong in both Akt1+/− and Akt1−/− MEFs, as Hoxc5 was expressed strongly in both cell types. In the Hoxd10 negative control, whose expression was not detected in both MEF types [21], the acetylation mark was not detected in either MEF type. Contrary to histone acetylation, histone H3 lysine 27 trimethylation (H3K27me3), a marker for transcriptional repression, was reduced in Akt1-null MEFs at Hoxc11 and Hoxc12 sites and levels were unchanged (or slightly reduced) at Hoxc5 and Hoxd10 sites compared with WT MEFs.

3.3. Histone deacetylase mediates the 5′ Hoxc gene repression when Akt1 is present

To examine whether Akt1-mediated repression of the 5′ Hoxc gene is due to the histone deacetylase (HDAC), WT MEFs were treated with trichostatin A (TSA), an inhibitor of class I/II/IV HDACs. Expression of the 5′ Hoxc gene was upregulated in a dose-dependent manner (Fig. 3A). Furthermore, the treatment of 5-aza-2′-deoxycytidine (Aza), a specific inhibitor of DNA methylation, in combination with TSA synergistically de-repressed the 5′ Hoxc genes. A control 3′ Hoxc gene, Hoxc5, showed no change in its expression. There was no effect on the de-repression of the 5′ Hoxc genes when MEFs were treated with the class III HDAC inhibitor, salermide (Fig. 3B).

To determine which HDAC is involved in the regulation of Hoxc gene expression, the relative expression level of class I, II and IV HDACs were examined in WT and Akt1-null MEFs. As shown in Fig. 3B, the expression levels of Hdac3, Hdac5, Hdac6, Hdac9 and Hdac11 were reduced in Akt1-null MEFs, and the protein levels of Hdac6 and Hdac9, whose expressions were significantly reduced in the absence of Akt1, were confirmed through Western blotting (Fig. 3C). To see whether specific Hdacs repressed the 5′ Hoxc genes, a knockdown experiment with siRNA against Hdac6 and Hdac9 was performed. As shown in Fig. 3D, Hoxc12 expression was recovered when Hdac6 siRNA was treated in

![Fig. 1. Effects of Akt1 on Hoxc gene expression.](image)

(A) Effects of Akt1 siRNA on Akt isoforms’ mRNA expression (left) and protein expression (right). (B) Quantitative analysis of 5′ Hoxc gene transcripts in Akt1 siRNA-transfected MEFs. Relative values ± S.E. were obtained from three independent assays. *p < 0.05; **p < 0.01, by one-way ANOVA.)
the presence of Aza. Hdac6 specifically repressed Hoxc12, but not Hoxc11 or Hoxc13, in MEF cells, which is most likely explained due to the differences in the genomic context of these genes. Since both siRNA and Aza treatment downregulated Hdac9 production, the association of Hdac6 along the Hoxc12 loci was investigated. When the direct binding of Hdac6 was examined using the ChIP-PCR method, the Hoxc12 promoter region was enriched with Hdac6 near the transcription start site (TSS) in WT MEFs (Fig. 3E).
To examine whether Hdac6 interacts with Akt1, a co-immunoprecipitation (Co-IP) analysis was performed with an antibody against Akt1 as well as Hdac6. As shown in Fig. 3F, direct association between the endogenous Hdac6 and Akt1 was detected. Although Akt1 is well known as a serine–threonine protein kinase, there is a still question whether Akt1 is recruited to the Hox promoters. To overcome the absence of ChIP-grade Akt1 antibody, a plasmid coding HA-tagged Akt1 was constructed and used for ChIP analysis using antibody against HA tag. Surprisingly, increased binding of overexpressed Akt1 was observed at both the Hoxc11 and Hoxc12 loci in wild type MEFs, while Akt1 was not enriched at Hoxc5 and Hoxd10 loci whose expression and repression were Akt1-independent, respectively (Fig. 3G and H).

3.4. Regulation of the H3K27me3 mark by Ezh2 and Kdm6b are Akt1-dependent

Since H3K27me3 repressive marks are positioned by the histone methyltransferase contained in PRC2 and removed by the histone demethylases Kdm6a (Utx) and Kdm6b (Jmjd3), the levels of PRC2 members (Ezh2 and Suz12), Kdm6a, and Kdm6b were analyzed using RT-PCR and Western blot analysis. As shown in Fig. 4A, the relative expression levels of Ezh2 and Suz12 were not changed in the presence or absence of Akt1; however, the expression levels of Kdm6a and Kdm6b were increased in Akt1-null MEFs. ChIP-PCR was used to test whether PRC2 proteins and histone demethylases were involved in H3K27me3 mark levels at the Hoxc11 and Hoxc12 gene loci. In WT MEFs, there was no significant change in the levels of PRC2; however, the binding of Ezh2 was increased at the Hoxc11 and Hoxc12 loci (Fig. 4B) while Suz12 remained unchanged (Fig. S3). Increased binding of the Kdm6b histone demethylase was observed at both the Hoxc11 and Hoxc12 loci in Akt1-null MEFs (Fig. 4B). Kdm6a binding levels were not changed along the Hoxc11 and Hoxc12 loci, which are similar to the Suz12 results in the presence or absence of Akt1 (Fig. S3).

To confirm that these results are due to the absence of Akt1 (i.e. Akt1 specific), not due to the differences between WT and Akt1-null MEF cells, WT MEFs were treated with Akt1 siRNA, and both mRNA and protein levels were analyzed. As shown in Fig. 4C, both histone methyltransferases (Ezh2 and Suz12) and demethylases (Kdm6a and Kdm6b) were detected at similar levels compared to the Akt1-null MEFs (Fig. 4A). Enrichment of Ezh2 in the WT and Kdm6b in the Akt1 siRNA-treated MEFs were detected at the 5′ Hox gene loci (Fig. 4D), which resulted in a significant reduction of the H3K27me3 mark in siAkt1-transfected cells (Fig. 4E) as detected in Akt1-null MEF cells (Fig. 2B).

4. Discussion

We previously reported that Akt1 is a potent repressor of the 5′ Hox genes in mouse embryonic fibroblast cells [21]. siRNA techniques confirmed that a loss of Akt1 caused the upregulation of the 5′ Hox genes such as Hoxc11 and Hoxc12 (Fig. 1). These results strongly suggested that upregulation of the 5′ Hox genes in Akt1-null MEFs was not a result of the different status of MEF cells (i.e., WT vs. Akt1−/− MEFs), but was due to direct regulation by Akt1.

In this study, we investigated how Hox gene expression was regulated by Akt1. In Akt1-null MEF cells, histone acetylation at H3K9, an active mark, was enriched at the 5′ Hoxc11 and Hoxc12 gene loci, which were coupled with their expression (Fig. 2). Since HDACs have been reported to be associated with many transcriptional repressive

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Fig. 4. H3K27me3 modification at the 5′ Hox loci through Ezh2 and Kdm6b in MEFs. Expression level of polycomb proteins (Ezh2 and Suz12) and H3K27 demethylases (Kdm6a and Kdm6b) at the mRNA (left) and protein (right) levels in WT vs. Akt1-null MEF cells (A), and control siRNA vs. siAkt1-treated cells (C). Quantitative ChIP-PCR at the Hoxc11 and Hoxc12 loci using anti-Ezh2 and anti-Kdm6b antibody in WT and Akt1-null MEFs (B), and Akt1-siRNA transfected MEFs (D), (E) Quantitative analysis of ChIP-PCR at the 5′ Hox genes, Hoxc11 and Hoxc12 in Akt1-siRNA transfected MEFs. Relative values ± S.E. were obtained from three independent assays. *p < 0.05, by Student’s t-test.
complexes and participate in transcriptional repression. We questioned whether HDACs were involved in 5′ Hoxc gene repression in Akt1+/− WT MEFs. Hdac6 was shown to participate in the transcriptional repression of Hoxc12 (Fig. 3). In the case of Hoxc11 and Hoxc13, different Hda(s) or locus/cellular context-dependency might be involved in their repression instead of Hdac6 alone. In previous reports, HDAC4 was shown to repress the expression of Hoxb3 in androgen receptor-negative prostate cancer cells [51], in which HDAC4 was recruited by transcription factor YY. Although the endogenous Akt1 seemed to interact with Hdac6 directly in MEFs and the exogenous Akt1 was enriched at the 5′ Hoxc genes (Hoxc11 and Hoxc12 loci), it is not clear how Akt1 is involved in Hdac recruitment and function. Because Hdac6 contains a potential Akt phosphorylation site, serine 129 (Fig. S4A), we wondered whether it could be modulated by Akt1. To determine whether Akt1 can phosphorylate Hdac6, we immunoprecipitated Hdac6 and probed it with an antibody that recognizes the phosphorylated form of Akt sub- strates. Surprisingly, the level of Hdac6 phosphorylation was enriched when Akt1 was absent (Fig. S4B). Since Akt2 and Akt3 isoforms are still in the cell, Hdac6 phosphorylation might be due to these isoforms. From these results along with the previous one (Fig. 3B), Akt1 seems to regulate the expression of Hdac6 (Fig. 3B) to repress 5′ Hoxc genes, rather than the direct phosphorylation of Hdac6. In Akt1-null MEFs, histone acetyltransferases (HATs) might be involved in increased acetylation levels at the 5′ Hoxc genes. Since histone acetylates such as Mo2 and Gcn5 have been reported to regulate Hox gene expression during development [52,53], we may need to discover which HAT is implicated in Hox gene repression in Akt1 signaling in the future.

In histone H3K27 methylation, the activation of P3K/Akt signaling has been reported to induce epigenetic silencing in which the enrichment of H3K27me3 in the promoter region repressed the CST6 tumor suppressor gene in breast cancer [46]. In another report, tumor suppressor C-terminal Src kinase (Csk)-binding protein (CBP) was downregulated by the enhanced enrichment of H3K27me3 by the MAPK/P3K pathway [54]. In a mammary epithelial cell line, the forced expression of Akt1 induced genome-wide reprogramming of H3K27me3 in many downstream genes, and several Hox genes were included in these targets [55]. Here, as one piece of the evidence that Akt1 regulates Hox gene expression through histone H3K27 trimethylation, we showed a predominant enrichment of H3K27me3 at the 5′ Hoxc11 and Hoxc12 loci in the presence of Akt1 in MEF cells (Fig. 2). Although the level of Ezh2 histone methyltransferase expression was similar in both WT and Akt1-null MEFs, the recruitment of Ezh2 at the 5′ Hoxc loci was reduced when Akt1 was absent (Fig. 4B). In contrast to our result, the presence of an Akt signal has been shown to de-repress silent target genes by phosphorylating EZH2 in cancer cell lines, impedes EZH2 binding to histone H3 and resulting in decreased trimethylation at H3K27 [48]. Although this discrepancy could be due to the cell type used, how Akt1 regulates Ezh2 binding or activity on the target sites in MEFs remains to be revealed.

Along with Ezh2, the histone demethylases, Kdm6a and Kdm6b, are also important regulators of gene expression through H3K27me3 and have been reported to regulate Hox genes during development and differentiation [17,56,57]. Interestingly, Lan et al. [57] showed that inhibition of Utx (Kdm6a) led to the mis-regulation of Hox genes and resulted in a concomitant posterior developmental defect in a Zebrafish model. Recently, Zhang et al. [58] showed that active Akt1 downregulated Kdm6a and Kdm6b expression, which is consistent with our finding here of both Kdm6a and Kdm6b gene upregulation in both Akt1-null and siAkt1-treated MEF cells (Fig. 4A,C). Furthermore, induced binding of Kdm6b was detected in the posterior 5′ Hoxc genes in both Akt1-null and siAkt1-treated MEFs (Fig. 4B,D).

In this study, we suggest that Akt1 mediates the posterior 5′ Hoxc gene expression through epigenetic modification such as histone methylation and acetylation, and partly through a direct binding to the promoter region of the 5′ Hoxc genes and/or Hdac6 in mouse embryonic fibroblast cells.
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