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The SNF2H chromatin remodeling enzyme has opposing effects on cytokine gene expression

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ABSTRACT

Cytokine gene expression is a key control point in the function of the immune system. Cytokine gene regulation is linked to changes in chromatin structure; however, little is known about the remodeling enzymes mediating these changes. Here we investigated the role of the ATP-dependent chromatin remodeling enzyme SNF2H in mouse T cells; to date, SNF2H has not been investigated in T cells. We found that SNF2H repressed expression of IL-2 and other cytokines in activated cells. By contrast, SNF2H activated expression of IL-3. The ISWI components SNF2H and ACF1 bound to the tested loci, suggesting the regulation was direct. SNF2H decreased accessibility at some binding sites within the IL2 locus, and increased accessibility within some IL3 binding sites. The changes in gene expression positively correlated with accessibility changes, suggesting a simple model that accessibility enables transcription. We also found that loss of the ISWI ATPase SNF2H reduced binding to target genes and protein expression of ACF1, a binding partner for SNF2H, suggesting complex formation stabilized ACF1. Together, these findings reveal a direct role for SNF2H in both repression and activation of cytokine genes.

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1. Introduction

Cytokine genes play pivotal roles in determining and executing the function of the immune system, so their regulation is of central importance. Much has been learned of the signaling pathways regulating these genes. A good deal is also known about the transcription factors that regulate cytokines. Activation specific factors, such as the NFAT and rel families, appear to function in activated T cells to directly regulate cytokine transcription. NFAT factors work on different genes in different T helper lineages (Avni et al., 2002; Wurster and Pazin, 2008). Lineage specific factors, such as GATA3 and T-bet, primarily function in particular T helper subsets (Wilson et al., 2009). STAT transcription factors are coupled to cytokine receptors to allow rapid response to the extracellular cytokine milieu. Together, these factors determine cytokine expression through combinatorial control.

Changes in cytokine gene expression frequently correlate with changes in chromatin structure. Changes in chromatin structure have been detected by nuclease accessibility, primarily DNase I hypersensitivity (DHS). Changes in histone modifications have also been detected. The IL3/GMCSF locus is an early example of successfully using DHS mapping to identify regulatory regions (Cockerill, 2004). In the IL2 locus, there are also reports of chromatin structure changes linked to gene regulation (Attema et al., 2002; Garrity et al., 1994; Yui et al., 2001). Perhaps the best-studied example in lymphocytes is the IL4/IL13/IL5 locus, referred to here as the Th2 locus, approximately 700 kb away from the murine IL3/GMCSF locus. As naïve murine CD4 T cells adopt the T helper 1 (Th1) or T helper 2 (Th2) fates, chromatin structure changes are detected by DNase I hypersensitivity (DHS) over nearly 150 kb, including a central shared locus control region (LCR) (Ansel et al., 2006; Lee et al., 2006). More recently, chromatin structure changes have been found in the IL17A/IL17F locus in the context of Th17 lineage commitment (Akimzhanov et al., 2007; Zhang et al., 2008). DHS mapping has been a general tool to characterize these loci, and in several instances has been used to identify cis-acting regulatory elements.

Though chromatin structure changes in cytokine genes are well documented, little is known about how those changes occur. Unless catalyzed by chromatin remodeling enzymes, changes in chromatin structure are slow. ATP-dependent chromatin remodeling enzymes can rapidly reposition, assemble and disassemble nucleosomes, while other classes of remodeling enzymes covalently modify histone proteins or DNA (Lusser and Kadonaga, 2003; Saha et al., 2006). ATP-dependent remodeling enzymes are often multi-protein complexes, classified by their ATPase subunit into subfamilies such as ISWI, SWI/SNF and Mi2 (Lusser and Kadonaga, 2003). Remodeling ATPases are generally broadly expressed, and primary CD4 T cells express several remodeling ATPases (Wurster and Pazin, 2008).

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SWI/SNF complexes are regulators of T cell development (Chi, 2004). SWI/SNF complexes contain 10–15 proteins, including the ATPases BRG1 and Brm. SWI/SNF plays an important role in activating T helper 2 (Th2) gene expression (Wurster and Pazin, 2008) and T helper 1 (Th1) gene expression (Letimier et al., 2007; Zhang and Boothby, 2006). BRG1 is dynamically recruited to the Th2 locus by transcription factors, during differentiation and again during activation (Wurster and Pazin, 2008). Thus, SWI/SNF components activate gene expression to regulate T cell development, differentiation and function.

Mi2 remodeling ATPases, found in NuRD complexes containing histone deacetylases, are also important in T cells. Mi2β/CHD4 has been found to be important for CD4 expression and T cell development (Williams et al., 2004). Mi2β can form stable complexes with the transcription factor Ikaros, and Mi2β and Ikaros appear to antagonize each other (Naito et al., 2007). In macrophages, SWI/SNF and Mi2β have opposing effects on gene regulation (Ramirez-Carrozzi et al., 2006). Thus the Mi2 remodeling family represses gene expression in the immune system.

Little is known about the role of the ISWI subfamily of remodeling ATPases in T cells, by contrast to the examples above. ISWI complexes are frequently dimeric complexes containing the SNF2H or SNF2L ATPase and one of several accessory proteins (Corona and Tamkun, 2004; Lusser and Kadonaga, 2003). We previously found that SNF2H mRNA is abundant in primary T cells (Wurster and Pazin, 2008). SNF2H is an essential gene with an embryonic lethal phenotype in mice (Stopka and Skoultchi, 2003). ACF1, a SNF2H binding partner, has been implicated in DNA replication and chromatin assembly (Collins et al., 2002; Fyodorov et al., 2004). The ACF1 subunit has been found to alter ISWI activity (Fyodorov et al., 2004; He et al., 2008, 2006). Here, we test the role of SNF2H in cytokine gene expression. We find SNF2H plays a direct role in transcription of cytokine genes. SNF2H and ACF1 bind to cytokine genes in cells. SNF2H protein is apparently required for ACF1 stability. SNF2H can increase or decrease gene expression, depending on the cytokine. This activity correlates with changes in SNF2H-mediated chromatin accessibility.

2. Materials and methods

2.1. Cells

Murine EL4 T cell line was obtained from ATCC, grown in RPMI/10% FBS/L-glutamine, penicillin and streptomycin.

Primary CD4 T cells were prepared from mice and differentiated in culture as described (Wurster and Pazin, 2008). Animal approval was from the NIA ACUC, protocol ASP-365-MJP-Mi, and all experiments conform to the relevant regulatory standards.

2.2. Knockdown

EL4 cells were transduced at high efficiency using nucleofection solution V and program T-14, according to the manufacturer's instructions. Typically, 65–85% of the cells become GFP+ in parallel transfections under these conditions (data not shown). Plasmid encoding SNF2H shRNA was cotransfected with a plasmid encoding a truncated version of the low-affinity NGF receptor (Miltenyi). Transduced cells were enriched 1 day after transfection using magnetic bead purification for NGFr+ cells (Miltenyi), fed on the second day, and analyzed on the third day. SNF2H shRNA plasmid (pBS/U6-606) was constructed by annealing GAGGAGGATGAAGAGCTATCTCGAGATAGCTCTTCATCCTCCTCTTTTTG (MP 606) and AATTCAAAAAGAGGAGGATGAAGAGCTATCTCGAGATAGCTCTTCATCCTCCTC (MP 607), followed by ligation into pBS/U6 (Sui et al., 2002). To date, we have not been able to silence SNF2H expression in primary T cells. SNF2H knockdown did not significantly alter proliferation or apoptosis (Fig. S1). SNF2H knockdown did not obviously alter cell recovery or cell morphology, relative to control shRNA transduction.

2.3. mRNA quantitation

Total RNA was purified using RNeasy columns, including DNase treatment on columns (Qiagen). cDNA was made using iScript (Bio-Rad) according to the manufacturer's instructions. The mRNA levels of chromatin remodeling factors, cytokines and transcription factors were determined by real-time PCR using SYBR green (Qiagen) on an ABI 7500. Expression levels were normalized to TBP or β-actin as indicated. Oligo sequences are in supplementary tables. We also confirmed that IL7ra and GADD45 are repressed by SNF2H while FKBp3 and LEF1 were activated, but did not study these genes further.

2.4. Chromatin immunoprecipitation (ChIP) assay

Chromatin immunoprecipitation was performed using methods similar to those described previously (Wurster and Pazin, 2008); details are available on request. Approximately 20 million cells (enough for 5 immunoprecipitations using different antibodies) were crosslinked with 1% formaldehyde and quenched with glycine. Nuclei were prepared with buffer containing 1% Triton X-100, treated with micrococcal nuclease, sonicated using a Biorup-

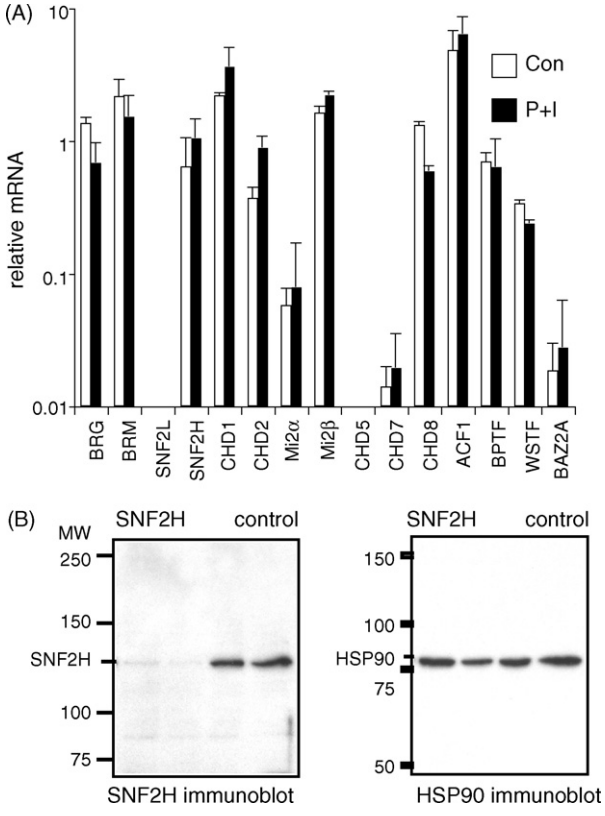

Fig. 1. Efficient silencing of SHF2H protein expression. (A) Several remodeling ATPases are expressed in EL4 cells. mRNA was harvested from EL4 cells, resting or stimulated (5 h PMA and ionomycin) as indicated. cDNA was synthesized and analyzed by Q-RT-PCR for the indicated genes; reported values are normalized to a housekeeping gene (TBP). (B) Cells were transduced in duplicate with SNF2H shRNA plasmid or pBS-U6 vector (as indicated) using nucleofection, transduced cells were enriched (as described in Section 2), whole cell lysates were made, and 15 μg clarified lysate were loaded per lane, probed with SNF2H antibody and reprobed with HSP90 antibody (loading control), as indicated.