Knockdown of ACTL6A promotes promyelocyte differentiation via the Notch1 signaling pathway post-print

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Abstract

To investigate the role of ACTL6A and its associated mechanisms in the differentiation of human leukemia NB4 cells. NB4 cells were induced to differentiate with ATRA, and Western blotting was performed to detect the expression changes of ACTL6A and CD11b. ACTL6A was knocked down, and morphological changes in NB4 cells were observed via Wright staining; Western blotting was used to detect the expression changes of ACTL6A, CD11b, and related proteins. Concurrent ACTL6A knockdown and ATRA treatment of NB4 cells were carried out, and flow cytometry was employed to detect the positive rate of the differentiation marker CD11b. Immunofluorescence was utilized to determine the spatial localization of ACTL6A in NB4 cells. The results demonstrated that CD11b protein expression was elevated following ACTL6A knockdown in NB4 cells; differentiation changes were observed by Wright staining; immunofluorescence revealed that ACTL6A was predominantly localized in the nucleus; Western blotting showed that the protein expression levels of Notch1, Hes1, and Sox2 were significantly downregulated. This study indicates that ACTL6A knockdown can promote the differentiation of human leukemia NB4 cells, and the mechanism involves inhibition of the Notch1 signaling pathway.

Full Text

Knock-down of ACTL6A Promotes Differentiation of NB4 Cells via the Notch1 Signaling Pathway

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Abstract

This study investigated the role of ACTL6A in the differentiation of human leukemia NB4 cells and its underlying mechanisms. We induced NB4 cell differentiation using all-trans retinoic acid (ATRA) and monitored changes in ACTL6A and CD11b expression levels via Western blotting. After knocking down ACTL6A, we observed morphological changes in NB4 cells through Wright staining and assessed expression levels of ACTL6A, CD11b, and related proteins by Western blotting. We also treated ACTL6A-knockdown NB4 cells with ATRA and measured the positive rate of the differentiation marker CD11b using flow cytometry, while immunofluorescence was employed to determine the subcellular localization of ACTL6A in NB4 cells. The results demonstrated that ACTL6A knockdown led to elevated CD11b protein expression and morphological changes indicative of differentiation. Immunofluorescence revealed that ACTL6A was predominantly localized in the nucleus, while Western blotting showed significant downregulation of Notch1, Hes1, and Sox2 protein expression. These findings suggest that knockdown of ACTL6A promotes differentiation of human leukemia NB4 cells, potentially through inhibition of the Notch1 signaling pathway.

Keywords: ACTL6A, NB4 cells, differentiation, Notch1 signaling pathway

Introduction

Acute promyelocytic leukemia (APL) is a malignant neoplasm of the bone marrow classified as M3 in the internationally recognized FAB classification system [1]. APL is characterized by the t(15;17) translocation resulting from chromosome translocation between chromosomes 15 and 17 [2, 3]. Pharmacological doses of all-trans retinoic acid (ATRA) induce clinical remission in APL patients by promoting promyelocyte maturation and degradation of the PML/RARα fusion protein [4]. Nevertheless, ATRA does not eliminate malignant myeloid clones in APL, and most relapsed APL patients develop resistance to further treatment with this drug [5, 6]. Therefore, investigating the pathogenesis of APL is critically important.

Granulocytic differentiation is an essential component of hematopoiesis controlled by a complex network of various regulatory factors, including cytokines [7], transcription factors [8], and non-coding RNAs [9]. Alterations in any of these critical factors can lead to dysregulated differentiation and severe consequences, including hematopoietic malignancies.

Actin-like 6A (ACTL6A), also widely known as BAF53a/Arp4/INO80K, is an

ATP/SNF-dependent BAF chromatin remodeling complex member that encodes a group of actin-related proteins [10, 11]. Previous reports have shown that ACTL6A participates in various cellular processes, including chromatin remodeling, transcriptional regulation, vesicular trafficking, and nuclear transfer [12, 13]. Recent studies have demonstrated that ACTL6A is associated with differentiation in multiple tumors and tissues, such as neural progenitor cell differentiation [14] and differentiation in epidermal and squamous cell carcinomas [15]. However, the role of ACTL6A in APL has not been reported. This study primarily investigated the function of ACTL6A during APL differentiation and its associated mechanisms.

Materials and Methods

1.1 Materials miR-302a mimic, lentivirus for ACTL6A knockdown, and negative controls were obtained from Shanghai GenePharma. The NB4 cell line was obtained from our laboratory. RPMI 1640 medium (Gibco), fetal bovine serum (Gemini), flow cytometry reagents for CD11b detection (Invitrogen), ACTL6A, CD11b, and Notch1 antibodies (Abcam), Hes1 antibody (CST), β-Actin antibody (Wuhan Boster Bio), and secondary antibodies (Beijing Zhongshan Jinqiao) were used.

1.2 NB4 Cell Recovery and Culture Cryopreserved NB4 cells were recovered and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C with 5% CO_2. NB4 cells in logarithmic growth phase were used for all experiments.

1.3 Wright Staining NB4 cell suspensions were collected, washed three times with ice-cold PBS, and resuspended in an appropriate volume of PBS. Five microliters of cell suspension were evenly smeared onto glass slides to a diameter of approximately 1 cm. After air drying, slides were stained with Wright' s stain, dried, and examined under a microscope for cellular morphology.

1.4 Flow Cytometry Detection of CD11b Positive Rate ACTL6A-knockdown NB4 cells and control NB4 cells were treated with ATRA for 72 hours. Cells were then collected, washed three times with ice-cold PBS, and resuspended in 100 L of PBS. Five microliters of CD11b flow cytometry antibody were added and incubated on ice in the dark for 30 minutes. After washing, NB4 cells were resuspended in PBS and analyzed by flow cytometry.

1.5 Immunofluorescence Detection of ACTL6A Distribution NB4 cell suspensions treated with ATRA (1 mol/L) for 72 hours and untreated control cells were evenly smeared onto coverslips and air-dried. Cells were fixed with 4% formaldehyde at room temperature for 22 minutes, washed three times with PBS, and permeabilized with 0.1% Triton for 18 minutes. After blocking with 10%

goat serum at room temperature for 30 minutes, cells were incubated with rabbit anti-human ACTL6A monoclonal antibody (1:200) overnight at 4°C. TRITC-labeled goat anti-rabbit IgG (1:200) was added and incubated at 37°C in the dark for 1 hour. Following DAPI staining in the dark for 5 minutes, coverslips were mounted with 70% glycerol and examined under a fluorescence microscope to assess ACTL6A expression patterns.

1.6 Western Blotting Treated cells were collected, washed, and lysed to extract total protein. After electrophoresis and membrane transfer, membranes were incubated with primary antibodies at 4°C for 16–18 hours. Membranes were washed with TBST and TBS, then incubated with secondary antibodies at room temperature for 1.5 hours. Protein bands were visualized using ECL chemiluminescence imaging.

1.7 Statistical Analysis All experimental data are presented as mean ± standard deviation. Statistical analysis was performed using SPSS 17.0 software. All experiments were repeated at least three times, and $p < 0.05$ was considered statistically significant.

Results

2.1 ACTL6A Expression Is Downregulated During ATRA-Induced NB4 Cell Differentiation NB4 cells were treated with ATRA (1 mol/L) for 72 hours, and changes in ACTL6A and CD11b expression levels were detected by Western blotting. The results showed that ACTL6A expression was downregulated during ATRA-induced NB4 cell differentiation [Figure 1: see original paper].

2.2 Knockdown of ACTL6A Promotes NB4 Cell Differentiation Using lentivirus as a vector, we established an ACTL6A-knockdown NB4 cell line and observed morphological changes through Wright staining. The results revealed that ACTL6A knockdown induced morphological changes characteristic of differentiation in NB4 cells [Figure 2: see original paper].

2.3 Knockdown of ACTL6A Enhances ATRA-Induced NB4 Cell Differentiation After successfully establishing the ACTL6A-knockdown NB4 cell line, we treated both knockdown and control NB4 cells with ATRA (1 mol/L) for 72 hours and measured changes in the differentiation marker CD11b by flow cytometry. The results demonstrated that CD11b expression was elevated compared to the control group, indicating that ACTL6A knockdown promotes ATRA-induced NB4 cell differentiation [Figure 3: see original paper].

2.4 Intracellular Localization of ACTL6A Following treatment of NB4 cells with ATRA (1 mol/L) for 72 hours, the subcellular localization of ACTL6A

was examined by immunofluorescence. The results showed that in NB4 cells, ACTL6A was predominantly distributed in the nucleus with a small amount in the cytoplasm. ATRA treatment did not alter the spatial localization of ACTL6A but did downregulate its fluorescence intensity [Figure 4: see original paper].

2.5 miR-302a Can Downregulate ACTL6A Expression We overexpressed miR-302a using a mimic and detected changes in ACTL6A and CD11b expression levels by Western blotting. The results showed that miR-302a overexpression decreased ACTL6A expression but did not significantly affect CD11b levels [Figure 5: see original paper].

2.6 Knockdown of ACTL6A Inhibits Notch1 Signaling and Promotes NB4 Cell Differentiation After establishing the ACTL6A-knockdown NB4 cell line, we examined expression levels of ACTL6A, CD11b, Notch1, and Hes1 by Western blotting. The results demonstrated that successful ACTL6A knockdown led to decreased Notch1 signaling pathway activity and increased CD11b expression [Figure 6: see original paper].

Discussion

APL is a malignant tumor of the bone marrow characterized by acute onset, dangerous clinical course, and high early mortality. Although pharmacological doses of ATRA can induce remission in APL patients, the side effects of ATRA treatment remain inadequately addressed. Therefore, investigating the pathogenesis of APL may provide better solutions and approaches.

ACTL6A is a multifunctional protein that functions as a transcriptional activator and repressor of specific genes through chromatin remodeling [16]. The association between ACTL6A and certain diseases is manifested through its abnormal regulation in various pathological conditions controlled by numerous genes related to cell differentiation. However, the role of ACTL6A in APL development and progression has not been previously reported.

Our study found that ACTL6A knockdown increased expression of the myeloid differentiation marker CD11b and induced morphological changes characteristic of differentiation as observed through Wright staining. Following ATRA treatment, ACTL6A-knockdown NB4 cells exhibited more pronounced differentiation compared to control cells. Additionally, we detected downregulation of the Notch1 signaling pathway by Western blotting. We also analyzed the distribution of ACTL6A in NB4 cells and found it was primarily localized in the nucleus with minor cytoplasmic distribution. ATRA treatment did not alter the spatial distribution pattern of ACTL6A, suggesting that ATRA-induced differentiation may not be directly related to ACTL6A subcellular localization. Notably, although miR-302a mimic did not cause CD11b elevation, its role in

APL cell proliferation warrants further investigation. These results confirm that knockdown of ACTL6A promotes APL differentiation, likely through the Notch1 signaling pathway.

Author Contributions

Peng-Qiang Zhong designed and executed the experiments, performed data analysis, and wrote the initial manuscript. Juan-Juan Yao and Dong-Dong Liu participated in experimental design and results analysis. Zhen Yuan and Jun-Mei Liu contributed to experimental data recording. Bei-Zhong Liu and Min Chen provided guidance on experimental design. Liang Zhong conceived and supervised the project, guided data analysis, and revised the manuscript. All authors read and approved the final text.

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References

[1] Lcs T, Pombodeoliveira M S. Acute promyelocytic leukaemia is highly frequent among acute myeloid leukaemias in Brazil: a hospital-based cancer registry study from 2001 to 2012. Annals of Hematology, 2016, 96(3):1-8.
[2] Lafagepochitaloff M, Alcalay M, Brunel V, et al. Acute promyelocytic leukemia cases with nonreciprocal PML/RARa or RARa/PML fusion genes. Blood, 1995, 85(5):1169-1174.
[3] Mozziconacci M-J, Liberatore C, Brunel V, et al. In vitro response to all-trans retinoic acid of acute promyelocytic leukemias with nonreciprocal PML/RARA or RARA/PML fusion genes. Genes Chromosomes & Cancer, 1998, 22(22):241-250.
[4] Gianni M, Fratelli M, Bolis M, et al. RARalpha2 and PML-RAR similarities in the control of basal and retinoic acid induced myeloid maturation of acute myeloid leukemia cells. Oncotarget, 2017, 8(23):37041-37060.
[5] Marasca R, Zucchini P, Galimberti S, et al. Missense mutations in the PML/RARalpha ligand binding domain ATRA-resistant As(2)O(3) sensitive relapsed acute promyelocytic leukemia. Haematologica, 1999, 84(11):963-968.
[6] Roussel M J, Lanotte M. Maturation sensitive and resistant t(15;17) NB4 cell lines as tools for APL physiopathology: nomenclature of cells and repertory of their known genetic alterations and phenotypes. Oncogene, 2001, 20(49):7287-7291.
[7] Metcalf D. Hematopoietic cytokines. Blood, 2008, 111(2):485-491.
[8] Friedman A D. Transcriptional control of granulocyte and monocyte development. Oncogene, 2007, 21(21):3377-3390.
[9] Lee S, Seo H H, Lee C Y, et al. Human Long Noncoding RNA Regulation of

Stem Cell Potency Differentiation. Cells International, 2017, (2017-8-30), 2017, 2017(5):1-10.
[10] Nishimoto N, Watanabe M, Watanabe S, et al. Heterocomplex formation by Arp4 and β-actin is involved in the integrity of the Brg1 chromatin remodeling complex. Journal of Cell Science, 2012, 125(16):3870-3882.
[11] Wu J I. Diverse functions of ATP-dependent chromatin remodeling complexes in development and cancer. 生物化学与生物物理学报 (英文), 2012, 44(1):54-69.
[12] Prasad P, Rönnerblad M, Arner E, et al. High-throughput transcription profiling identifies putative epigenetic regulators of hematopoiesis. Blood, 2014, 123(17):46-57.
[13] Vogelciernia A, Matheos D P, Barrett R M, et al. The neuron-specific chromatin regulatory subunit BAF53b is necessary for synaptic plasticity and memory. Nature Neuroscience, 2015, 16(5):552-561.
[14] Krasteva V, Buscarlet M, Diaztellez A, et al. The BAF53a subunit of SWI/SNF-like BAF complexes is essential for hemopoietic stem cell function. Blood, 2012, 120(24):4720-4732.
[15] Saladi S V, Ross K, Karaayvaz M, et al. ACTL6A is co-Amplified with p63 in Squamous Cell Carcinoma to Drive YAP Activation, Regenerative Proliferation and Poor Prognosis. Cancer Cell, 2016, 31(1):35-49.
[16] Wade S L, Langer L F, Ward J M, et al. MiRNA-mediated regulation of the SWI/SNF chromatin remodeling complex controls pluripotency and endodermal differentiation in human ES cells. Stem Cells, 2015, 33(10):2925-2935.

Note: Figure translations are in progress. See original paper for figures.