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The human SWI/SNF complex associates with RUNX1 to control transcription of hematopoietic target genes

Rachit Bakshi¹, Mohammad Q. Hassan¹, Jitesh Pratap¹, Jane B. Lian¹, Martin A. Montecino², Andre J. van Wijnen¹, Janet L. Stein¹, Anthony N. Imbalzano¹, and Gary S. Stein^{1,*}

¹ Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, MA 01655, USA

² Centro de Investigaciones Biomedicas, Facultad de Ciencias Biologicas y Facultad de Medicina, Universidad Andres Bello, Republica 217, Santiago, Chile

Abstract

The acute myeloid leukemia 1 (AML1, RUNX1) transcription factor is a key regulator of hematopoietic differentiation that forms multi-protein complexes with co-regulatory proteins. These complexes are assembled at target gene promoters in nuclear microenvironments to mediate phenotypic gene expression and chromatin related epigenetic modifications. Here, immunofluorescence microscopy and biochemical assays are used to show that RUNX1 associates with the human ATP-dependent SWI/SNF chromatin remodeling complex. The SWI/SNF subunits BRG1 and INI1 bind in vivo to RUNX1 target gene promoters (e.g., GMCSF, IL3, MCSF-R, MIP and p21). These interactions correlate with histone modifications characteristic of active chromatin, including acetylated H4 and dimethylated H3 Lysine 4. Down-regulation of RUNX1 by RNA interference diminishes the binding of BRG1 and INI1 at selected target genes. Taken together, our findings indicate that RUNX1 interacts with the human SWI/SNF complex to control hematopoietic-specific gene expression.

Keywords

leukemia; BRG1; INI1; AML1; chromatin remodeling; histone modification

INTRODUCTION

Hematopoiesis is a multi-step process that generates multiple distinct mature cell types. Transcriptional regulation and epigenetic control of lineage-specific genes are required for the progression of undifferentiated hematopoietic progenitor cells into mature lymphoid, myeloid and erythroid lineages [Cantor and Orkin, 2001; Rothenberg, 2007; Iwasaki and Akashi, 2007; Rice et al., 2007; Metcalf, 2007]. For instance, PU.1 commits cells to the myeloid lineage and GATA-1 is known to play an essential role in erythropoietic and megakaryocytic differentiation [Friedman, 2007; Sauntharajah et al., 2006; Cantor and Orkin, 2002; Koschmieder et al., 2005; Rosenbauer and Tenen, 2007]. The Ikaros family of transcription factors plays a major role in lymphoid development [Rebollo and Schmitt, 2003; Ng et al., 2007]. RUNX1 (AML1/Cbfa2), a member of the runt-related transcription factor family, is a key regulator of definitive hematopoiesis in embryos and normal

*Corresponding author: Gary S. Stein, Ph.D., ¹Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, USA, Tel: 508-856-5625/Fax: 508-856-6800/ gary.stein@umassmed.edu.

hematopoiesis in adults [Kurokawa, 2006; Speck, 2001; Yokomizo et al., 2008]. Furthermore, the RUNX1 gene is the most common target of chromosomal translocations in acute human leukemias [Lutterbach and Hiebert, 2000; Downing et al., 2000; Pabst and Mueller, 2007; Peterson and Zhang, 2004]. Its crucial importance for normal blood cell development is most evident in AML1 null mice, which display no definitive hematopoiesis [Okuda et al., 1996; Wang et al., 1996]. RUNX1 regulates promoters and enhancers of a number of myeloid and lymphoid related genes including IL3 (interleukin 3), GM-CSF (colony-stimulating factor [granulocyte-macrophage]), myeloperoxidase (MIP), macrophage colony-stimulating factor 1 receptor (M-CSF-R), CD4 (CD4 antigen), and Tcr-d (T-cell receptor delta chain) [Cameron et al., 1994; Lauzurica et al., 1997; Takahashi et al., 1995; Zhang et al., 1994; Vradii et al., 2006; Michaud et al., 2008].

In addition to regulating hematopoiesis-specific genes, RUNX1 also regulates cell cycle related genes, including p21^{CDKN1A} (also known as WAF1/CIP1), which encodes a cyclin-dependent kinase inhibitor important for checkpoint controls and terminal differentiation [Lutterbach et al., 2000; Peterson et al., 2007b]. Levels of RUNX1/AML1 increase as cells progress into S phase and are downregulated at the G2/M transition [Bernardin-Fried et al., 2004; Biggs et al., 2006]. Furthermore, RUNX1/AML1 controls cell cycle progression by shortening the G1/S phase in hematopoietic cells and is negatively regulated by cyclin D3 [Strom et al., 2000; Peterson et al., 2005].

RUNX proteins either activate or repress transcription in a promoter- or enhancer-specific context [Lian et al., 2004]. The RUNX1 protein contains multiple domains that are structurally and functionally conserved among the three family members. These include the N-terminal CBF β heterodimerization and DNA-binding runt homology domain (RHD), a nuclear localization signal, a C-terminal nuclear matrix targeting signal (NMTS), as well as context-dependent transcriptional activation/repression domains [Zeng et al., 1997; Zeng et al., 1998; Li et al., 2005]. C-terminal functions of RUNX1 including subnuclear targeting are important for myeloid differentiation, and chromosomal translocations that result in loss of the C-terminus have been linked to acute myeloid leukemias [Zeng et al., 1997; Zeng et al., 1998; Li et al., 2005; Peterson and Zhang, 2004; Vradii et al., 2005]. A large number of co-regulatory proteins associate physically with both the N-terminal DNA binding and C-terminal transcriptional regulatory domains of RUNX1 [Wotton et al., 1994; Giese et al., 1995; Hiebert et al., 1996; Rhoades et al., 1996; Petrovick et al., 1998; Rubnitz and Look, 1998; Osato et al., 1999]. RUNX proteins functionally control transcription of phenotypic target genes by supporting modifications in nucleosomal structure and epigenetic marks in chromatin [Young et al., 2007a; Gutierrez et al., 2007; Young et al., 2007b].

Mammalian SWI/SNF enzymes are evolutionarily conserved, multi-protein complexes that contain one of two closely related ATPases, BRM or BRG1, and utilize the energy of ATP to remodel chromatin structure [Roberts and Orkin, 2004; Martens and Winston, 2003; de la Serna et al., 2006; Imbalzano, 1998]. Brg1 and other SWI/SNF subunits, including Ini1/Snf5 and Srg3/Baf155, have been shown to be essential for mouse development [Bultman et al., 2000; Kim et al., 2001; Guidi et al., 2001; Roberts et al., 2000; Klochendler-Yeivin et al., 2000]. SWI/SNF enzymes have been shown to both activate and repress a subset of genes in yeast and mammals [Martens and Winston, 2003; Sif, 2004]. In vitro and in vivo evidence indicates that SWI/SNF enzymes can promote binding of transcriptional activators and the TATA box Binding Protein, as well as support formation of RNA polymerase II related pre-initiation and elongation complexes [Brown et al., 1996; Corey et al., 2003; Kwon et al., 1994; Imbalzano et al., 1994]. The selectivity of SWI/SNF function at specific genes is attributable to its recruitment by promoter-bound regulatory proteins through physical interactions of SWI/SNF subunits with different activators or repressors [Roberts and Orkin, 2004; Li et al., 2007].

ATP-dependent chromatin-remodeling complexes contribute to spatio-temporal regulation of gene expression during development. The essential role of BRG1 in hematopoietic development has previously been established [Vradii et al., 2006; Griffin et al., 2008; Bultman et al., 2005], but the mechanistic connection between SWI/SNF-mediated chromatin remodeling and RUNX1 regulation of hematopoietic genes remains to be explored. Here, we have used a combination of experimental approaches to examine interactions between RUNX1 and the core hSWI/SNF subunits BRG1 and INI1. Our results demonstrate that both BRG1 and INI1 associate with RUNX1 and are recruited to RUNX1 target gene promoters. We propose that RUNX1 and SWI/SNF complexes support transcriptional regulation and chromatin remodeling of genes during myeloid differentiation.

MATERIAL AND METHODS

Cell culture

The human Jurkat cells were cultured in RPMI supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/ml streptomycin. Cells were maintained at 37°C in a humidified atmosphere with 95% air, 5% CO₂ at a concentration between 0.5 and 1×10⁶ cells/ml.

Immunofluorescence microscopy

Cells were grown in regular growth medium for 1 to 2 days and then processed for in situ immunofluorescence. Cell suspension (500 µl) was deposited onto glass slides in a Shandon Cytospin 2 centrifuge (Thermo Shandon, Pittsburgh, PA). Cells were rinsed with ice-cold phosphate buffered saline (PBS) and fixed in 3.7% formaldehyde in PBS for 10 min on ice. After rinsing once with PBS, the cells were permeabilized in 0.25% Triton X-100 in PBS, rinsed twice in PBSA (0.5% bovine serum albumin (BSA) in PBS) and stained with antibodies.

The following primary antibodies and dilutions were used: BRG1 rabbit polyclonal (1:100; H-88, Santa Cruz Biotechnology); AML1 mouse monoclonal (1:100; 2B5 generous gift from Yoshiaki Ito, National University of Singapore, Singapore). For localization of antigen/antibody complexes, we used the following complementary fluorescent secondary antibodies: Alexa-488 goat anti-rabbit IgG, and Alexa-594 goat anti-mouse IgG (1:800; Molecular Probes, Eugene, OR).

Staining of cell preparations was recorded with a CCD camera (Hamamatsu Photonics, Bridgewater, NJ, USA; Cat.No. C4742-95) attached to an epifluorescence Zeiss Axioplan 2 (Zeiss Inc., Thorwood, NY) microscope. For interphase studies single image planes were acquired and deconvoluted using the Metamorph Imaging Software (Universal Imaging, Downingtown, PA).

Chromatin immunoprecipitation and analysis

Chromatin immunoprecipitation assays (ChIPs) were performed by crosslinking asynchronously growing cells with 1% formaldehyde in RPMI for 10 min at room temperature. Crosslinking was quenched by adding glycine to a final concentration of 250 mM for 10 min. Cells were collected and washed twice with PBS. Cell pellets were resuspended in 2.5 ml of lysis buffer (150 mM NaCl, 50 mM Tris-HCl pH 8.0, 1% NP-40, 25 µM MG-132, and 1X Complete® Protease inhibitor cocktail (Roche)). After 10 min on ice, cells were sonicated to obtain DNA fragments of ~500 bp as determined by agarose gel electrophoresis with ethidium bromide staining. Protein-DNA complexes were isolated by centrifugation at 15,000 rpm for 20 min. Supernatants with protein-DNA complexes were incubated for 16 h with 3 µg rabbit polyclonal antibody directed against each protein. The

following primary antibodies were used: BRG1 rabbit polyclonal (H-88, Santa Cruz Biotechnology), INI1 rabbit polyclonal and AML1 rabbit polyclonal (Active Motif, Carlsbad CA). The antibodies used to detect histone modifications were as follows: Acetylated histone H4, dimethyl H3K4 and dimethyl H3K27 (Upstate Biotechnology, Lake Placid, NY). Antibody-Protein-DNA complexes were further incubated with 50–60 μ l 30% protein A/G beads (Santa Cruz Biotechnology) to isolate antibody bound fractions of chromatin. Immuno-complexes were washed with the following buffers: low salt (20 mM Tris-Cl, pH 8.1, 150 mM NaCl, 1% Triton X-100, 2 mM EDTA, 1X Complete protease inhibitor), high salt (20 mM Tris-Cl, pH 8.1, 500 mM NaCl, 1% Triton X-100, 2 mM EDTA), LiCl (10 mM Tris-Cl, pH 8.1, 250 mM LiCl, 1% deoxycholate, 1% NP-40, 1 mM EDTA) and twice in TE (10 mM Tris-Cl, pH 8.1, 1 mM EDTA). Protein-DNA complexes were eluted in 1% SDS and 100 mM NaHCO₃. Crosslinks of pulldown fractions and inputs (2% of total IP fraction) were reversed by incubation overnight in elution buffer and 0.2M NaCl. DNA then was extracted, purified, precipitated, and resuspended in TE for qPCR. Gene-specific primers were used to amplify precipitated DNA and quantified by real time qPCR (Table 1).

Co-immunoprecipitation analysis

Jurkat cells (50–70% confluent) were used for co-immunoprecipitation studies as described previously [Hassan et al., 2004]. Equal amounts of cell lysate were immunoprecipitated with antibodies for INI1 (612111, BD Transduction Laboratories, San Jose, CA), BRG1 (H-88, Santa Cruz Biotechnology) and AML1 (39000, Active Motif), overnight in phosphate buffered saline with 5 mM EDTA. After a 2 h incubation with protein A/G beads followed by 3 washes with PBS, the immunocomplexes were separated in 10% SDS-PAGE and western blotted with the indicated antibodies.

RNA interference

Exponentially growing Jurkat cells were electroporated using the Nucleofector device (Amaxa, Gaithersburg, MD) according to the manufacturer's protocol, with siRNAs against RUNX1/AML1 (Dharmacon, Lafayette, CO). A non-silencing siRNA (Qiagen) was used as a negative control. Total RNA and protein were isolated for further analysis.

Quantitative Reverse Transcription-PCR (RT-qPCR)

RNA was extracted from all samples using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Purified total RNA was subjected to DNase I digestion, followed by column purification using the DNA Free RNA Kit (Zymo Research, Orange, CA). Eluted total DNA-free RNA was quantitated by spectrophotometry, and 1 μ g was added to a reverse transcription reaction using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA) with a mixture of random hexamers and oligo(dT) primers. Relative quantitation was determined using the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA) measuring real-time SYBR Green supermix fluorescence. The relative level of each mRNA was determined using the comparative CT method for relative quantitation with GAPDH as an endogenous reference.

RESULTS

RUNX1 interacts with subunits of the human SWI/SNF complex in vivo

In this study, we address the biological question whether the human SWI/SNF complex supports the lineage-specific induction of RUNX1 target genes during hematopoiesis. Because RUNX1 proteins reside at specific subnuclear domains, we first examined whether SWI/SNF proteins are targeted to the same locations. Immunofluorescence microscopy was

used to monitor the subcellular localization of endogenous RUNX1 and BRG1 in Jurkat cells (Figure 1). DNA was counterstained with DAPI to confirm that the co-localized signals were present in the nucleus. Double label immunofluorescence microscopy for RUNX1 and BRG1 revealed that both proteins display similar nuclear distribution patterns and considerable co-localization. We measured the extent of signal overlap by counting the number of foci with both red and green immunofluorescence signals (yellow in merged images). We observed that there is ~20% overlap of immunofluorescence signals in all interphase cells examined, while mitotic Jurkat cells exhibited limited co-localization (less than 5% overlap of immunofluorescence signals). Because a subset of RUNX1 and BRG1 are targeted to the same subnuclear domains, we considered that these proteins may function together in the regulation of hematopoietic-specific genes.

RUNX1 is required for transcription of multiple hematopoietic-specific target genes and SWI/SNF is required for chromatin remodeling. Therefore, we assessed whether these events may be mechanistically coupled through protein-protein interactions. The association of RUNX1 with core subunits of the human SWI/SNF complex was examined by biochemical assays. Co-immunoprecipitation assays were carried out with endogenous RUNX1 and the SWI/SNF components BRG1, INI1 and BAF155 in Jurkat cells. Immuno-complexes obtained with RUNX1 antibody clearly reveal that RUNX1 associates with the three SWI/SNF subunits (Figure 2A). To validate these results, reciprocal co-immunoprecipitation assays with antibodies raised against BRG1 and INI1 were performed. Indeed, RUNX1 is present in immuno-precipitates obtained with either antibody (Figure 2B). These results demonstrate that the RUNX1 protein associates with the human SWI/SNF complex.

Human SWI/SNF subunits are associated with RUNX1 target gene promoters in vivo

Chromatin immunoprecipitation (ChIP) assays were performed to determine in vivo occupancy and functional regulation of selected known target genes of RUNX1 by BRG1 and INI1, two core components of the human SWI/SNF complex. We performed ChIP assays with primers spanning RUNX binding motifs on target gene promoters. Quantitative PCR data show that RUNX1, as well as BRG1 and INI1, associate with the promoters of the genes for the CDK inhibitor p21^{CDKN1A}, IL-3, GMCSF, MCSF-R and MIP (Fig. 3). We also examined the promoter occupancy of the human osteocalcin (hOC) gene, which has validated RUNX elements but is not known to be expressed in Jurkat cells. Interactions of RUNX1, BRG1 and INI1 with the hOC gene promoter are only detectable at levels near the IgG background values (Fig. 3). Thus, our data show that BRG1 and INI1 associate with RUNX1 target genes in hematopoietic cells.

We also performed ChIP assays to investigate histone modifications characteristic of transcriptionally active or inactive chromatin on the RUNX1-dependent GMCSF and IL3 promoters, or the silent hOC promoter in Jurkat cells. ChIP analyses were carried out using two antibodies recognizing active epigenetic marks (i.e., acetylated H4 [H4-Ac] and H3 dimethyl lysine 4 [H3K4me2]), and one antibody recognizing a repressive histone modification (H3 dimethyl lysine 27 [H3K27me2]). We observed that the active GMCSF and IL3 promoters are associated with H3K4me2 and H4-Ac, but not with H3K27me2 (Figure 4). These two genes interact robustly with RNA polymerase II, as well as with RUNX1, BRG1 and INI1. In contrast, the inactive hOC gene promoter is associated predominantly with H3K27me2 and exhibits background binding for RNA polymerase II and the other factors. Taken together, these results demonstrate that BRG1 and INI1 subunits of the human SWI/SNF complex are associated with active RUNX1 target gene promoters in hematopoietic cells.

Recruitment of SWI/SNF complexes to the GMCSF and IL3 gene promoters is RUNX1 dependent

RUNX proteins function as transcriptional scaffolds that organize the assembly of transcriptional activation complexes and facilitate chromatin modifications. We investigated whether the recruitment and association of the BRG1 and INI1 subunits of the human SWI/SNF complex to RUNX target genes is RUNX1 dependent. We directly addressed this question by using RNA interference in Jurkat cells. We used two distinct small interfering RNAs (siRNAs) specific for RUNX1, as well as a non-silencing siRNA, thus accounting for the possibility of off-target effects (by the specific siRNA) or non-specific effects (from siRNA transfection). Both RUNX1 siRNAs specifically down-regulate RUNX1 mRNA levels ~ 2-fold and diminish protein levels by ~ 3-fold (Figures 5A and 5B). Thus, our approach utilizes two effective siRNAs that selectively deplete RUNX1 levels in Jurkat cells.

We performed ChIP assays in RUNX1 depleted cells with antibodies specifically recognizing RUNX1, BRG1 and INI1, as well as non-specific IgG. The results clearly show that interactions of BRG1 and INI1 with the GMCSF and IL-3 gene promoters are reduced upon siRNA mediated RUNX1 knockdown (Figures 5C). This decreased association of RUNX1 and human SWI/SNF subunits to the GMCSF and IL3 promoters correlates with a reduced expression of these genes as measured by RT-qPCR (Figure 5D). These data indicate that RUNX1 supports recruitment of BRG1 and INI1 to target gene promoters to regulate their expression.

DISCUSSION

RUNX proteins are cell fate determining factors that control cell growth and differentiation and are principal regulators of phenotype-specific genes in different developmental lineages. RUNX proteins modulate epigenetic histone marks on their target genes through interactions with histone modifying enzymes. However, whether RUNX proteins participate in SWI/SNF mediated enzymatic steps involved in chromatin remodeling has not yet been resolved. SWI/SNF complexes have been shown to participate in developmentally regulated transcription. For example, SWI/SNF complexes are involved in the transcriptional activation of genes in multiple lineages including erythroid [Armstrong et al., 1998; Lee et al., 1999; O'Neill et al., 1999], myeloid [Kowenz-Leutz and Leutz, 1999; Vradii et al., 2006], adipocytic [Pedersen et al., 2001; Salma et al., 2004], myoblastic [de la Serna et al., 2001] and osteoblastic [Young et al., 2005a] cell types. In this study, we have shown that RUNX1 interacts in immuno-precipitable complexes with several core subunits of the hSWI/SNF chromatin remodeling complex. Our data indicate that RUNX1 is required for the association of hSWI/SNF subunits BRG1 and INI1 to transcriptionally active promoters of RUNX1 target genes (e.g., GMCSF and IL-3) to modulate their expression. The results presented in this study suggest that RUNX1 links transcriptional regulation of hematopoietic genes with the chromatin remodeling activity of the human SWI/SNF complex in the myeloid lineage.

The induction of lineage-specific differentiation and phenotypic gene expression requires the combinatorial activities of master regulatory factors (e.g., RUNX proteins, C/EBP proteins, homeodomain proteins, Ets-like factors, MyoD, PPAR γ) and the remodeling of chromatin organization and topology by SWI/SNF and analogous enzymatic complexes. In the osteoblastic lineage, RUNX2 is essential for early stages of phenotype commitment and activation of bone-specific genes [Young et al., 2005b; Javed et al., 1999; Stein et al., 2004]. The osteoblast-specific induction of the OC gene is mediated by RUNX2 but SWI/SNF recruitment to the OC promoter appears to be mediated by C/EBP β , a factor that synergizes with RUNX2 and forms stable complexes with SWI/SNF subunits [Gutierrez et al., 2004].

One fundamental finding of the present study is that, in contrast to RUNX2, RUNX1 is capable of interacting with the SWI/SNF complex and directly required for the recruitment of the core subunits BRG1 and INI1 to several genes involved in hematopoietic differentiation.

Apart from the key roles of SWI/SNF chromatin remodeling complexes during normal tissue development and cellular differentiation, SWI/SNF proteins are linked to the molecular pathology of human cancer [Versteeg et al., 1998; Bultman et al., 2000; Bochar et al., 2000; Klochendler-Yeivin et al., 2000; Guidi et al., 2001; Roberts et al., 2000; Bultman et al., 2008]. Absence of properly formed SWI/SNF complexes contributes to tumorigenicity either by deregulating expression of or disrupting interactions with tumor suppressors (e.g., pRB and p53) [Halliday et al., 2008]. The BAF57 subunit of SWI/SNF has been implicated in both androgen-dependent prostate tumors and estrogen-dependent breast tumors [Garcia-Pedrero et al., 2006; Link et al., 2005; Link et al., 2008]. Importantly, deletions of INI1 have also been reported in the chronic phase and blast crisis of chronic myeloid leukemia [Grand et al., 1999; Guidi and Imbalzano, 2004]. Similarly, RUNX1 is frequently targeted by chromosomal translocation in acute myeloid leukemias (AML) [Peterson and Zhang, 2004]. The t(8;21) RUNX1(AML1)-ETO fusion protein blocks differentiation of myeloid progenitors [Peterson and Zhang, 2004; Peterson et al., 2007a]. For example, the RUNX1(AML1)-ETO fusion protein has been shown to aberrantly recruit co-repressor complexes (e.g., N-CoR/Sin3/HDAC1) to actively shut down transcription from RUNX1 target genes important for normal hematopoiesis [Hiebert et al., 2001]. Because our data show that RUNX1 interacts with the SWI/SNF complex, future studies should address whether SWI/SNF complexes remain associated with the leukemia-related RUNX1(AML1)-ETO fusion protein.

In conclusion, our study shows that RUNX1 interacts with components of the human SWI/SNF complex and supports binding of this complex to RUNX1 target genes related to hematopoietic lineage progression. Compared to the bone-related RUNX2 protein that supports induction of osteoblast-specific gene expression through the indirect recruitment of SWI/SNF by C/EBP β [Gutierrez et al., 2002; Villagra et al., 2006], RUNX1 has the distinct molecular ability to associate with the human SWI/SNF complex. We propose that the resulting RUNX1-SWI/SNF containing promoter complex may directly facilitate structural alterations in chromatin organization to support hematopoietic differentiation.

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Figure 1. Co-localization of RUNX1 and BRG1 proteins in Jurkat Cells

Immunofluorescence microscopy was performed to detect endogenous RUNX1 and BRG1 in Jurkat cells, and images were merged to determine co-localization. Immunofluorescence images were obtained using antibodies against RUNX1 (green) and BRG1 (red). The insets at top left of each panel show the phase contrast image. DAPI staining (blue) was used to visualize the nucleus (top row). RUNX1 and BRG1 proteins are predominantly present in the nucleus and display significant co-localization in interphase cells. Mitotic cells exhibit limited co-localization (less than 5% overlap in immunofluorescence signals).

Figure 2. Endogenous RUNX1 interacts with multiple subunits of the human SWI/SNF complex
 A) Immunoprecipitation analysis was carried out with an antibody for RUNX1 in Jurkat cells. The precipitates were subjected to immunoblot analysis with BRG1, INI1, BAF155 and RUNX1 specific antibodies. All three proteins BRG1, INI1 and BAF155 were detected in western blot analysis as indicated in top three panels. B) Endogenous BRG1 and INI1 were immunoprecipitated from Jurkat cells using rabbit polyclonal antibodies. The precipitates were subjected to western blot analysis with RUNX1, BRG1 and INI1 antibodies. RUNX1 is present in immunoprecipitates obtained with either BRG1 or INI1 antibody.

Figure 3. RUNX1, BRG1 and INI1 associate with Runx target genes in interphase Jurkat cells
Chromatin immunoprecipitation was done with asynchronously growing Jurkat cells using RUNX1, BRG1, INI1 and IgG antibodies. Quantitative PCR was performed with specific primers from promoter regions of the following genes: CDK inhibitor p21 (CDKN1A), granulocyte-macrophage colony-stimulating factor (GMCSF), myeloperoxidase (MIP), interleukin 3 (IL-3), macrophage colony-stimulating factor 1 receptor (MCSF-R) and human osteocalcin (OC). The data show that endogenous RUNX1, BRG1 and INI1 bind to the promoters of the target genes in Jurkat cells. There is no significant binding of these proteins on the OC promoter, which is not expressed in these cells. Quantitative PCR data are normalized to genomic DNA and denoted as percent input.

Figure 4. RUNX1, BRG1 and INI1 occupancy of the GMCSF and IL3 promoters is associated with active histone modifications

Chromatin immunoprecipitations with RUNX1, BRG1, INI1, Pol II, IgG and histone modification antibodies were performed in Jurkat cells. The antibodies used to detect histone modifications were as follows: Acetylated histone H4 (H4-Ac), dimethylated H3 lysine 4 (H3K4me2) and dimethylated H3 lysine 27 (H3K27me2). The GMCSF and IL3 promoters are associated with dimethylated H3K4 and acetylated H4, but not dimethylated H3K27 (top panel). These two genes interact robustly with RNA polymerase II (lower panel), as well as with RUNX1, BRG1 and INI1 (middle panels). The human OC gene promoter is predominantly associated with dimethylated H3K27 and exhibits background binding for RNA polymerase II and the other factors. Quantitative PCR data are normalized to genomic DNA and denoted as percent input.

Figure 5. RUNX1 supports BRG1 and INI1 association with the GMCSF and IL3 promoters
 Jurkat cells were transfected with two independent RUNX1 siRNAs and non-silencing (NS) Si-RNA control. A). To check the efficiency of knockdown, RUNX1 mRNA expression was measured by RT-qPCR relative to GAPDH in equal numbers of cells. B) Protein expression of RUNX1 was examined in siRNA treated samples as well as non silencing controls by western blot analysis using specific antibody. Immunoblotting with antibody against α -tubulin was performed as loading control. C). ChIP assays were performed in RUNX1 knockdown and control Jurkat cells with antibodies against RUNX1, BRG1, INI1 and IgG. Quantitative PCR data show that interactions of BRG1 and INI1 with the GMCSF and IL3 promoters are significantly reduced upon siRNA mediated RUNX1 knockdown. Quantitative PCR data are normalized to genomic DNA and denoted as percent input. D). Expression levels of GMCSF and IL3 mRNAs were analyzed by real-time quantitative RT-PCR relative to GAPDH in control and siRNA treated samples. Decreased association of RUNX1, BRG1 and INI1 to the GMCSF and IL3 promoters correlates with a reduced expression of these genes as measured by qRT-PCR.

Table 1

Primers Used In Real Time PCR.

Gene product	Forward primer	Reverse primer
GMCSF	TTCCCATGTGTGGCTGATAA	CTGTGTACTGGGCTCACTGG
IL3	CCCATCTCTCATCCTCCTTG	GGCGTCGGAAGGATCTTTAT
p21	CTCGTCAAATCCTCCCTTC	AAAAGGGCAACCTGATCTCC
MIP	CATTCAGTTCTTTGCCTCTGG	ATGAACTCTCCAGCCCCATT
MCSF-R hOC	TGGGTTCAAGGCTTTGTTTT CAGTCTGATTGTGGCTCACC	GGACACACGTTCTCTCCTC GAATCTGCCAGGGCTATTTG