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Role of Sug1, a 19S proteasome ATPase, in the transcription of MHC I and the atypical MHC II molecules, HLA-DM and HLA-DO

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Abstract

The 19S proteasome regulatory particle plays a critical role in cellular proteolysis. However, emerging evidence suggests roles for 19S proteasome subunits in regulating yeast and mammalian transcription. It has been previously shown that Sug1 is important for the transcription of MHC II molecules. We report here that Sug1 also has a role in regulating transcription of class I MHC and the MHC II-like molecules, HLA-DM and HLA-DO. Reduction of Sug1 expression causes a decrease in the transcription of MHC I and MHC II-like molecules. In addition, we show that association of Sug1 with MHC promoters is followed by the recruitment of the CREB-binding protein (CBP) and the class II transactivator (CIITA). Reduction of Sug1 expression is accompanied by decreased recruitment of CBP and CIITA to the MHC promoters and decreased histone H3 acetylation in these promoters. These studies suggest that Sug1 plays a critical role in transcription of MHC class I, and the MHC class II-like molecules, HLA-DM and HLA-DO.

Keywords

19S ATPase; HLA-DM; HLA-DO; MHC class I; transcriptional regulation; Sug1

1. Introduction

The major histocompatibility complex (MHC) is an extensive multigene family that encodes surface glycoproteins that are essential for the development and function of the immune system by presenting antigenic peptides to T cells. They are encoded by two main families of genes: class I MHC (MHC I) and class II MHC (MHC II). MHC II, in association with the accessory molecules invariant chain (Ii), human leukocyte antigen (HLA)-DM and HLA-DO proteins, facilitate the presentation of extracellular antigens to CD4+ T lymphocytes [1]. HLA-DM promotes the dissociation of the CLIP peptide (which is part of the invariant chain) from MHC II molecules and is responsible for editing the peptide

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repertoire to favor stable peptide-MHC II complexes [2]. HLA-DO, in turn, modulates the peptide loading activity of HLA-DM [3]. MHC I present cytosol-derived peptides to CD8+ T lymphocytes.

The most important regulatory factor of MHC II expression is the class II transactivator (CIITA) molecule that functions as an integrator of most, if not all, pathways that influence the expression of MHC II [4]. Although it does not bind directly to the MHC II promoter, CIITA is associated with the pre-assembled enhanceosome complex [5–8], with the basal transcription machinery [9–12], and with chromatin remodeling enzymes [13] allowing MHC II transcription initiation. A role for CIITA has been demonstrated in MHC I [14], HLA-DM [15,16] and HLA-DO [17,18] gene induction.

The MHC II promoter contains a regulatory module, which consists of four consensus sequences (S, X, X2 and Y boxes) that are tightly constrained in order, orientation and spacing [30]. Alignment of the MHC I and MHC II promoter DNA sequences revealed similarities in structure [31]. The promoters of MHC II-like genes, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB and Ii, all of which produce proteins that play an important role in assembly and transport of MHC II to the cell surface, contain the conserved X and X2 sequence motif, which explains their inducibility by CIITA.

The 26S proteasome consists of a central 20S proteolytic complex capped at both ends by 19S regulatory complexes. These 19S caps serve to recognize ubiquitinated proteins and presumably convert them into a form suitable for degradation by the 20S complex. The 19S cap contains at least 18 proteins, including six adenosine triphosphatases (ATPases) of the AAA (ATPases associated with various cellular activities) family [19]. Recent biochemical studies have provided evidence that the 19S complex is directly involved in transcription [20–25]. The 19S proteasome subunit, Sug1 has been implicated in the regulation of yeast and mammalian transcription. Studies in yeast show the recruitment of Sug1 to the stress-induced genes promoter [25]. In mammalian cells, Sug1 is recruited to *p21waf1* promoter after cells were exposed to UV for 2h [26]. Recently, it was found that Sug1 is recruited to MHC II proximal promoter and plays a role recruiting and/or stabilizing CIITA in MHC II transcription [20]. Sug1 was also shown to be important in recruiting CREB-binding protein (CBP) and to be important for regulating histone H3 acetylation at the MHC class II proximal promoter [27].

CBP is thought to have various functions on MHC II promoter, as a histone acetyltransferase and as an integrator that links CIITA with enhanceosome complex [28, 29]. Sug1 binds to CBP and is important for the recruitment of CBP to the MHC II proximal promoter [27].

Here we show that Sug1 is necessary for optimal mRNA expression of MHC I and the MHC II-like molecules, HLA-DM and HLA-DOB in B cells. In addition, we demonstrate that Sug1 is recruited to the proximal promoter of MHC I and II as well as HLA-DM and the beta chain of HLA-DO (HLA-DOB). Sug1 recruitment to MHC promoters is inducible after 2 hours of interferon-gamma (IFN- γ) treatment, followed by CBP and CIITA. Furthermore, Sug1 is required for recruiting CBP and CIITA to MHC I, MHC II and MHC II-like promoters. The expression of Sug1 is also essential for regulating H3 acetylation at the MHC I and II and HLA-DM and HLA-DO promoter. The results show the involvement of the 19S proteasome subunit, Sug1, in MHC I, HLA-DM and HLA-DOB transcription and therefore in regulating antigen presentation.

2. Materials and Methods

2.1. Cell culture

LG-2 EBV B cells kindly provided by Dr. Lawrence J. Stern (University of Massachusetts Medical School, Worcester, MA) were maintained at 37°C with 5% CO₂ using Roswell Park Memorial Institute (RPMI) media with HEPES and L-glutamine (Sigma), and supplemented with 10% FBS, 50 U/mL of penicillin (Pen) and 50 µg/ml of streptomycin (Strep).

2.2. Antibodies

Anti-Sug1, anti-CBP, anti-HLA-DRA, anti-HLA-A, and anti-GAPDH antibodies were obtained from Santa Cruz Biotechnologies. Anti-CIITA, anti-HLA-DMA, anti-HLA-DMB, anti-HLA-DOA, and anti-HLA-DOB antibodies were obtained from Abcam. Anti-histone H3 and anti-acetylated histone H3 K18 antibodies were obtained from Millipore.

2.3. Chromatin immunoprecipitation (ChIP)

LG-2 EBV cells were plated at a cell density of 1×10^6 cells/mL in a T75 flask, and were activated with 500 ng/mL IFN- γ for 24 h. At the end of the activation period, cells were crosslinked with 1% formaldehyde for 15 min at room temperature (RT). The crosslinking reaction was stopped with 0.125 M glycine for 10 min at RT. The assay was performed according to the manufacturer's instructions (chromatin immunoprecipitation (ChIP) assay kit from Millipore). Briefly, cells were lysed in sodium dodecyl sulfate (SDS) lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris, pH 8.1 and protease inhibitors) and sonicated for 30 min to generate an average of 100 – 200 base pairs (bp) sheared DNA. The sonicated lysates were pre-cleared with blocked protein G beads and then immunoprecipitated with 10 µg of an antibody against Sug1 (Santa Cruz Biotechnology) or no antibody overnight at 4°C. Blocked protein G beads were added to the samples and incubated for 1 h at 4°C. Samples were washed for 5 min at 4°C with the following buffers: low salt buffer (0.1% SDS, 1% Triton-X, 2 mM EDTA, 20 mM Tris, pH 8.1, 150 mM NaCl), high salt buffer (0.1% SDS, 1% Triton-X, 2 mM EDTA, 20 mM Tris, pH 8.1, 500 mM NaCl), lithium chloride (LiCl) buffer (0.25 M LiCl, 1% NP-40, 1% deoxycholic acid, 1 mM EDTA, 10 mM Tris, pH 8.1) and 1× TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). Samples were eluted with SDS elution buffer (1% SDS, 0.1 M sodium carbonate (NaHCO₃)). Following elution, crosslinked materials were reversed with 5 M NaCl at 65°C for 5 h and immunoprecipitated DNA was isolated using phenol:chloroform:isopropanol mix (Invitrogen) and analyzed by real-time PCR (StepOne Plus, Applied Biosystems).

2.4. Sug1 knockdown and RNA isolation

LG-2 EBV cells were plated at a cell density of 2.5×10^5 cells/well in a six-well plate using media without FBS or antibiotic. Transfection of Sug1 siRNA (Applied Biosystems) was carried out using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. Twenty four hours following siRNA transfection, cells were stimulated with IFN- γ (500 ng/µL) for 24 h. Cells were harvested, centrifuged at 1500 revolution per minutes (rpm) for 5 min at RT, and 15% of the cells were lysed with lysis buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1% NP40, 1 mM EDTA, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, dH₂O) with protease inhibitors and were analyzed by western blot for Sug1 knockdown. The remaining fraction of cells was subjected to RNA extraction, and total RNA was prepared with 1 mL of TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The Phusion reverse transcriptase PCR kit (New England Biolabs) was used to make 20 µL of cDNA from 1 µg of RNA.

Gene expression was analyzed using real-time PCR using primers for HLA-A2, HLA-DRA, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB, GAPDH (Applied Biosystems).

2.5. Western Blot

LG-2 EBV cells transfected with control or Sug1 siRNA were lysed at 0 and 24 h post IFN- γ stimulation. Protein samples were electrophoresed on a SDS-PAGE gel and transferred onto a PDVF membrane (Invitrogen). The blots were probed with antibodies specific for HLA-DRA, HLA-A2, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB, CIITA or GAPDH using standard methods after blocking with 5% BSA. Proteins were visualized by using Opti-4CN kit (Bio-Rad).

2.6. ChIP in Sug1 knockdown

LG-2 EBV cells were transfected with Sug1 siRNA (Applied Biosystems) or control siRNA (Applied Biosystems). After 24 hours, cells were treated with 500 ng/ μ L IFN- γ as indicated. Subsequent to 18 hours of treatment, cells were subjected to a ChIP assay.

2.7. Statistical analysis

Data were analyzed by two-way ANOVA with Bonferroni post-test and the *p*-value was set to be <0.05. Data are expressed as mean $\pm$ SD of (n=3) independent experiments.

3. Results

3.1. Sug1 is required for MHC Class I and II optimal expression

In order to study whether Sug1 is essential for MHC I, MHC II, HLA-DM and HLA-DO protein expression, we employed Sug1 siRNA knockdown in LG-2 EBV cells. After 24h of Sug1 or control siRNA transfection, LG-2 EBV cells were stimulated with IFN- γ . The effect of Sug1 siRNA on HLA-DRA, HLA-A2, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB and CIITA protein levels was examined by Western blot analysis of samples at 0 and 24 hours after IFN- γ stimulation. After 24 hours post-stimulation, the levels of HLA-DRA, HLA-A2, HLA-DMA, HLA-DMB and HLA-DOB decreased significantly when compared to the levels of the proteins in cells transfected with control siRNA. Levels of MHC I (HLA-A2), MHC II (HLA-DRA), HLA-DM and HLA-DO mRNA levels were analyzed by real-time PCR. Sug1 protein level in LG-2 EBV cells transfected with Sug1 siRNA was reduced by 90% (Fig. 1A) after IFN- γ treatment while its mRNA expression was reduced by 75% (Fig. 1B). As shown in Figure 1, reduction of Sug1 expression led to a significant reduction in the mRNA expression of MHC II (HLA-DRA, by 73%, Fig. 1C), MHC I (HLA-A, by 75%, Fig. 1D), HLA-DMA (by 64%, Fig. 1E), HLA-DMB (by 61%, Fig. 1F) and HLA-DOB (by 70%, Fig. 1G). In contrast, mRNA expression of HLA-DOA and CIITA (Fig. 1H and 1I) was not affected by the reduction of Sug1 expression. Consequently, the effects of decreased expression of Sug1 on physiologically induced MHC I and II, and HLA-DM and HLA-DOB expression were not due to decreased expression of CIITA as the expression of CIITA is not altered in Sug1 siRNA treated cells. These results demonstrate that Sug1 not only regulates the expression of class II MHC molecules [20] but that it is also important for the regulation MHC I, HLA-DM and HLA-DOB.

3.2. Sug1 is recruited to the MHC Class I and Class II-like promoters

To determine if Sug1 directly associates with class I MHC, HLA-DM and HLA-DO promoters, we performed chromatin immunoprecipitation (ChIP) assays on endogenous MHC I (HLA-A2), HLA-DMA, HLA-DMB, HLA-DOA and HLA-DOB promoters. LG-2 EBV cells were stimulated with IFN- γ for 24h, immunoprecipitated with Sug1 antibody and analyzed by real-time PCR using primers for the W-X-Y box of the promoters for MHC II

(HLA-DRA and HLA-DRB), MHC I (HLA-A2), HLA-DMA, HLA-DMB, HLA-DOA and HLA-DOB. As shown in Figure 2, Sug1 is recruited to MHC I, MHC II, HLA-DMA, HLA-DMB, HLA-DOA and HLA-DOB promoters with $p < 0.01$ upon 24h of IFN- γ treatment. Amplification of GAPDH promoter shows no increase after IFN- γ treatment, which confirms the specificity of Sug1 interaction with MHC promoters after IFN- γ treatment. These findings indicate that Sug1 is recruited to MHC class I and II, HLA-DM and HLA-DO proximal promoters and that Sug1 may be involved in the expression of these molecules.

3.3. Sug1 is recruited to MHC promoters after 2 hours of IFN- γ treatment

To compare the association of Sug1 with MHC class I, class II and MHC II-like promoters with that of CIITA and CBP, we performed ChIP assays on an endogenous MHC class I (HLA-A2), MHC class II (HLA-DRA) and MHC class II-like (HLA-DMA) promoters (Fig. 3). After 2 h of IFN- γ , Sug1 is associated with MHC promoter and this association peaked at 4 h (Fig. 3A, 3D and 3G). Association of endogenous CBP with the MHC promoters is detectable at 4 h of post-stimulation, but this association increased significantly after 8 h and peaked at 24 h post-stimulation (Fig. 3B, 3E and 3H). Association of endogenous CIITA to MHC promoters also peaked after 24 h post-stimulation (Fig 3C, 3F and 3I). These findings indicate that both CBP and CIITA are recruited to the MHC promoters after the association of Sug1 with these promoters.

3.4. Sug1 knockdown decreases CBP and CIITA recruitment to MHC promoters

To investigate potential roles for Sug1 in recruiting CIITA and CBP to MHC class I and II and atypical MHC II proximal promoters, we performed ChIP experiments to look at the endogenous levels of CIITA and CBP at the MHC I (HLA-A2), MHC II (HLA-DRA) and HLA-DMA proximal promoters (Fig. 4). Upon IFN- γ stimulation, CBP and CIITA were recruited to MHC I, MHC II and HLA-DMA promoters in control siRNA-treated cells but their recruitment decreased in Sug1 siRNA-treated cells. Reduction of Sug1 expression led to a significant reduction of CBP recruitment to HLA-A2 (by 71%), HLA-DRA (by 60%) and HLA-DMA (by 70%) promoters (Fig. 4A, 4B and 4C respectively). Moreover, Sug1 knockdown also caused a reduction in the recruitment of CIITA to HLA-A2 (by 69%), HLA-DRA (by 69%) and HLA-DMA (by 75%) promoters (Fig. 4D, 4E and 4F respectively). Sug1 knockdown does not affect the expression of CBP (Fig. 4G) and CIITA (Fig. 4H), indicating that the reduction in the recruitment of CBP and CIITA to the MHC promoters is in fact due to the absence of Sug1. These results demonstrate that Sug1 is essential for CBP and CIITA recruitment to the MHC proximal promoters.

3.5. Sug1 knockdown decreases histone H3 acetylation at Lys18 within MHC Class I and II-like promoters

To study the requirement of Sug1 for appropriate histone acetylation in the MHC class I, class II and atypical MHC II proximal promoters, we performed ChIP experiments to detect endogenous levels of histone H3 acetylation at lysine 18 in the MHC I and MHC II-like (HLA-DM and HLA-DO) proximal promoters (Fig. 5). Upon IFN- γ stimulation, H3 Lys 18 acetylation was enhanced in control siRNA-treated cells at the MHC II (HLA-DRA and HLA-DRB, Fig. 5A and 5B respectively), HLA-DMA (Fig. 5C), HLA-DMB (Fig. 5D), HLA-DOA (Fig. 5E), HLA-DOB (Fig. 5F) MHC I (HLA-A2, Fig. 5G) promoters. However, enhancement of H3 Lys 18 acetylation at the HLA-DOA promoter was not statistically significant. In Sug1 siRNA-treated cells, H3 Lys 18 acetylation at all tested promoters, except for HLA-DOA, was drastically decreased. IFN- γ treatment does not change H3 acetylation at Lys 18 of the GAPDH promoter in the presence or absence of Sug1 (Fig. 5H).

4. Discussion

In this study we have investigated a general role for Sug1 in the expression of classical MHC molecules and the atypical MHC II proteins, HLA-DM and HLA-DO. Our studies, while confirming the role of Sug1 in regulating the expression of MHC class II molecules [20], demonstrates an important and crucial role for the expression of the other class II-like molecules HLA-DM and HLA-DO. These proteins are very important for peptide loading and exchange on HLA-DR. In addition, we also show that Sug1 influences the expression of class I MHC proteins. The role of the immune proteasome in generating peptides for MHC-class I presentation upon IFN γ treatment is well known [32]. This new observation that a non-catalytic subunit of the proteasome is able to control the expression of MHC-class I molecules is likely to be important in coordinating regulation of antigen presentation.

The most important regulatory factor of MHC II expression is CIITA, which functions as an integrator of most pathways that influence the expression of MHC II [4]. A role for CIITA has been demonstrated in MHC I [14], HLA-DM [15,16] and HLA-DO [17,18] gene induction. The MHC II promoter contains a regulatory module tightly conserved in order, orientation and spacing, which consist of four consensus sequences (S, X, X2 and Y boxes) [30]. Alignment of the MHC I and MHC II promoter DNA sequences show similarities in structure [31]. In MHC I, site α has the same function of X2 box in MHC II promoter. Upstream of site α , DNA sequences in the MHC I promoter are homologous to the S and X boxes of the MHC II promoter. Downstream of that same site, the inverted CCAAT box in enhB of the MHC I promoter is identical to the Y box in the MHC II promoter [31]. The MHC II-like molecules, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB and Ii produce proteins that play an important role in assembly and transport of MHC II to the cell surface and their promoters contain the conserved X and X2 sequence motif, which explains their inducibility by CIITA. Recently, it was found that Sug1 is recruited to MHC II proximal promoter and plays a role recruiting and/or stabilizing CIITA in MHC II transcription [20]. Sug1 was also shown to be important in regulating histone H3 acetylation at the MHC class II proximal promoter [27]. In the same way, we show that Sug1 is important in the regulation of MHC I and the atypical class II MHC molecules, HLA-DM and HLA-DO. Although Sug1 is recruited to HLA-DOA proximal promoter after IFN- γ stimulation, siRNA-mediated suppression of Sug1 did not impact HLA-DOA transcription. We speculate that the expression of HLA-DOA is been induced in these cells by a different mechanism that does not involve Sug1. There is an Interferon Regulatory Factor-7 binding site in the HLA-DOA promoter, but not the others promoters studied here, that could cause differential expression of HLA-DOA independently of Sug1 suppression.

In conclusion, we believe that Sug1 plays an important role the transcription of classical and atypical MHC molecules by controlling the activity of CBP and CIITA and modulating histone H3 acetylation of the promoters. How other 19S subunits are recruited to these promoters and what is the exact role of these other 19S subunits in transcription events is still unknown. These findings contribute to our knowledge of the role of Sug1 in mammalian histone modification and antigen presenting pathways.

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Highlights

- Sug1 is required for optimal gene expression of MHC I, and the atypical MHC II proteins HLA-DM and HLA-DO.
- Sug1 binds directly and specifically to the HLA promoters.
- Histone H3 acetylation at the HLA promoters decreases in the absence of Sug1.
- Sug1 is essential for CBP and CIITA recruitment to the MHC promoters.

Figure 1.

Sug1 is required for optimal gene expression of MHC I and atypical MHC II proteins. (A) Western blots showed 90% of Sug1 knockdown efficiency. Sug1 protein suppression decreases the protein levels of HLA-DRA, HLA-A2, HLA-DMA, HLA-DMB and HLA-DOB. Reduced expression of Sug1 in siRNA transfected LG-2 EBV cells decrease (C) HLA-DRA, (D) HLA-A2, (E) HLA-DMA, (F) HLA-DMB and (G) HLA-DOB mRNA expression after 24 hours of IFN- γ treatment but does not affect mRNA expression of HLA-DOA and CIITA (H and I respectively). (mRNA levels of Sug1 in IFN- γ -treated LG-2 cells are shown in part B.) Levels of mRNA were measure by real-time PCR and were normalized to GAPDH. mRNA levels are presented as the fold difference in RNA level relative to unstimulated sample values and represent the mean $\pm$ SD of (n=3) independent experiments. **, $p < 0.01$ versus control siRNA; ***, $p < 0.001$ versus control siRNA.

Figure 2.

Sug1 associates with MHC I, MHC II and atypical MHC II promoters. LG-2 EBV cells were stimulated with IFN- γ for 24 hours. Lysates were immunoprecipitated with antibody against Sug1, and DNA was isolated and analyzed via real-time PCR. Real-time PCR values were normalized to the total amount of promoter DNA added to the reaction (input). Input values represent 5% of the total cell lysate. Immunoprecipitated values represent mean $\pm$ SD of ($n = 3$) independent experiments. **, $p < 0.01$ versus untreated sample; ***, $p < 0.001$ versus untreated sample.

Time Course ChIP in HLA-DRA Promoter

Time Course ChIP in HLA-A2 Promoter

Time Course ChIP in HLA-DMA Promoter

Figure 3.

Sug1 associates with MHC I, MHC II and MHC II- like proximal promoters before CBP and CIITA. (A, D, G) Sug1 is associated with MHC promoter after 2 h post-stimulation. (B, E, H) Association of endogenous CBP with the MHC promoters is increased after 8 h post-stimulation. (C, F, I) Association of endogenous CIITA to MHC promoters peaked after 24 h post-stimulation. LG-2 EBV cells were stimulated with IFN- γ for 0, 2, 4, 8, 24 or 48 hours. Lysates were immunoprecipitated with antibody against Sug1, CBP, CIITA or no antibody. DNA was isolated and analyzed via real-time PCR. Real-time PCR values were normalized to input. Input values represent 5% of the total cell lysate. Immunoprecipitated values represent mean $\pm$ SD of ($n = 3$) independent experiments. *, $p < 0.05$ versus untreated sample; **, $p < 0.01$ versus untreated sample; ***, $p < 0.001$ versus untreated sample.

Endogenous CBP ChIP in Presence of Sug1 knockdown

Endogenous CIITA ChIP in Presence of Sug1 knockdown

Figure 4.

CBP and CIITA recruitment to the MHC I, MHC II and MHC II-like proximal promoter is decreased in the absence of Sug1. Sug1 knockdown decreases levels of CBP and CIITA recruited to (A,D) HLA-DRA, (B,E) HLA-A2 or (C,F) HLA-DMA promoters. LG-2 EBV cells were transfected with control siRNA or with Sug1 specific siRNA and 24 h later were stimulated with IFN- γ for 18 h or left untreated (0). The lysate was immunoprecipitated with CBP or CIITA antibody or no antibody and associated DNA was isolated and analyzed via real-time PCR. Real-time PCR values were normalized to the total amount of *HLA-DRA*, *HLA-A2* or *HLA-DMA* promoter DNA added to the reaction (input). (G) CBP and (H) CIITA mRNA level were not affected in the presence or absence of Sug1. (mRNA levels of Sug1 in IFN- γ -treated LG-2 cells are shown in part I.) Immunoprecipitated values represent mean $\pm$ SD of ($n = 3$) independent experiments. ***, $p < 0.001$ versus control siRNA.

Endogenous Acetylated H3 ChIP in the Presence of Sug1 Knockdown

Figure 5.

Sug1 knockdown decreases histone H3 acetylation at the HLA promoters studied. Sug1 knockdown decreases levels of acetylated histone at the (A) HLA-DRA, (B) HLA-DRB, (C) HLA-DMA, (D) HLA-DMB, (E) HLA-DOA, (F) HLA-DOB, and (G) HLA-2 promoters, although the difference is not statistically significant for HLA-DOA (E). Histone H3 acetylation of the (H) GAPDH promoter was not affected in the presence or absence of Sug1. (mRNA levels of Sug1 in IFN- γ -treated LG-2 cells are shown in part I.) ChIP assays were carried out in LG-2 EBV cells transfected with control siRNA or Sug1-specific siRNA and 24 h later stimulated with IFN- γ for 0 or 18 hours. Lysates were subjected to IP with antibody to endogenous acetylated histone H3 K18 or no antibody. Associated DNA for was isolated and analyzed via real-time PCR. Real-time PCR values were normalized to the total amount of promoter DNA added to the reaction (input). Input values represent 5% of the total cell lysate. Immunoprecipitated values are presented as the ratio between acetylated histone H3 and histone H3 IP sample values and represent the mean $\pm$ SD of (n=3) independent experiments. **, $p < 0.01$ versus control siRNA; ***, $p < 0.001$ versus control siRNA.