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ACTL6A interacts with p53 in acute promyelocytic leukemia cell lines to affect differentiation via the Sox2/Notch1 signaling pathway

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ABSTRACT

Actin-like 6A (ACTL6A), a component of BAF chromatin remodeling complexes, is important for cell differentiation. Nevertheless, its role and mechanism in acute promyelocytic leukemia (APL) has not been reported. To identify the genes that may participate in the development of APL, we analyzed data from an APL cDNA microarray (GSE12662) in the NCBI database, and found that ACTL6A was up-regulated in APL patients. Subsequently, we investigated the function and mechanisms of ACTL6A in myeloid cell development. The expression of ACTL6A was gradually decreased during granulocytic differentiation in all-trans retinoic acid-treated NB4 and HL-60 cells, and phorbol myristate acetate-treated HL-60 cells. We also found that knockdown of ACTL6A promoted differentiation in NB4 and HL-60 cells, and decreased the levels of Sox2 and Notch1. Mechanistically, ACTL6A interacted with and was co-localized with Sox2 and p53. Meanwhile, CBL0137, an activator of p53, decreased the expression of ACTL6A and promoted differentiation in NB4 and HL-60 cells. These findings suggest that the inhibition of ACTL6A promotes differentiation via the Sox2 and Notch1 signaling pathways. Furthermore, the differentiation promoted by inhibiting ACTL6A could be regulated by p53 via its physical interaction with ACTL6A.

1. Introduction

Acute promyelocytic leukemia (APL) is a malignancy of the bone marrow that is classified as the M3 variant of acute myelocytic leukemia (AML) in the internationally accepted French-American-British system [1,2]. In bone marrow, there are mature, and many immature, blood cells called promyelocytes [3]. APL is characterized by the t (15;17) mutation which is due to a translocation between chromosomes 15 and 17 [4,5]. Pharmacological doses of all-trans retinoic acid (ATRA) produce clinical remission in APL patients by inducing the maturation of promyelocytes and degradation of the PML/RARα fusion protein [6,7]. Nevertheless, ATRA does not eliminate the malignant myeloid clone in APL, and most relapsed APL patients are resistant to further treatment with this drug [8,9]. Thus, research on the pathogenesis of APL is exceedingly important.

Differentiation of granular leukocytes is an important part of hematopoietic functions. It is controlled by a complex network composed of a variety of regulatory factors, including cytokines [10], transcription factors [11], and noncoding RNAs [12]. Changes in any of these important factors can result in deregulation of differentiation and lead to serious consequences, including hematopoietic malignancies.

Actin-like 6A (ACTL6A), an actin-like protein, is also widely known as BAF53a/Arp4/ INO80K. It is a member of ATP-dependent SWI/SNF-like BAF chromatin remodeling complexes that encode a family of actin-related proteins [13,14]. Previous reports revealed that ACTL6A was involved with varying cellular processes including chromatin remodeling, transcriptional regulation, vesicular transport, and nuclear migration [14–16]. A recent study reported that ACTL6A was relevant to neural progenitor cell differentiation and proliferation [17]. Another study found that ACTL6A exerted a significant role in the proliferation of squamous cell carcinoma cells. Additionally, ACTL6A participated in the differentiation process in the epidermis and squamous cell

Abbreviations: AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; DAPI, 4,6-diamidino-2-phenylindole; PMNs, polymorphonuclear leukocytes; ACTL6A, actin-like 6A; ATRA, all-trans retinoic acid; PMA, phorbol 12-myristate 13-acetate; shACTL6A, knockdown of ACTL6A by shRNA; DAPT, N-[N-(3, 5-difluorophenacetyl)-l-alanyl]-*S*-phenylglycine t-butyl este; CBL0137, curaxin-137 hydrochloride; Sox2, sex determining region Y-box2

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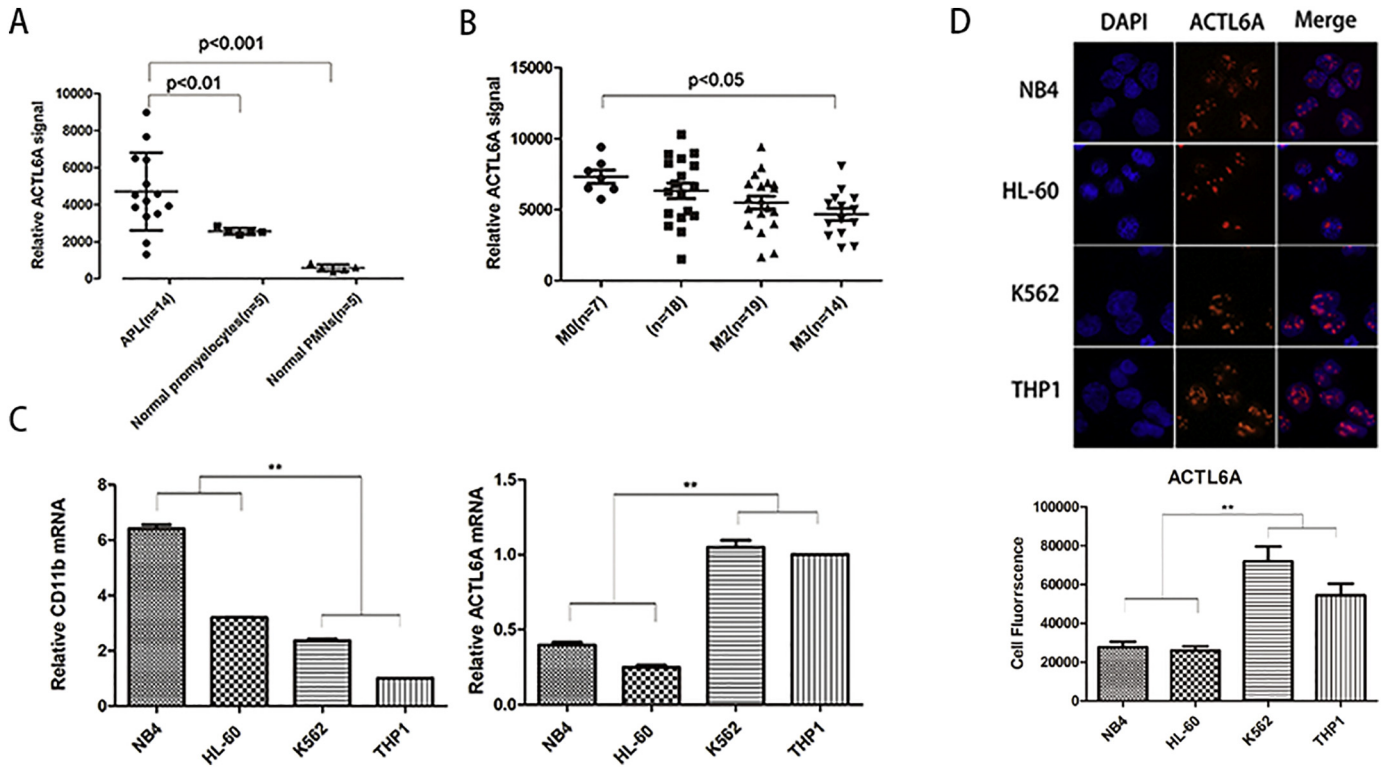

Fig. 1. Expression levels of ACTL6A in AML patients, normal persons, and in vitro. (A–B) Comparison of ACTL6A mRNA levels in APL patients, normal persons, and in different Fab typing AML patients. These data have been obtained from the cDNA microarrays available on the NCBI GEO database. The signal values of ACTL6A mRNA expression were obtained from GEO2R (online analysis software of NCBI: https://www.ncbi.nlm.nih.gov/geo/info/geo2r.html). (C) qRT-PCR analysis of the mRNA expression levels of ACTL6A and CD11b in AML cells. (D) Immunofluorescence analysis (×400) of endogenous ACTL6A (red) localization in AML cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

carcinoma [18]. Nevertheless, what role ACTL6A plays in APL has not been reported.

Sox2 (SRY-Box 2) is a protein-coding gene that reportedly participates in the differentiation of diverse diseases. Recent reports illustrated that Sox2 could regulate the activity of the Notch1 signaling pathway [19–21]. Thus, the expression level of Sox2 is relevant to ACTL6A. Here, we researched the association between ACTL6A and the Notch1 signaling pathway.

P53 is a tumor suppressor discovered 40 years ago [22]. It is widely believed that p53 functions as a tumor suppressor by virtue of its diverse activities including inducing cell apoptosis and cell cycle arrest [23]. It is also becoming increasingly clear that p53 regulates diverse cellular signaling pathways, and these pathways implement its tumor suppressor activity [24]. A recent study demonstrated that p53 performed a significant function in leukemia differentiation [25]. Another report indicated that p53 could interact with BAF53 [26]. Thus, we hypothesized that p53 also interacted with ACTL6A, a subtype of BAF53.

In the current study, we researched the association between ACTL6A, p53, and differentiation. Our research revealed an abnormal up-regulation of ACTL6A mRNA expression in APL patients, and identified that decreased ACTL6A released the inhibition on Sox2 and the Notch1 signaling pathway, which promoted cell differentiation. We also found that p53 promoted granulocytic differentiation via its physical interaction with ACTL6A, thereby decreasing active levels of this protein.

2. Materials and methods

2.1. Acquisition and processing of public microarray data

Public microarray data was obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). The APL cDNA microarray data of the normal control that were uploaded by Payton were downloaded from the NCBI GEO database (Affymetrix Human Genome U133 + 2.0 array, https://www.ncbi.nlm.nih).The expression level of differentially expressed genes in bone marrow cells of APL patients (M0, M1, M2) and healthy donors was analyzed through the GEO2R software. GEO2R is an interactive web tool that allows users to compare two or more sample groups in the GEO series to identify genes that are expressed differently under different experimental conditions.

2.2. Quantitative real-time polymerase chain reaction (qRT-PCR)

Following the designated treatments, total RNA was isolated using trizol reagent (Takara, Japan) and reverse transcribed into cDNA using the PrimeScript™ RT reagent Kit (Takara, Japan). The cDNA was amplified and relative expression levels in different samples were detected through quantitative real-time PCR (qRT-PCR) using the SYBR Green PCR Kit (TAKARA, Dalian, China) and a PRISM 7300 Sequence Detection System (Invitrogen, Carlsbad, California). β-actin was used as an internal control. Experiments were performed at least in triplicates. All primers were synthesized at and purchased from the TSINGKE Company (Shanghai, China). The sequences of primers used were as follows: ACTL6A forward F, 5′-ATGTGTGATATTGACATCAGACCAG-3′, ACTL6A reverse R, 5′-CGCCAATCCATGAGCTAAACC-3′; p53 F, 5′-CCT GAGGTTGGCTCTGACTGTA-3′, p53 R, 5′-AAAGCTGTTCCGTCCCAGT AGA-3′; CD11b F, 5′-ACTGGTGAAGCCAATAACGCA-3′, CD11b R, 5′-TCCGTGATGACAACTAGGATCTT-3′; and β-actin F, 5′-TGACGTGGA CATCCGCAAAG-3′, β-actin R, 5′-CTGGAAGGTGGACAGCGAGG-3′.

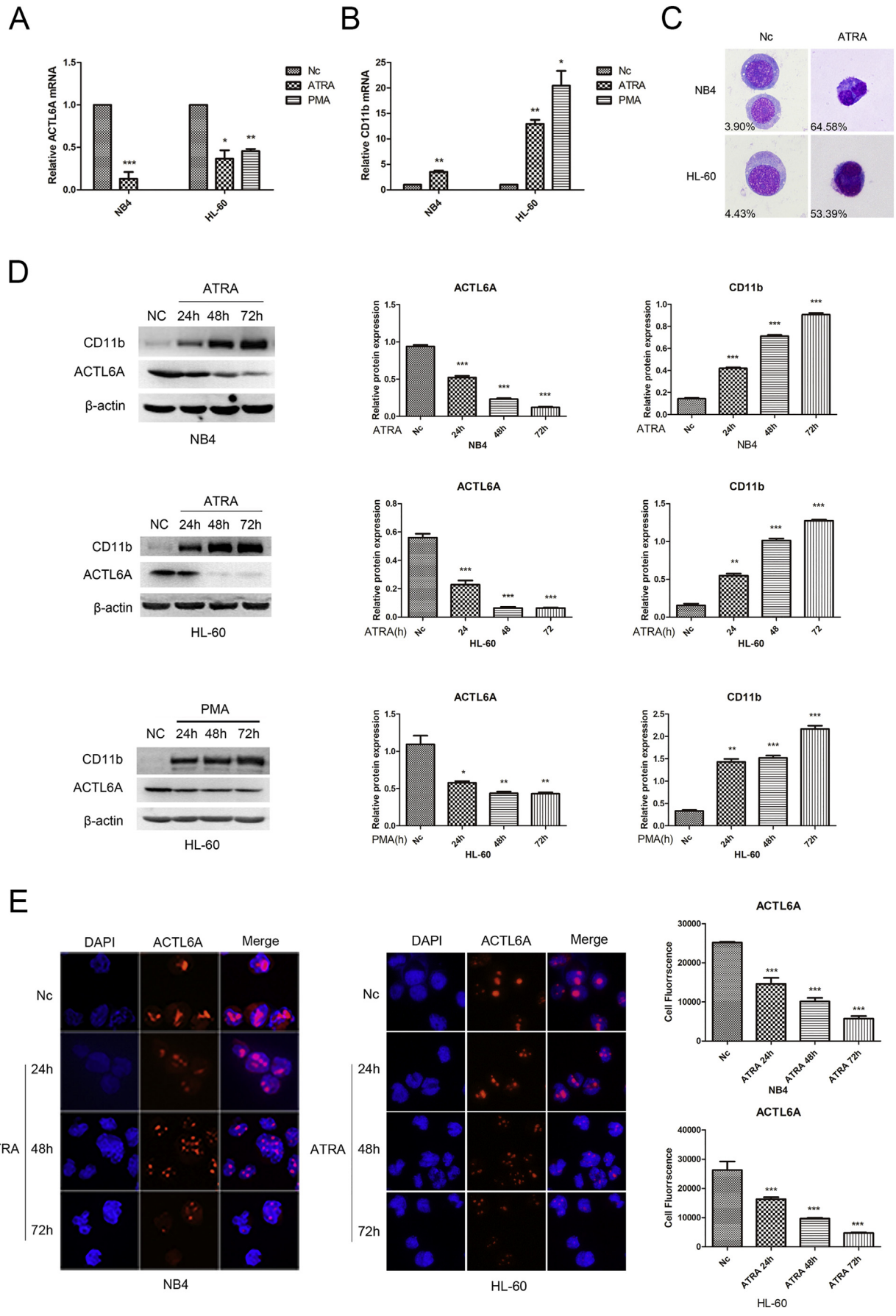

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Fig. 2. Expression levels of ACTL6A during ATRA-induced granulocytic differentiation of NB4 and HL-60 and PMA-induced granulocytic differentiation of HL-60. (A-B) qRT-PCR analysis of the mRNA expression levels of ACTL6A and CD11b in NB4 cells treated with ATRA and HL-60 cells treated with ATRA or PMA. **(C)** Morphological analysis of NB4 and HL-60 cells treated with ATRA by Wright staining. (D) Western blot analysis of the protein expression levels of ACTL6A and CD11b in NB4 and HL-60 cells treated with ATRA at 0 h, 24 h, 48 h, and 72 h. (E) Immunofluorescence analysis (×400) of endogenous ACTL6A (red) localization and expression in NB4 and HL-60 cells treated with ATRA at 0 h, 24 h, 48 h, and 72 h. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Western blot analysis

Cells were divided into several groups following the designated treatments and were washed thrice with pre-chilled phosphate buffer saline (PBS). The cells were then harvested and paddy cracking was carried out at 4 °C for 30 min in a lysis buffer to which the protease and phosphatase inhibitor phenyl methane sulfonyl fluoride (PMSF, Cell Signal, USA) and the phosphatase inhibitors NaF and Na3VO4 were added. Clear fluid was collected after centrifugation at 4 °C for 15 min at 13,000 ×*g*. Protein concentration was determined through the BCA method. Later, the same amount of protein (50 μg) obtained from each sample was run in a 10% SDS-PAGE gel and transferred onto a PVDF membrane (United States, Boston, MA). Blocking was done in 5% skimmed milk, incubation; blots were incubated with the appropriate primary antibody at 4 °C for 16 h. This was followed by incubation with an HRP-conjugated secondary antibody for 1 h at room temperature. Protein signals were detected using the enhanced chemiluminescent substrate (ECL). The signals were visualized and analyzed using biological rad gel imaging system on the cooling image workstation II (Viagene, USA). The primary antibodies used were as follows: Anti-ACTL6A, Anti-Notch1, and Anti-CD11b (Abcam, Cambridge, UK); Anti-p53, Anti-cleaved Notch1, and Anti-Hes1 (Cell Signaling Technology, Boston, USA); and Anti-Sox2 (GeneTex, St. Louis Park, MN, USA).

2.4. Cell line and culture

Suspension cultures of the human AML cell lines, HL-60, NB4, K562, and THP-1 were established in RPMI-1640 medium (Gibco-Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Melbourne, Australia) in an environment with 5% CO2 at 37 °C. Cell density was maintained within the range 1–10 × 10^5 cells/ml. Cells were counted routinely using a Coulter Counter. To induce differentiation, 1 μM ATRA was added to cells (1 × 10^5/mL), which were then incubated for 72 h. DAPT (10 μM), an inhibitor of the Notch1 signaling pathway, was added to the cells (1 × 10^5 cells/mL), which were then incubated for 72 h. Similarly, CBL0137 (1 μM), an activator of p53, was added to the cells (1 × 10^5 cells/mL), which were then incubated for 24 h.

2.5. Co-immunoprecipitation

After harvesting the cells, a suitable amount of cell lysis buffer (including protease inhibitors) was added to the cells, which were then incubated on ice for 30 min; the supernatant was obtained after centrifuging the cell suspension at maximum speed for 30 min. A small volume of pyrolysis liquid was used for western blot analysis of residual cracking liquid; the appropriate antibody (1 μg) was added to each cell lysis solution, and solutions were incubated overnight at 4 °C with slow shaking. Twenty five microliters of protein A agarose beads were washed thrice by suspending them in lysis buffer, centrifuging for 10 min and discarding the supernatant. 10 μl of pretreated protein A agarose beads were mixed with antibody and incubated at 4 °C in cell lysis buffer with slow shaking for 5 h. After immunoprecipitation, protein A agarose beads were collected by centrifugation at 4 °C, 3000 rpm for 3 min followed by careful suction. The beads were then washed 3–4 times with 1 ml of cleft buffer. Finally, 15 ml of 2× sample buffer containing SDS was added and boiled in the waterbath for 5 min. The enhanced chemiluminescent substrate (ECL) was used to analyze the test.

2.6. Cell morphological staining

After the designated treatment, cells were collected and washed thrice with pre-chilled PBS and then re-suspended in fresh PBS. The cell suspension (10 μl) was daubed onto glass slides, and then air-dried. The cells were stained with Wright staining fluid.

2.7. Immunofluorescence

Cellular immunofluorescence was performed based on the protocol suggested by Abcam. Cells were first coated onto a glass slide; they were then fixed using a 4% solution of polyformaldehyde in phosphate buffer saline (PBS), with 0.2% of Trion. Blocking was carried out for 1 h using 1% caprine serum albumin. The appropriate dilutions of antibodies (please refer to section 2.3) were added to the slides, which were then incubated at 4 °C. Cells were finally washed and incubated with the appropriate secondary antibodies and DAPI before visualization under a fluorescence microscope.

2.8. Establishment of ACTL6A knockdown cells

The ACTL6A knockdown lentivirus and the negative control (NC) lentivirus were synthesized by and purchased from GenePharma (Shanghai, China). Lentiviruses containing short hairpin RNA (shRNA) targeting ACTL6A were transfected into NB4 and HL-60 cells according to the manufacturer's instructions. Cells transfected with an empty vector were used as controls. Puromycin (final concentration: 5 μg/ml) was used to select stable clones. The shRNA target sequence for ACTL6A was GCGTGTTCCGAGGGAGAATAT. The shRNA negative control (NC) sequence was TTCTCCGAACGTGTCACGT.

2.9. Data analysis

The data are reported as the mean ± SEM. Student's *t*-test or one-way analysis of variance (ANOVA) was used to test the statistical significance of the differences observed between experimental means. All analyses were performed using GraphPad Prism 5 ("*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$).

3. Results

3.1. ACTL6A is abnormally up-regulated in APL patients

We analyzed differentially expressed genes in bone marrow mononuclear cells derived from 14 APL patients and five healthy donors by GEO2R. These genes were downloaded from cDNA microarray data (Affymetrix Human Genome U133 Plus 2.0 Arrays) in the NCBI GEO database. The data showed that ACTL6A was up-regulated abnormally in APL patients compared to healthy controls (Fig. 1A). This demonstrated that ACTL6A could function as an oncogene in APL development.

3.2. ACTL6A is related to differentiation in AML

To investigate the relationship between ACTL6A and AML differentiation, we analyzed differentially expressed genes by GEO2R in bone

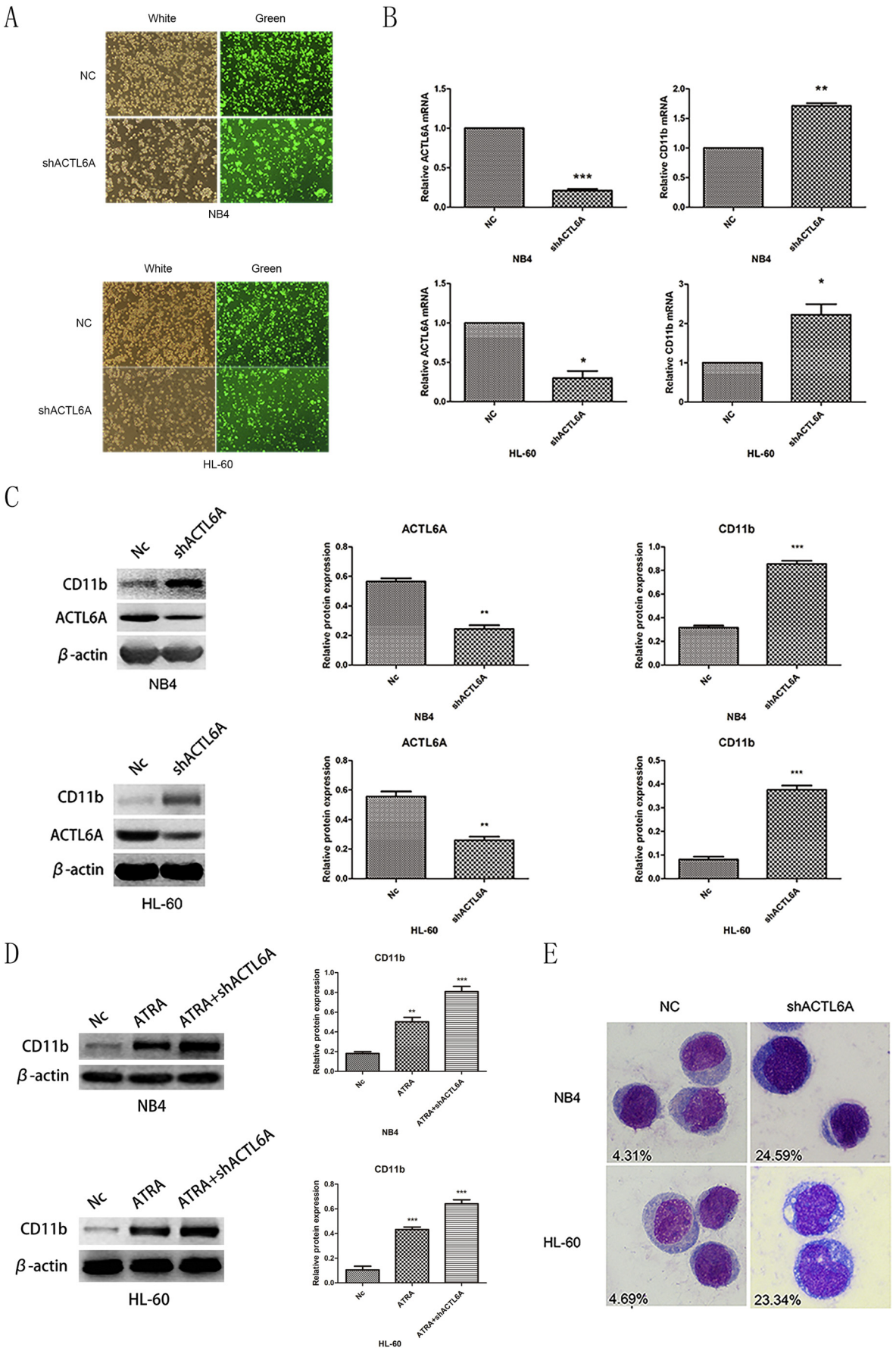

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Fig. 3. Promotion of granulocytic differentiation in NB4 and HL-60 cells through ACTL6A knockdown. (A) GFP fluorescence (green) in cells transfected with ACTL6A shRNA and control cells was detected through fluorescence microscopy (×100). (B) The mRNA expression levels of ACTL6A and CD11b were detected by qRT-PCR in NB4 and HL-60 cells after knock down of ACTL6A. (C) Western blot analysis of ACTL6A and CD11b expression in NB4 and HL-60 cells in which ACTL6A was knocked down. (D) The protein levels of CD11b in ACTL6A shRNA-transfected and control cells treated with ATRA were detected through western blotting. (E) Morphological observation of ACTL6A shRNA-transfected cells through Wright staining. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

marrow mononuclear cells derived from 76 AML patients that were divided into four groups. The cDNA microarray data were the same as before. From these results, we discovered that the mean level of ACTL6A expression decreased gradually from M0 to M3, and was remarkably decreased at M3 (Fig. 1B). We also assessed the ACTL6A mRNA expression levels in AML cells with different expression levels of CD11b mRNA (a marker of granulocytic differentiation). These results demonstrated that low ACTL6A-expressing NB4 and HL-60 cells had higher CD11b levels as compared to high ACTL6A-expressing K562 and THP1 cells (Fig. 1C). Similar results were obtained for protein levels by immunofluorescence analyses, as indicated by the accumulation of ACTL6A puncta in NB4 and HL-60 cells (Fig. 1D). These results suggest that ACTL6A is related to differentiation in AML.

3.3. ACTL6A expression is decreased gradually during ATRA- or phorbol myristate acetate (PMA)-induced differentiation in NB4 and HL-60 cells

To analyze whether ACTL6A participated in APL myeloid differentiation, we first measured its levels during granulocytic differentiation in the NB4 and HL-60 cell lines. The quantitative reverse transcription polymerase chain reaction (qRT-PCR) showed a significant decrease of ACTL6A mRNA (Fig. 2A) and increase of CD11b mRNA in cells treated with ATRA or PMA (Fig. 2B). Morphological observations showed differentiation of NB4 and HL-60 cells treated with ATRA (Fig. 2C). Western blotting demonstrated a gradual decrease of ACTL6A and increase of CD11b protein levels during ATRA-induced granulocytic differentiation of NB4 and HL-60 cells, or PMA-induced differentiation of HL-60 cells (Fig. 2D). Similar results were obtained by immunofluorescence analyses; there was a decreased accumulation of ACTL6A puncta in cells treated with ATRA (Fig. 2E). These results revealed that ACTL6A might have a role in granulocyte differentiation promoted by ATRA.

3.4. Knockdown of ACTL6A promotes granulocytic differentiation of NB4 and HL-60 cells

To examine the function of ACTL6A in granulocyte differentiation, endogenous ACTL6A was knocked down in NB4 and HL-60 cells via lentiviral shRNA. Cells containing ACTL6A shRNA were identified by visualizing their green fluorescent protein content, and the knockdown of ACTL6A in cells transfected with lentiviral shRNA was confirmed (Fig. 3A). qRT-PCR analyses revealed a significant increase of CD11b mRNA in the ACTL6A shRNA-transfected cells compared to the control cells (Fig. 3B). Western blots showed that the protein level of CD11b in the ACTL6A shRNA-transfected cells was up-regulated compared to the control cells (Fig. 3C). The protein level of CD11b was increased in ACTL6A shRNA-transfected cells treated with ATRA compared to the control cells treated with ATRA (Fig. 3D). Wright staining revealed a greater fraction of more mature granulocytic cells in NB4 and HL-60 cell populations transfected with ACTL6A shRNA (Fig. 3E). These results suggest that ACTL6A could function as a negative regulator in granulocytic differentiation.

3.5. Knockdown of ACTL6A promotes differentiation via Sox2 and the Notch1 signaling pathway

An interaction between ACTL6A and Sox2 has been reported in hepatocellular carcinoma cells [24]. We propose that ACTL6A also interacts with Sox2 in APL cell lines. To assess this possibility, the association between ACTL6A and Sox2 was analyzed by immunoprecipitation experiments and immunofluorescence analyses. As shown in Fig. 4A, co-localization of ACTL6A and Sox2 was detected, and ACTL6A was expressed primarily in the nucleus. Fig. 4B shows that ACTL6A co-immunoprecipitated with Sox2, whereas no ACTL6A could be detected in the IgG control. Correspondingly, Sox2 was found in the ACTL6A immunoprecipitated complexes. We also found that ACTL6A knockdown decreased the Sox2 expression level (Fig. 4C). Thus, Sox2 plays a crucial role in the Notch1 signaling pathway and we propose that ACTL6A regulates this pathway to drive differentiation in APL cell lines.

As expected, ACTL6A knockdown decreased Notch1 and Hes1 expression (Fig. 4D). Subsequently, we treated NB4 and HL-60 cells with DAPT, an inhibitor of the Notch1 signaling pathway, for three days. The results demonstrated that CD11b was up-regulated, and Notch1, Cleaved Notch1, and Hes1 were decreased (Fig. 4E). However, ACTL6A expression was not affected by DAPT. Increased level of CD11b and decreased level of Notch1 and Hes1 were detected in ACTL6A shRNA-transfected cells treated with DAPT compared with the control cells treated with DAPT (Fig. 4F). These results indicate that ACTL6A promotes differentiation via Sox2 and the Notch1 signaling pathway.

3.6. CBL0137, a p53 activator, promotes differentiation via a physical interaction between p53 and ACTL6A

As reported previously, BAF53 interacts with p53 in U2OS cells [27]. Thus, we proposed that ACTL6A, a subtype of BAF53a, also interacted with p53. To address this possibility, the association between ACTL6A and p53 was analyzed by immunoprecipitation and immunofluorescence analyses. As shown in Fig. 5A, ACTL6A co-localized with p53. Fig. 5B shows that ACTL6A co-immunoprecipitated with p53, whereas no ACTL6A was detected in the IgG control. Correspondingly, p53 was also found in the ACTL6A immunoprecipitated complexes.

Subsequently, we treated NB4 and HL-60 cells with CBL0137, a p53 activator, and performed qRT-PCR analyses. The results confirmed that the mRNA levels of p53 and CD11b were up-regulated, and the mRNA level of ACTL6A was decreased (Fig. 5C, D). Western blots revealed that the protein levels of ACTL6A, Sox2, and Notch1 decreased, and CD11b increased (Fig. 5E). Similar results were obtained by immunofluorescence analyses, as indicated by the decreased number of ACTL6A puncta in NB4 and HL-60 cells (Fig. 5F). Additionally, the expression of endogenous Sox2 decreased in APL cells treated with CBL0137 as detected by immunofluorescence (Fig. 5G). Overall, these results indicate that p53 drives differentiation via a physical interaction with ACTL6A, and decreases both the mRNA and protein levels of ACTL6A.

4. Discussion

ACTL6A is a multifunctional protein Involved in transcriptional activation and repression of selected genes by chromatin remodeling [27,28]. Nevertheless, the role played by ACTL6A in APL development has not been reported. An association of ACTL6A with some disorders is indicated by its abnormal up-regulation in diverse diseases that are regulated by numerous genes related to cell differentiation or proliferation [29]. We found abnormal up-regulation of ACTL6A in APL patients from APL cDNA microarray data (GSE12662). Therefore, we conjectured that ACTL6A might participate in the APL disease process.

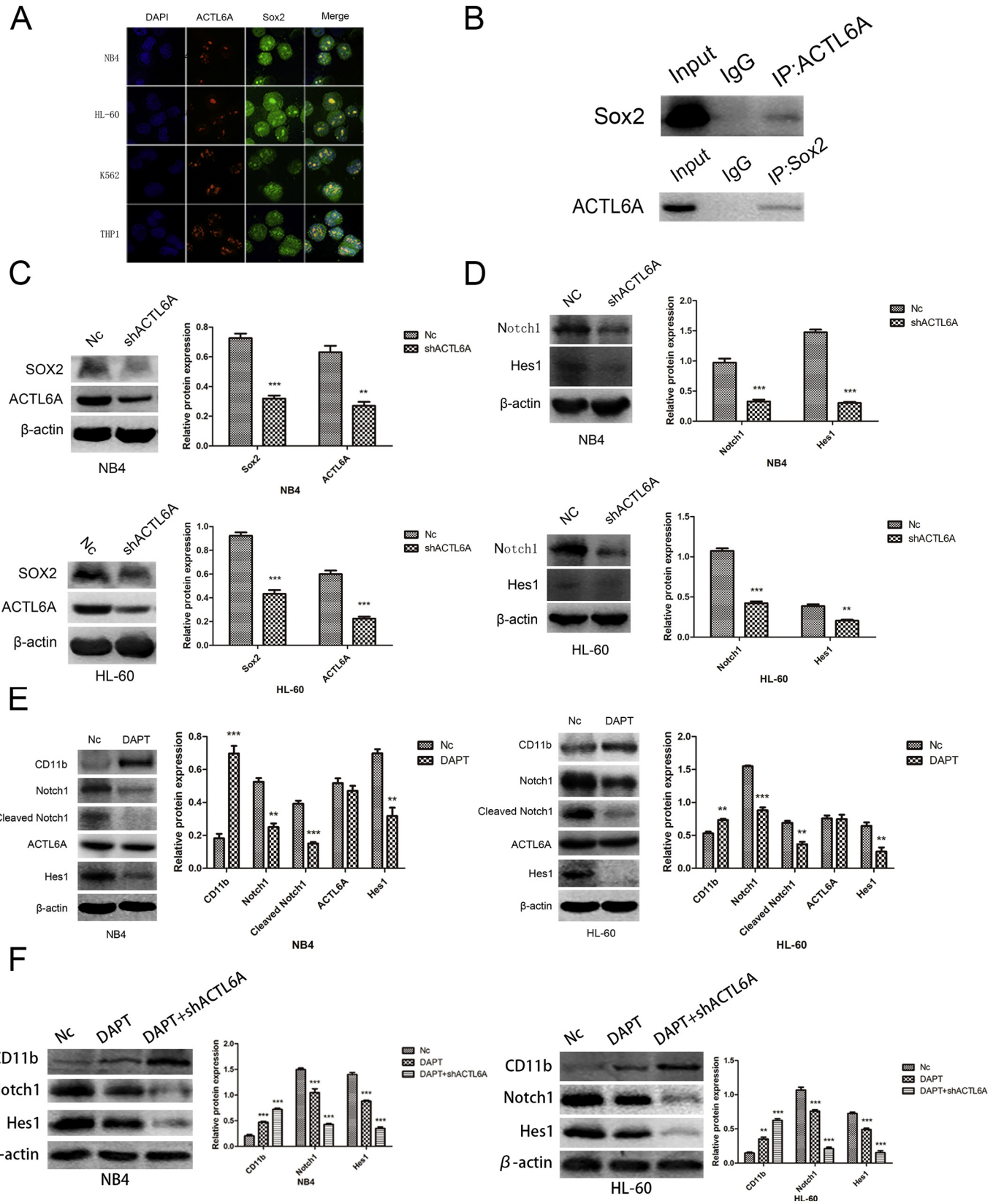

Fig. 4. Promotion of differentiation via Sox2 and Notch1 signal pathway through the knockdown of ACTL6A. (A) Immunofluorescence analysis (×400) of endogenous ACTL6A (red) and Sox2 (green) localizations in AML cells. (B) The interaction between ACTL6A and Sox2 in NB4 cells was detected by co-immunoprecipitation. (C) Western blot analysis of the protein levels of Sox2 in shACTL6A, NB4, and HL-60 cells. (D) Western blot analysis of the protein levels of Notch1 in shACTL6A, NB4, and HL-60 cells. (E) Western blot analysis of the protein levels of CD11b and Notch1 in APL cells treated with DAPT. (F) Western blot analysis of the protein levels of CD11b, Notch1, and Hes1 in shACTL6A APL cells treated with DAPT. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

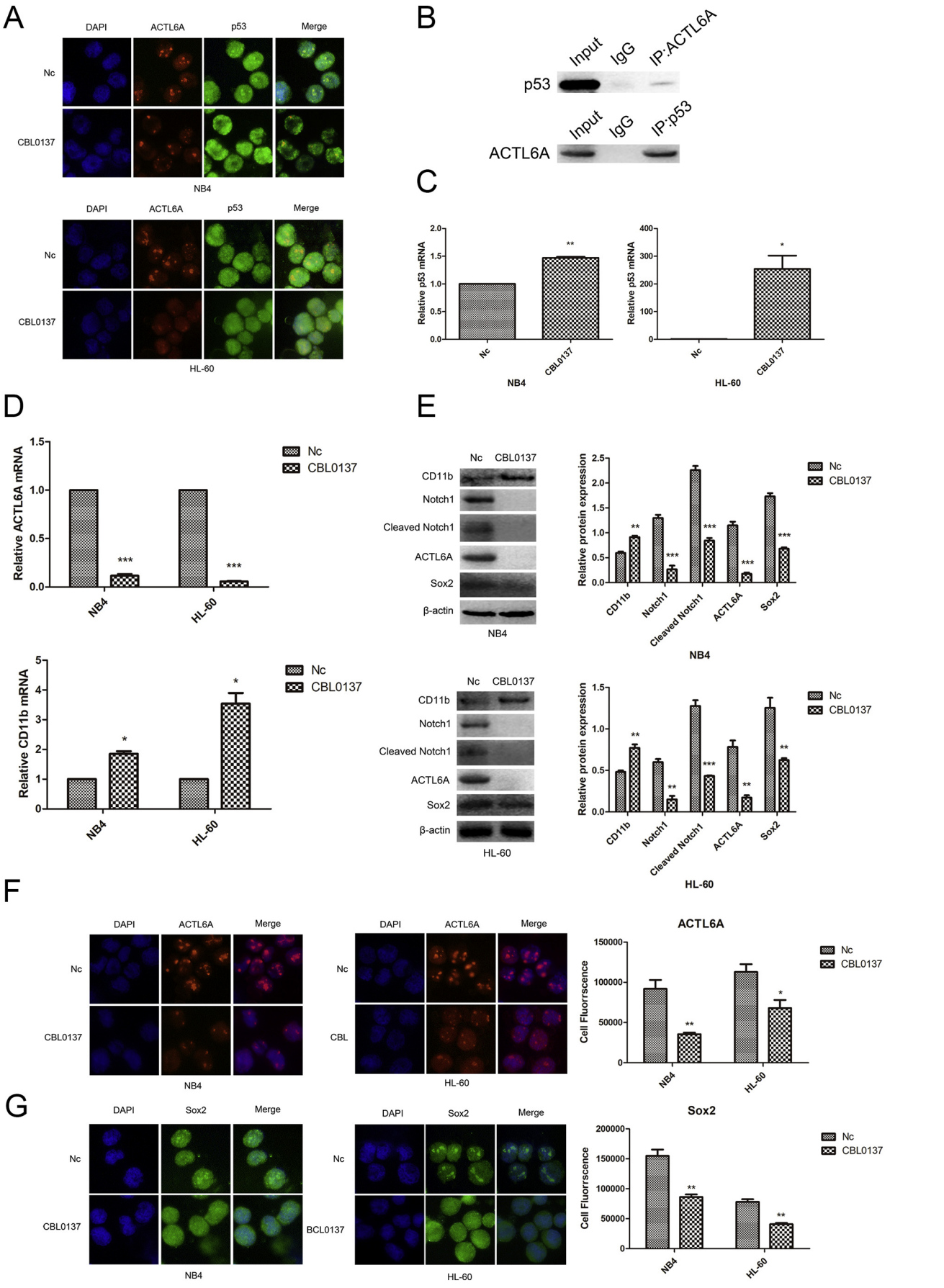

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Fig. 5. Promotion of differentiation by CBL0137 via physical interaction with ACTL6A. (A) Immunofluorescence analysis (×400) of endogenous ACTL6A (red) and p53 (green) localizations in APL cells. (B) The interaction between ACTL6A and p53 in NB4 cells was detected through co-immunoprecipitation. (C) mRNA levels of p53 were detected by qRT-PCR in APL cells treated with CBL0137. (D) qRT-PCR analysis of the mRNA levels of ACTL6A and CD11b in APL cells treated with CBL0137. (E) Western blot analysis of the protein levels of CD11b, Notch1, cleaved Notch1, ACTL6A, and Sox2 in APL cells treated with CBL0137. (F) Immunofluorescence analysis (×400) of the expression and localization of endogenous ACTL6A (red) in APL cells treated with CBL0137. (G) Immunofluorescence analysis (×400) of the expression and localization of endogenous Sox2 (green) in APL cells treated with CBL0137. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ATRA, a classic clinical drug for treating APL, acts mainly via inducing the differentiation of APL cells. Consistent with this mechanism, we detected a gradual decrease in ACTL6A levels during ATRA-induced granulocytic differentiation of NB4 and HL-60 cells, confirming the function of ACTL6A in APL cells. Furthermore, we observed its inhibitory role in the differentiation of APL cells by lentivirus knockdown. The level of CD11b increased in ACTL6A shRNA-transfected cells treated with ATRA compared with that in the control cells treated with ATRA, confirming that the knockdown of ACTL6A could promote the ATRA-induced differentiation of APL cells. These results revealed that ACTL6A had a significant role in APL differentiation.

Notch, an important signaling pathway composed of several transmembrane ligands and receptors, has functions including cell fate, apoptosis, and differentiation. As reported previously, ACTL6A participates in diverse cellular processes including vesicular transport [30], spindle orientation [31], nuclear migration [32], and chromatin remodeling [33]. In the present study, our data revealed that the knockdown of ACTL6A reduced Sox2; the product of this gene is required for stem-cell maintenance and the Notch1 signaling pathway. Inhibition of the Notch1 signaling pathway by DAPT promoted the differentiation of NB4 and HL-60 cells. These results suggest that the Notch1 signaling pathway could function as a negative regulator in granulocytic differentiation. Meanwhile, we demonstrated that ACTL6A co-localized and interacted with Sox2. We also found that immunofluorescence puncta of ACTL6A were mainly in the cell nucleus, but Sox2 puncta were localized not only in the nucleus but also in the cytoplasm. Furthermore, our study revealed that the knockdown of ACTL6A specifically inhibited the Notch1 signaling pathway. As reported, sox2 regulates the activity of the Notch1 signaling pathway [19]. Thus, we believe that ACTL6A regulates this pathway by decreasing and interacting with Sox2. However, the exact mechanism through Sox2 regulates the Notch1 signaling pathway in APL cells requires further verification. DAPT, an inhibitor of the Notch1 signaling pathway, downregulated the levels of Notch1, Cleaved Notch1, and Hes1. However, this inhibition had no effect on ACTL6A. These results are consistent with previous reports [19]. Thus, we believe that ACTL6A and the Notch1 signaling pathway have a regulatory relationship of superiors and subordinates.

P53, a classical tumor suppressor, encodes a protein involved in transcriptional activation [34], DNA binding [35], and oligomerization domains [36,37]. A recent report demonstrated that p53 interacted with BAF53 [26]. Thus, we researched the relationship between ACTL6A, a subtype of BAF53, and p53. We found that p53 interacted with ACTL6A and reduced the expression of ACTL6A while promoting differentiation of APL cells. We also found co-localization of p53 and ACTL6A by immunofluorescence. In addition, we detected that the knockdown of ACTL6A inhibited the protein expression of components of the Notch1 signaling pathway. Thus, we explored the association between p53 and the Notch1 signaling pathway. We found that the activation of p53 by CBL0137 also inhibited the protein expression of components of the Notch1 signaling pathway. These results revealed that p53 promoted differentiation via the Notch1 signaling pathway and inhibited the Notch1 signaling pathway via inhibition of ACTL6A.

Taken together, our results revealed new functions and mechanisms of ACTL6A in APL pathology for the first time. This included defining a functional role for p53, ACTL6A, Sox2, and the Notch1 signaling pathway in the APL differentiation process. Collectively, these results indicate that pharmacological inhibitors of ACTL6A and Notch1 signaling are potential therapeutics for APL.

Acknowledgements

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