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BCL11A-dependent recruitment of SIRT1 to a promoter template in mammalian cells results in histone deacetylation and transcriptional repression

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Abstract

The B cell leukemia 11A protein (BCL11A/Evi9/CTIP1) has been implicated in hematopoietic cell development and malignancies. BCL11A is a transcriptional repressor that binds directly to a GC-rich motif and is also recruited to a promoter template via interaction with the orphan nuclear receptor, chicken ovalbumin upstream promoter transcription factor II. In both cases, BCL11A-mediated transcriptional repression is only minimally reversed by trichostatin A, suggesting the possible lack of involvement of class I or II histone deacetylases. Nonetheless, chromatin immunoprecipitation assays revealed that expression of BCL11A in mammalian cells resulted in deacetylation of histones H3 and/or H4 that were associated with the promoter region of a reporter gene. BCL11A-mediated transcriptional repression, as well as deacetylation of histone H3/H4 in BCL11A-transfected cells, was partially reversed by nicotinamide, an inhibitor of class III histone deacetylases such as SIRT1. SIRT1 was found to interact directly with BCL11A and was recruited to the promoter template in a BCL11A-dependent manner leading to transcriptional repression. These findings define a role for SIRT1 in transcriptional repression mediated by BCL11A in mammalian cells.

Keywords: SIRT1; Bcl11a; CTIP1; Evi9; Transcriptional repression; Histone deacetylase

BCL[1]11A was originally identified as a protein that interacted with and stimulated the transcriptional repression activity of chicken ovalbumin transcription factor II (COUP-TFII), and was therefore named COUP-TF-interacting protein 1 (CTIP1 [1]). BCL11A was also independently identified by Copeland's group as ecotropic viral integration site 9 (Evi9), the locus of which was demonstrated to be a site of proviral integration resulting in acute myeloid leukemia in BXH2 mice [2]. Subsequently, the human locus of BCL11A was shown to be involved in a translocation event, t(2; 14)(p13; q32.3), that may underlie some forms of chronic lymphocytic leukemia (CLL) and immunocytoma, linking the gene to human disease [3,4]. However, the mechanistic basis for the contribution of BCL11A to neoplastic processes in hematopoietic cells of murine or human origin remains unclear.

Although BCL11A has been shown to interact directly with COUP-TF II [1], as well as BCL6 [2], BCL11A also binds directly to a GC-rich motif and represses transcription of a downstream reporter gene in

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[1] *Abbreviations used:* BCL, B cell leukemia; bp, base pair; CAT, chloramphenicol acetyltransferase; COUP-TF, chicken ovalbumin upstream promoter transcription factor; CTIP 1 and 2, COUP-TF-interacting proteins 1 and 2; GST, glutathione *S*-transferase; HA, hemagglutinin; HDAC, histone deacetylase; HEK293, human embryonic kidney 293 cells; IgG, immunoglobulin G; kDa, kilodalton; NAM, nicotinamide; Sir2, silent information regulator 2; SIRT1, Sir2-like protein 1 or sirtuin 1; TSA, trichostatin A; WT, wild type.

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the absence of overexpressed COUP-TF family members or BCL6 [5]. This finding suggests that COUP-TF- and BCL6-independent mechanisms of BCL11A-mediated transcriptional repression may be operant on some cell types and/or promoter contexts.

The analysis of BCL11A-null mice has demonstrated the role for BCL11A in both hematopoiesis and postnatal development [6]. BCL11A and its paralog BCL11B/CTIP2 are similar in sequence, DNA binding specificity, and interacting partners (i.e., COUP-TF proteins). However, the lymphoidal defects resulting from disruption of each locus differ. BCL11A is essential for B cell development [6], whereas BCL11B is required for αβ T cell development [7].

Previous studies in transiently transfected cells revealed that both BCL11A and BCL11B mediated transcriptional repression of reporter gene in a manner that was only partially reversed by trichostatin A (TSA) [1,5,8]. More recently, BCL11B was demonstrated to interact with and recruit the class III HDAC, SIRT1, to a promoter template resulting in deacetylation of histones H3 and/or H4 and transcriptional repression in transiently transfected cells [8]. This finding implicated SIRT1 in the transcriptional activity of BCL11B in mammalian cells, and at least in part, may explain the TSA-insensitive nature of BCL11B-mediated transcriptional repression.

The structural and biochemical relatedness of BCL11A and BCL11B prompted us to speculate that the histone deacetylase SIRT1 may also underlie the mechanism of BCL11A-mediated transcriptional repression. Six lines of evidence, described herein, indicate that SIRT1 is involved in BCL11A-mediated transcriptional repression in transfected cells: (1) overexpression of BCL11A resulted in deacetylation of histones H3 and/or H4 that were associated with the promoter region of a target gene, (2) both the deacetylation of histone H3/H4 in BCL11A-transfected cells and BCL11A-mediated transcriptional repression were found to be partially reversed by nicotinamide, an inhibitor SIRT1, (3) endogenous SIRT1 was specifically recruited to the reporter gene template by overexpressed BCL11A, (4) SIRT1, but not a catalytically inactive mutant, stimulated transcriptional repression mediated by BCL11A, (5) endogenous BCL11A and SIRT1 were found to coimmunoprecipitate from nuclear extracts prepared from untransfected 70z/3 cells, and (6) BCL11A and SIRT1 were found to participate in a direct, physical interaction in vitro. Collectively, these findings implicate the histone deacetylase SIRT1 in the transcriptional repression activity of BCL11A in mammalian cells.

Materials and methods

Constructs

The (17-mer)$_4$-*tk*-CAT reporter construct was a kind gift from Dr. Ming-jer Tsai (Baylor College of Medicine). Flag-BCL11A was prepared by PCR amplification of the BCL11A open reading frame [1] with appropriate primers and insertion into pcDNA3(+) (Invitrogen). The Gal4 DBD-BCL11A construct was prepared by PCR amplification with appropriate primers followed by insertion into pM (Clontech). Myc-SIRT1, Myc-SIRT1 H363Y, and GST-SIRT1 constructs [9] were kind gifts from Dr. T. Kouzarides (University of Cambridge, Cambridge, UK). All vectors encoding GST fusion proteins were prepared by PCR amplification of appropriate templates followed by insertion into pGEX-2T (Amersham–Pharmacia Biotech). The constructs used for generating [^{35}S]methionine-labeled proteins were prepared by PCR amplification with primers containing appropriate restriction sites for insertion into pcDNA3(+) or pcDNA3.1/His (Invitrogen). All constructs were verified by complete DNA sequence analysis.

Antibodies

Purified rabbit anti-Sir2α, mouse anti-SIRT1, and rabbit anti-acetylated-histone H3 and -histone H4 antibodies were obtained from Upstate. Mouse anti-Flag and -Myc monoclonal antibodies were purchased from Sigma and Oncogene, respectively. Mouse anti-Gal4 was obtained from Santa Cruz Biotechnology. The mouse anti-BCL11A monoclonal antibody was raised by Dr. Michael Marusich (Monoclonal Antibody Facility, Institute for Neuroscience, University of Oregon, Eugene, Oregon) against a cocktail of GST-BCL11A fusion proteins and recognizes an epitope located within the central region of the protein, amino acids 171–434 (data not shown).

Chromatin immunoprecipitation assays

Chromatin immunoprecipitation (ChIP) assays were performed on transfected cells essentially as described previously [8] with the slight modifications. HEK293 cells were co-transfected at 60% confluency (10 cm plates) with 3 μg of the (17-mer)$_4$-*tk*-CAT reporter, 5–20 μg Gal4-BCL11A, 0.5 μg Myc-SIRT1, and/or the corresponding parental vectors using the calcium-phosphate method. Cells were washed twice with phosphate buffered saline (PBS) after 48 h, and cross-linked with 1% formaldehyde in PBS at room temperature for 10 min. Cells were then washed twice with ice-cold PBS buffer and collected in harvesting buffer (100 mM Tris–HCl, pH 9.4, containing 10 mM DTT). The cells were lysed in lysis buffer (50 mM Tris–HCl, pH 8.1, containing 1% SDS, 10 mM EDTA, and a protease inhibitor cocktail). The sonicated lysates were then cleared by centrifugation and diluted 2.5- to 3.75-fold with ChIP dilution buffer (16.7 mM Tris–HCl, pH 8.1, containing 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 167 mM NaCl,