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Epigenetic regulation of CIITA expression in human T-cells

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ABSTRACT

In humans, T-cells accomplish expression of MHC-II molecules through induction of CIITA upon activation. Here we show that CIITA promoter accessibility in T-cells is epigenetically regulated. In unstimulated T-cells, CIITA-PIII chromatin displays relative high levels of repressive histone methylation marks (3Me-K27-H3 and 3Me-K20-H4) and low levels of acetylated histones H3 (Ac-H3) and H4 (Ac-H4). These repressive histone marks are replaced by histone methylation marks associated with transcriptional active genes (3Me-K4-H3) and high levels of Ac-H3 and Ac-H4 in activated T-cells. This is associated with concomitant recruitment of RNA polymerase II. In T-leukemia cells, devoid of CIITA expression, similar repressive histone methylation marks and low levels of acetylated histone H3 correlated with lack of CIITA expression. This in contrast to CIITA expressing T-lymphoma cells, which display high levels of Ac-H3 and 3Me-K4-H3, and relative low levels of the 3Me-K27-H3 and 3Me-K20-H4 marks. Of interest was the observation that the levels of histone acetylation and methylation modifications in histones H3 and H4 were also noted in chromatin of the downstream CIITA-PIV promoter as well as the upstream CIITA-PI and CIITA-PII promoters both in normal T-cells and in malignant T-cells. Together our data show that CIITA chromatin in T-cells expressing CIITA display similar histone acetylation and methylation characteristics associated with an open chromatin structure. The opposite is true for T-cells lacking CIITA expression, which display histone modifications characteristic of condensed chromatin.

1. Introduction

The class II transactivator (CIITA) is essential for transcriptional activation of all MHC-II genes and plays an ancillary function in the control of MHC-I gene transcription (with the exception of HLA-G) [1–3]. This is clearly illustrated in a subgroup of MHC-II-deficiency patients with a defect in CIITA, which fail to express MHC-II molecules [4]. Furthermore, CIITA is also involved in the transcription regulation of the MHC-II accessory genes (HLA-DM, HLA-DO and invariant chain) and of the gene encoding β_2-microglobulin [1,5]. CIITA is expressed in a constitutive fashion in antigen presenting cells of the immune system, while its expression can be induced in a variety of other cell types in an environment rich in inflammatory cytokines, of which IFN-γ is the most potent, or upon activation such as in human T-cells [6–8].

Transcriptional regulation of *MHC2TA*, the gene encoding CIITA, is mediated through the activity of independent promoter units (CIITA-PI through CIITA-PIV) [9]. These promoter units are employed in a cell type- and activation-specific manner. CIITA-PI and CIITA-PIII are used for the constitutive expression in dendritic and B cells, respectively [9]. CIITA-PIII has also been shown to be employed by human T-cells upon activation [7,8]. CIITA-PIV has been shown to be the promoter predominantly involved in IFN-γ inducible expression [10–12]. In addition, in human non-B cells, CIITA-PIII can also be activated by IFN-γ through an element located 2 kb upstream of the core CIITA-PIII promoter [12–14]. The promoter function of CIITA-PII is still ill-defined. The various CIITA promoters each transcribe a unique first exon and are located within a region of approximately 14 kb [9].

Previously others and we have shown that the ubiquitously expressed transcription factor CREB-1 plays a key role in the transcriptional activation of *MHC2TA* mediated by CIITA-PIII in activated T-cells [7,8]. Furthermore, we have shown that the lack of MHC-II molecule expression in human T-leukemia cells is due to lack of CIITA expression which correlated with hypermethylation of CIITA-PIII and -PIV [15,16] (unpublished observations). Besides in T-leukemia, hypermethylation of CIITA promoters has been

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linked to lack of CIITA and MHC-II molecule expression in various other types of cancer [17–22].

It is widely accepted that DNA and histone methylation modifications in gene silencing are functionally linked [23]. Histone methylation plays an important role in chromatin dynamics and gene expression [24–26]. The mechanisms that underlie gene repression by histone methylation are known to involve tri-methylation of histone H3 at lysine 9 (3Me-K9-H3) and at lysine 27 (3Me-K27-H3), and of histone H4 at lysine 20 (3Me-K20-H4), which are catalyzed by the conserved SET domain of respectively the lysine methyltransferases (KMTases) SUVAR39H1 (hKMT1A), enhancer of Zeste homolog 2 (EZH2, hKMT6), a subunit of the polycomb repressive complex (PRC) 2, and SUV4-20H1/H2 (hKMT5B/C) [25,27–29]. The KMTase hSet1 (hKMT2F/G) catalyses tri-methylation of K4-H3 and this modification is associated with transcriptionally active chromatin and gene expression [29].

In the current study, we have evaluated the contribution of epigenetic DNA and histone modifications in transcriptional activation of *MHC2TA* in human T-cells to relate with similar epigenetic modifications in T-leukemia and T-lymphoma cells. The results of these analyses show that histone acetylation and methylation modifications, that are respectively associated with transcriptional inactive or active genes, correlate with lack of CIITA expression in unstimulated T-cells and with CIITA expression in activated T-cells. Besides CIITA-PIII, the principal promoter employed in T-cells, the various histone modifications were noted also in chromatin of the other CIITA promoters. No methylation of CpG residues in DNA of the investigated CIITA-PIII and -PIV promoters was found both in unstimulated and in activated normal T-cells. When compared with CIITA-expressing T-lymphoma cells, CIITA-deficient T-leukemia cells displayed high levels of the repressive 3Me-K27-H3 and, albeit to a lesser extent, also 3Me-K20-H4 at CIITA-PIII chromatin. In addition, higher levels of EZH2 were also observed in CIITA-PIII chromatin in leukemic T-cells. Similar to normal T-cells these features were also noted in chromatin of the other CIITA promoters.

2. Materials and methods

2.1. Cell culture

The following cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA): T-leukemia; Jurkat clone E6-1 ($CD4^+$, TIB-152), HSB-2 ($CD4^-$, CCL-120.1) and Molt-4 ($CD4^{+/-}$, CRL-1582), and T-lymphoma; HUT-78 ($CD4^+$, TIB-161) and HH ($CD4^+$, CRL-2105). The T-lymphoma Karpas-299 ($CD4^+$, ACC 31) was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany). HUT78, HH and HSB-2 were cultured in IMDM (Lonza Verviers, Verviers, Belgium) supplemented with 10% heat-inactivated FBS (PAA Laboratories, GmbH, Cölbe, Germany), 100 U/ml streptomycin, 100 U/ml penicillin and 2 mM L-glutamine. All other cell lines were cultured in RPMI 1640 (Life Technologies, Breda, The Netherlands) supplemented with 10% heat-inactivated FBS (PAA Laboratories), 100 U/ml streptomycin, 100 U/ml penicillin and 2 mM L-glutamine. For re-expression of CIITA, cells were exposed to 100 μM of zebularine [V.E. Marquez, 30], for 96 h followed by 2 μM of 3-deazaneplanocin A (DZNep [V.E. Marquez, 31]) for 72 h and 0.5 μM MS-275 (Sigma–Aldrich; Steinheim, Germany [32]) for 48 h in IMDM (HSB) or RPMI (Jurkat and Molt-4) with supplements as described above.

Human CD4+ T-cells of two different donors were sorted from freshly isolated PBMC using a FACSAria Flow Cytometer (BD Biosciences, Mountain View, CA, USA) and activated in vitro as described earlier [7]. Shortly, naive T-cells were stimulated twice with 1 μg/ml PHA (Welcome Diagnostics, Dartford, UK) and 20 U/ml IL-2 (Novartis Pharma B.V., Basel, Switserland) in the presence of irradiated (3000 rad) allogeneic PBMCs. After 21 days of culture cells were harvested for ChIP and bisulfite sequencing analysis. HLA-DR (PE-labeled, #347367, BD Pharmingen) expression was determined by flow cytometry.

2.2. Bisulfite sequencing

Total genomic DNA was isolated from Jurkat, unstimulated and stimulated T-cells and 1 μg of genomic DNA was used to convert unmethylated CpG's using the EZ DNA Methylation kit (Zymo Research, Orange, CA, USA). CIITA promoter DNA was then amplified using primer sets for specific CpG containing regions of CIITA-PIII (PIII.1 forward: 5′-GAGTAGGTATGGTAGAGGAGAG-TAGTATTT-3′; PIII.1 reverse: 5′-ATCAAATTTCTATTTCTAAACACC-CTC-3′; PIII.2 forward: 5′-GAGGGTGTTTAGAAATAGAAATTTGAT-3′; PIII.2 reverse: 5′-AAATAACAAAACCAACTAAAACTACAC-3′) and CIITA-PIV (PIV forward: 5′-TGGGGATAAGTTTTTTGTAATTTAGGA-3′; PIV reverse: 5′-CTACTAATAACCTCTCCCTCCCCACCAA-3′; [33]). PCR products were purified using the NucleoSpin extract II (Macherey-Nagel, Düren, Germany), cloned into pGEM-T Easy vector (Promega, Madison, WI, USA), and individual clones were sequenced at the Leiden Genome Technology Center (Leiden, The Netherlands).

2.3. Chromatin immunoprecipitation (ChIP)

Crosslinking was performed by adding formaldehyde (Sigma–Aldrich) to the cells at a final concentration of 1% for 10 min at room temperature. The crosslinking was quenched by the addition of 0.125 M glycine for 5 min. After washing with PBS, cells were resuspended at a concentration of 4×10^6 cells/ml in cell lysis buffer (5 mM PIPES [pH 8.0], 0.5% NP-40, 85 mM KCl) containing protease inhibitors (protease inhibitor cocktail (PIC, Sigma–Aldrich) and kept on ice for 10 min. After centrifugation at 3000 rpm for 5 min at 4 °C, the cell pellets were resuspended at a concentration of 5×10^6 cells/ml in nuclear lysis buffer (50 mM Tris [pH 8.1], 10 mM EDTA, 1% SDS) containing protease inhibitors and kept on ice for 10 min. Chromatin was then stored in 1 ml aliquots at −80 °C until use.

Chromatin samples were sonicated into fragments with an average length of 0.5–3 kb using a Branson 250 sonifier (Boom, Meppel, The Netherlands) and diluted 10 times using dilution buffer containing 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris–HCl [pH 7.9], 167 mM NaCl, and protease inhibitors. Sonicated chromatin was pre-cleared using pre-blocked protein A agarose beads (Upstate Biotechnology, Lake Placid, NY). Five microgram of antibody: Anti-Ac-H3 (#06-599, Upstate Biotechnology, Lake Placid, NY, USA); Anti-Ac-H4 (#06-866, Upstate); Anti-3Me-K4-H3 (Ab 8586, Abcam, Cambridge, UK); Anti-3Me-K9-H3 (#07-442, Upstate); Anti-3Me-K27-H3 (#07-449, Upstate); Anti-3Me-K20-H4 (ab9053, Abcam); Anti-RNA pol II (#sc-899x, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and Anti-EZH2 (#07-689, Upstate) or no antibody as background control, was added to 2 ml pre-cleared sonicated chromatin (equivalent to 1×10^6 cells) and allowed to bind overnight at 4 °C. 140 μl of a 25% slurry of pre-blocked protein A agarose beads were added and allowed to bind for 2 h at 4 °C. Beads were washed using low salt, high salt, LiCl, TE buffers, and chromatin complexes were released from the beads in elution buffer (0.1 M $NaHCO_3$, 1% SDS). Immune-precipitated chromatin complexes were de-cross-linked for 4 h at 65 °C using 0.2 M NaCl, and treated with 20 μg/ml RNAseI for 15 min at 37 °C. Proteins were removed by proteinase K (Invitrogen, Carlsbad, CA, USA) digestion at 45 °C for 1 h, followed by phenol/chloroform extraction. Finally, after precipitation, chromatin was resuspended in 30 μl distilled H_2O. One-tenth of the

immune precipitated chromatin was quantified by real-time PCR using an IQCycler and SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) using the primer sets: PI forward: 5′-AAAAGCCAATATC-CATCCGTTC-3′; PI reverse: 5′-GCATCCAAAACATGAAGTGAAAAC-3′; PII forward: 5′-GGGGGCCCCAGACAATATC-3′; PII reverse: 5′-GCCCATGTGCCAGTTCAAC-3′; PIII forward: 5′-AGAAACAGAAATCT-GACCGCTTG-3′; PIII reverse: 5′-TCATCACTAACCAGTCACCAGTTG-3′ [34]; and PIV forward: 5′-TCCTGGCCCGGGGCCTGG-3′; PIV reverse: 5′-CTGTTCCCCGGGCTCCCGC-3′; GAPDH forward: 5′-TACTAGCGGTTTTACGGGCG-3′; GAPDH reverse: 5′-TCGAACAG-GAGGAGCAGAGAGCGA-3′. The data presented are derived from two independent ChIP analyses with real-time PCR performed in duplicate for each ChIP.

2.4. RNA isolation and real-time PCR

After treatment of Jurkat, Molt-4 and HSB-2 cells with zebularine, DZNep and MS-275 [30–32], total RNA was isolated using the RNA-Bee extraction method (Tel-Test, Friendswood, TX, USA). cDNA was synthesized from 1 μg of each RNA sample using Superscript III (Invitrogen,) with random primers (Promega). CIITA and RNA pol II RNAs were quantified on an ICycler IQ system (Bio-Rad Laboratories) using the IQ SYBR Green Supermix and the following primers: panCIITA forward: 5′-CCGACACAGACACCAT-CAAC-3′; panCIITA reverse: 5′-CTTTTCTGCCCAACTTCTGC-3′; RNA pol II forward: 5′-CAGGAGTGGATCCTGGAGAC- 3′; RNA pol II reverse: 5′-GGAGCCATCAAAGGAGATGA-3′; Relative transcript levels of CIITA RNAs after exposure to zebularine, DZNep and MS-275 treatment were calculated with the comparative Ct method and related to RNA pol II transcript levels.

3. Results

3.1. Epigenetic DNA and histone modifications in CIITA promoter chromatin in normal T-cells

To investigate the association of epigenetic DNA and histone modifications with expression of CIITA in normal T-cells, we performed bisulfite sequencing and chromatin immunoprecipitation (ChIP) on unstimulated human CD4+ T-cells and on human CD4+ T-cells following activation. First, genomic DNA isolated from unstimulated and from activated T cells was treated with bisulfite and subsequently amplified with primer sets PIII.1 (for evaluation of the methylation status of CpG 1–3 in CIITA-PIII) and with PIII.2 (for evaluation of the methylation status of CpG 4–12 in CIITA-PIII). Following cloning into the pGEM-T Easy vector the sequence analysis of at least ten individual clones showed that CpG residues in CIITA-PIII, the principal T cell employed CIITA promoter, were unmethylated, both in unstimulated and in activated T-cells expressing CIITA and MHC-II molecules (Fig. 1). The adjacent CIITA-PIV promoter was also found to be devoid of methylation of CpG residues (Fig. 2).

Next the ChIP analyses revealed high levels of acetylated histones H3 and H4, and of 3Me-K4-H3 in CIITA-PIII chromatin in activated T-cells, while these histone marks associated with gene transcription were low in unstimulated T-cells (Fig. 3, upper row).

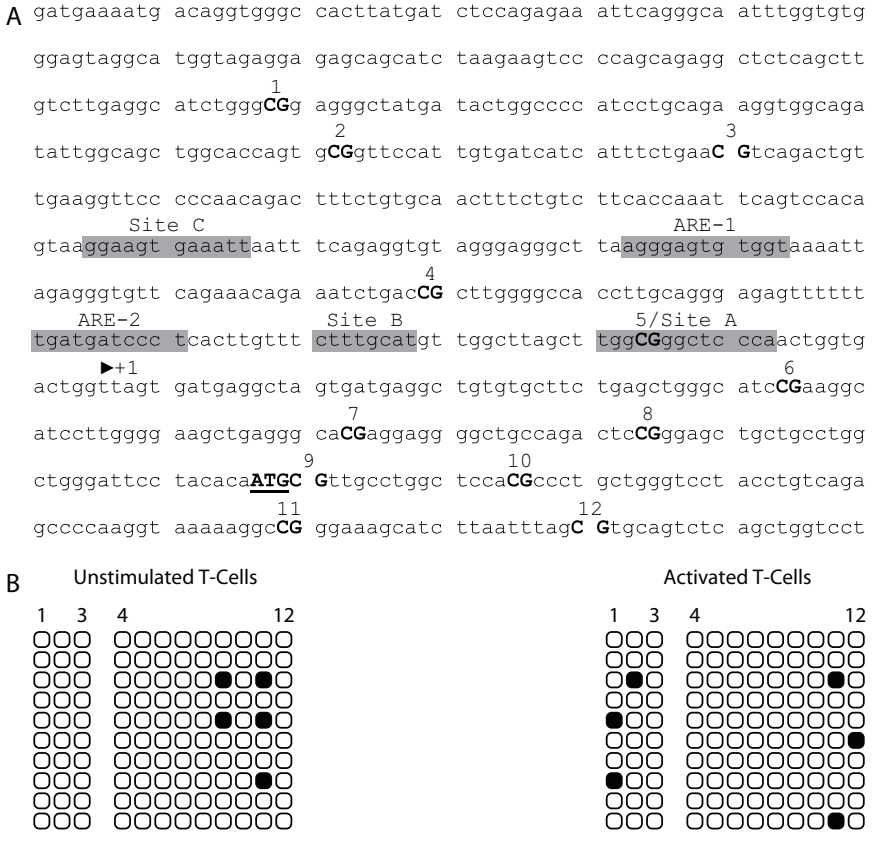

Fig. 1. CpG dinucleotide methylation pattern in unstimulated and in activated CD4+ T-cells. (A) Schematic overview of the CIITA-PIII region. CpG dinucleotides are indicated in bold (in capital letters) and numbered. Shaded regions indicate known transcription factor binding sites and critical regulatory elements [13,43]. (B) Bisulfite sequencing of the template strand of genomic DNA from unstimulated and in vitro activated CD4+ T-cells. Numbers refer to the CpG nucleotide numbers in (A). At least 10 individual CIITA-PIII sequences obtained after PCR with primer sets PIII.1 (CpG 1–3) or with PIII.2 (CpG 4–12) of bisulfite treated DNA and subsequent cloning into the pGEM-T Easy vector were examined in each case. Each sequence amplicon is represented by a horizontal row of boxes. Methylated CpGs are indicated by black boxes and unmethylated CpGs are indicated by white boxes.

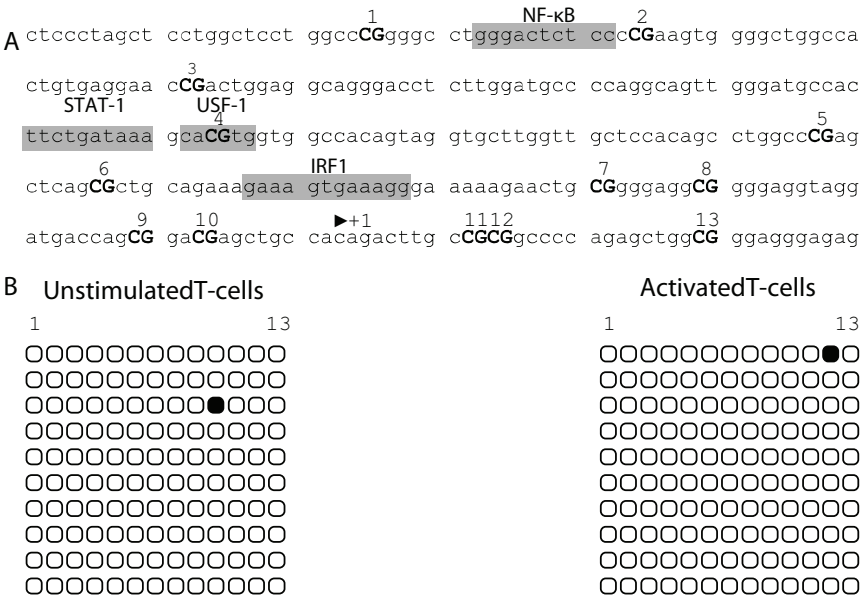

Fig. 2. CpG dinucleotide methylation pattern in unstimulated and in activated CD4+ T-cells. (A) Schematic overview of the CIITA-PIV region. CpG dinucleotides are indicated in bold (in capital letters) and numbered. Shaded regions indicate known transcription factor binding sites and critical regulatory elements [10]. (B) Bisulfite sequencing of the template strand of genomic DNA from unstimulated and in vitro activated CD4+ T-cells. Numbers refer to the CpG nucleotide numbers in (A). At least 10 individual CIITA-PIV sequences obtained after PCR with primer set PIV (CpG 1–13) of bisulfite treated DNA and subsequent cloning into the pGEM-T Easy vector were examined in each case. Each sequence amplicon is represented by a horizontal row of boxes. Methylated CpGs are indicated by black boxes and unmethylated CpGs are indicated by white boxes.

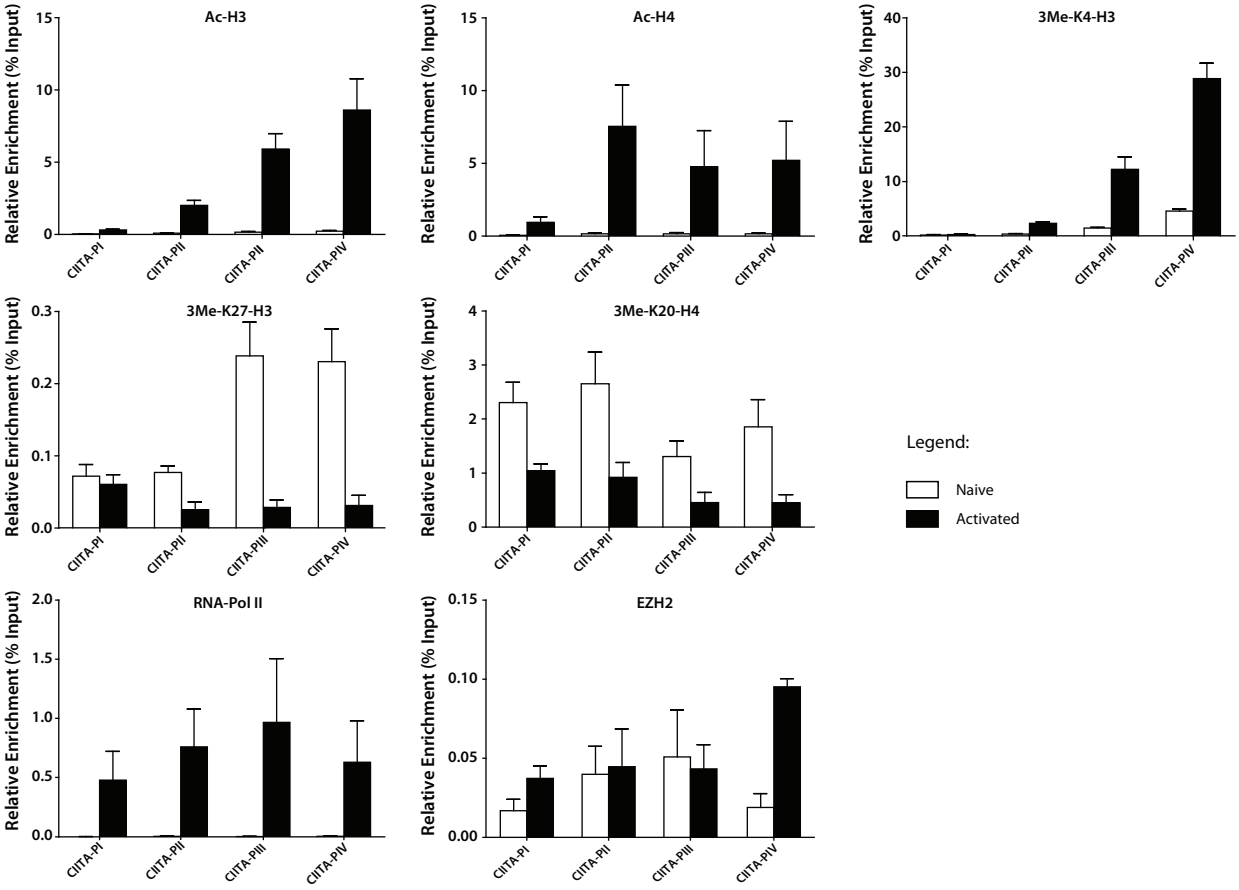

Fig. 3. Determination of histone H3 and H4 acetylation (Ac-H3 and Ac-H4) and methylation (3Me-K4-H3, 3Me-K27-H3 and 3Me-K20-H4) modifications associated with the various CIITA promoters in unstimulated (open bars) and activated (black bars) T-cells by ChIP. Upper row: levels of Ac-H3, Ac-H4 and 3Me-K4-H3. Middle row: levels of 3Me-K27-H3 and 3Me-K20-H4. Lower row: RNA polymerase II and EZH2 recruitment to the various CIITA promoters. Protein binding is indicated as relative enrichment (%) of input. Error bars represent SEM.

These histone marks were also abundantly present in chromatin of the other CIITA promoters in activated T-cells (Fig. 3, upper row). As expected, a reverse pattern was found for the repressive 3Me-K27-H3 and 3Me-K20-H4 marks in CIITA-PIII chromatin (Fig. 3, middle row). In unstimulated T-cells, these histone modifications were abundantly present in CIITA-PIII chromatin and also in chromatin of the other CIITA promoters, while reduced levels of these marks were noted in activated T-cells (Fig. 3, middle row). The 3Me-K9-H3 modification was barely detectable both in unstimulated and stimulated T-cells (results not shown). In activated T-cells RNA polymerase II was recruited to the various promoters in the *MHC2TA* multipromoter region with the highest levels of recruitment to CIITA-PIII and CIITA-PIV (Fig. 3, lower row, left).

Together, these observations show that in the absence of DNA methylation, the 3Me-K27-H3 and 3Me-K20-H4 repressive histone modifications are associated with the transcriptional silent state of *MHC2TA* in unstimulated T-cells, while in activated T-cells these repressive histone modifications are replaced by the activating histone 3Me-K4-H3 and acetylation modifications of histones H3 and H4. When we evaluated the presence of lysine methyltransferase EZH2 (hKMT6), we found low levels of EZH2 in the chromatin of the various CIITA promoters both in unstimulated and in activated T-cells (Fig. 3, lower row, right).

3.2. Defined histone acetylation and methylation modifications correlate with lack of transcriptional activity of MHC2TA in MHC-II-deficient T-leukemia cells

Previously we have shown that lack of CIITA expression in T-leukemia cells and in primary tumors correlated with hypermethylation of CIITA-PIII and CIITA-PIV [15,16] (unpublished observations). This hypermethylation of CpG residues in CIITA-PIII promoter DNA was confirmed by bisulfite sequencing (results not shown). To evaluate histone acetylation and methylation modifications involving the various CIITA promoters we have performed ChIP analyses in MHC-II-deficient T-leukemia cells and in MHC-II expressing T-lymphoma cells. Shown in Fig. 4 (upper row) is that the acetylation levels of histone H3 are significantly reduced in CIITA-PIII chromatin in the CIITA and MHC-II-deficient T-leukemia cells Jurkat, Molt-4 and HSB-2 in comparison with CIITA-PIII chromatin of the CIITA and MHC-II-molecule expressing T-lymphoma cells HH, HUT-78 and Karpas-299. Moreover, the other *MHC2TA* promoters (CIITA-PI, -PII and PIV) in CIITA T-leukemia cells also displayed significant lower levels of histone H3 acetylation in comparison to these promoters in the CIITA expressing T-lymphoma cell lines (Fig. 4, upper row). Notably, when the individual CIITA promoters in MHC-II expressing T-lymphoma cells were compared, the histone H3 acetylation levels were found enhanced in CIITA-PIV chromatin (Fig. 4, upper row).

We also investigated the presence of the repressive 3Me-K27-H3 and 3Me-K20-H4 histone methylation modifications. The 3Me-K27-H3 modification was abundantly present in CIITA-PIII chromatin in T-leukemia cells, when compared with T-lymphoma cells (Fig. 4, lower row). Furthermore, relative high levels of the 3Me-K27-H3 mark were also noted in chromatin of in particular CIITA-PIV in the leukemic T-cells (Fig. 4, lower row). CIITA-PIII in leukemic T-cells also displayed increased levels of 3Me-K20-H4. With respect to CIITA-PIV this mark displayed less distinctive features, whereas in CIITA-PII and CIITA-PI chromatin no differences were observed between CIITA expressing and non-expressing malignant T-cells (Fig. 4, lower row). The 3Me-K9-H3 modification was found negligible in chromatin of the various CIITA promoters both in T-leukemia and T-lymphoma cells (results not shown).

Subsequently, we investigated whether the high levels of 3Me-K27-H3 correlated with enhanced levels of EZH2 (hKMT6) in CIITA-PIII chromatin of the MHC-II deficient T-leukemia cells. The results of these analyses revealed that the T-leukemia cells displayed a significant increase in EZH2 levels in CIITA-PIII chromatin when compared with the CIITA expressing T-lymphoma cells (Fig. 4, upper row right). In line with enhanced levels of 3Me-K27-H3, enhanced levels of EZH2 were also noted in chromatin of the other CIITA promoters (Fig. 4). Together, in concordance with the

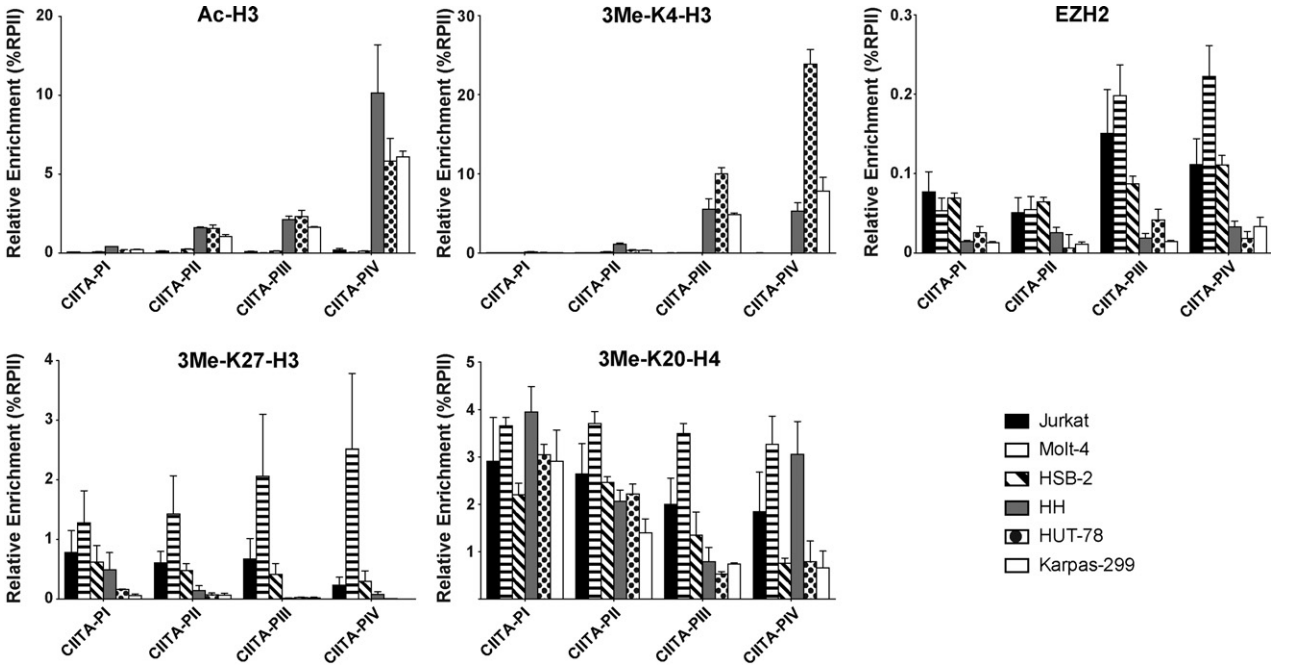

Fig. 4. Relative levels of histone H3 acetylation (Ac-H3) and methylation (3Me-K27-H3), and of histone H4 methylation (3Me-K20-H4) modifications associated with the various CIITA promoters in malignant T-cells. ChIP analysis was performed on genomic DNA of the CIITA-deficient T-leukemia cell lines Jurkat, Molt-4 and HSB-2, and of the CIITA expressing T-lymphoma cell lines HH, HUT-78 and Karpas-299 as indicated in the figure. Upper row: levels of Ac-H3, 3Me-K4-H3 and EZH2. Bottom row: levels of 3Me-K27-H3 and 3Me-K20-H4. Protein binding is indicated as relative enrichment (%) of input. Error bars represent SEM.

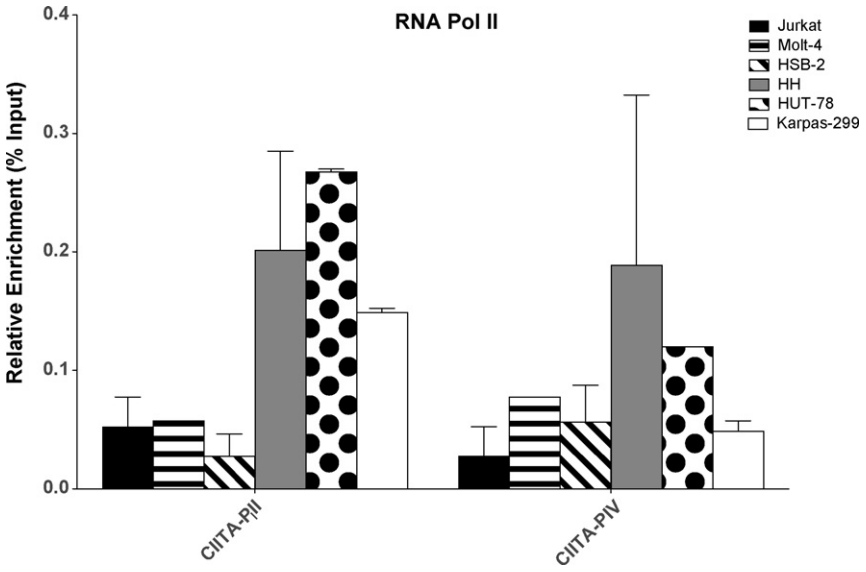

Fig. 5. Association of RNA polymerase II to CIITA-PIII (left panel) and CIITA-PIV (right panel) in T-leukemia and T-lymphoma cell lines indicated. Protein binding is indicated as relative enrichment (%) of input. Error bars represent SEM.

observed 3Me-K27-H3 modification, EZH2 was found to be associated with CIITA-PIII and also with CIITA-PI, -PII and -PIV chromatin in T-leukemia cell lines lacking expression of CIITA and MHC-II molecules.

The levels of RNA polymerase II recruitment to CIITA-PIII chromatin were severely reduced in T-leukemia cells in comparison with RNA polymerase II recruitment in T-lymphoma cells (Fig. 5). A similar observation was made for CIITA-PIV. Therefore we conclude from these analyses that the repressive chromatin environment in the T-leukemia cell lines investigated affects recruitment of RNA polymerase II to the CIITA promoters PIII and PIV investigated, which is in line with the observed lack of CIITA and MHC-II expression in these T cell malignancies [15].

3.3. Re-expression of CIITA in T leukemic lines

Next, we aimed to induce transcription of *MHC2TA* in Jurkat, Molt4 and HSB-2 T-leukemia cells through pharmacologic inhibition of the activities of the enzymes that modify histones and DNA. For the purpose of these investigations cells were treated with a combination of the DNMT inhibitor zebularine (100 μM, 96 h), the KMT inhibitor DZNep (2 μM, 72 h) and the KDAC inhibitor MS-275 (0.5 μM, 48 h) [30–32]. Shown in Fig. 6 is that exposure of the T leukemic cells to these small molecule inhibitors resulted in induction of CIITA expression.

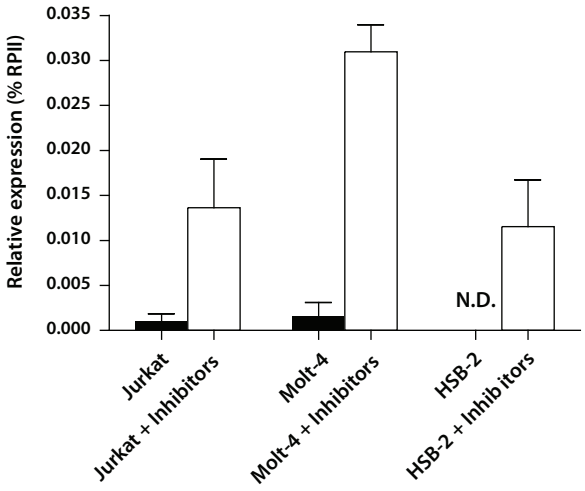

Fig. 6. Relative levels of CIITA re-expression in Jurkat, Molt4 and HSB T leukemic cells. Cells were exposed to zebularine (100 μM, 96 h), DZNep (2 μM, 72 h) and MS-275 (0.5 μM, 48 h). Re-expression of CIITA was monitored by real-time PCR using pan-CIITA primers and related to the expression levels of RNA pol II. N.D.: not detectable.

Together, these data show that both DNA methylation, and histone acetylation and methylation modification mechanisms contribute to the transcriptional control of CIITA in T leukemic cell lines.

4. Discussion

Previously we have shown that lack of MHC-II molecule expression in leukemic T-cells correlated with lack of *MHC2TA* transcription, which was found associated with hypermethylation of CIITA-PIII and of CIITA-PIV [15,16] (unpublished observations). In this study we show that lack of MHC-II molecule expression in T-leukemia cells is also associated with distinct epigenetic histone modifications. The lack of CIITA transcripts in the leukemic T cell lines investigated correlated with relative high levels of the repressive histone marks 3Me-K27-H3 and 3Me-K20-H4 in CIITA-PIII chromatin (Fig. 7). Moreover, we also found high levels of the lysine methyltransferase EZH2 (KMT6) which principally catalyses 3Me-K27-H3 [27]. The presence of the 3Me-K27-H3 was associated with impaired recruitment of RNA polymerase II into CIITA-PIII chromatin. Of interest is the observation that these epigenetic histone modifications and EZH2 were also present in chromatin of the other *MHC2TA* promoters (CIITA-PI, -PII and PIV) (Fig. 7). It reveals distribution of the found repressive chromatin mark over the entire *MHC2TA* multipromoter region, which spans about 14 kb on chromosome 16p13. The involvement of the entire multi-promoter region in silencing of *MHC2TA* transcription has previously also been observed in dendritic cell maturation and during B cell differentiation [34,35].

The observation that in T-leukemia DNA hypermethylation of the CIITA promoters investigated (CIITA-PIII and–PIV; [15,16] and unpublished observations) was associated with high levels of the 3Me-K27-H3 histone modification and the KMTase EZH2, is in line with the suggestion that EZH2 directly controls DNA methylation [36]. However, it should be noted that in several studies it was

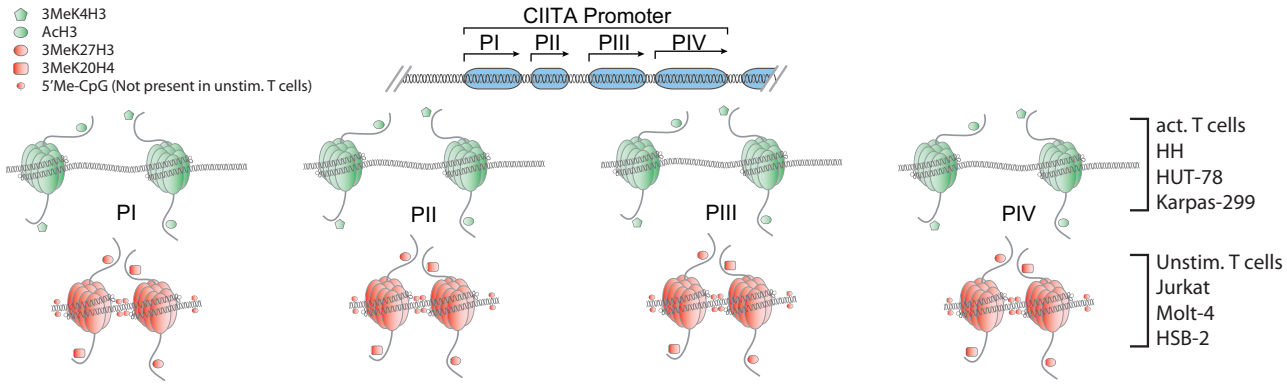

Fig. 7. Schematic overview of the epigenetic DNA and histone modifications encompassing the various CIITA promoters, which regulate transcription of *MHC2TA* in normal and malignant T cells. Expression of CIITA and resulting MHC-II molecules on the cell surface is central to MHC-II-mediated antigen recognition and intracellular cell signaling [16].

shown that depletion of EZH2 did not affect the levels of DNA methylation [37,38]. It has been suggested that the 3Me-K27-H3 histone modification premarks genes to become methylated at CpG residues in cancer cells [39]. In line with this notion is our current observation that both in unstimulated and in activated CD4+ human T-cells the CIITA promoters CIITA-PIII and CIITA-PIV are almost completely devoid of DNA methylation. In contrast to activated CD4+ T cells, which lack the 3Me-K27-H3 mark, the high levels of 3Me-K27-H3 found in CIITA-PIII chromatin and in chromatin of the other CIITA promoters in unstimulated CD4+ T cells could represent a premarking of the CIITA multipromoter region to become hypermethylated in leukemic T-cells. This is clearly the case for the investigated CIITA-PIII and CIITA-PIV promoters, which were found methylated in CIITA-deficient leukemic T cells, while these promoters were not methylated in CIITA-expressing T lymphoma cells [15,16] (unpublished observations).

The finding that RNA polymerase II in T-lymphoma cells is recruited to CIITA-PIII and -PIV chromatin is in line with the observation that both CIITA-PIII and -PIV isotypes can be detected in T-lymphoma cells as we have previously described [15]. Although we have not observed expression of the CIITA-PIV isotype in normal activated T-cells [7], the association of RNA polymerase II with CIITA-PIV could be the result of read-through from CIITA-PIII. Alternatively, this association and association with the other promoters could reflect stalling of RNA polymerase II at these promoters. Because the antibody used was raised against the N-terminus of RNA polymerase II we cannot exclude the possibility that the occupancy noted in the CIITA-PI and CIITA-PII regions is due to detection of the RNA polymerase II initiation complex only.

Of interest is the observation that EZH2, albeit at low levels, remains associated with chromatin of transcriptional active *MHC2TA* in the absence of the 3Me-K27-H3 mark. This could reveal an alternative role for EZH2 in transcriptional control of *MHC2TA* in T-cells. The unconventional associations of EZH2 and also of other members of the Polycomb Group proteins have previously been made for c-Myc and cyclin D1 in breast cancer cells [40], and for cytokine genes during Th1/Th2 differentiation [41]. In breast cancer cells it was revealed that EZH2 activates transcription of the c-Myc and cyclin D1 genes and that this activation activity is independent of the conserved SET domain, which plays a critical role in gene repression [40]. During Th1/Th2 differentiation various members of the Polycomb Group family of proteins, including EZH2, bound to actively transcribed IFN-γ and IL-4 genes in differentiating Th1 and Th2 cells [41]. Based on the unconventional association of Polycomb Group proteins with actively transcribed c-Myc, cyclin D1 and cytokine genes [40,41] and also with *MHC2TA* (this study), it could therefore be argued that in addition to suppressing gene transcription, EZH2 might also act as a facilitator of gene transcription and as such contributes to the processes involved in T lymphocyte differentiation and T cell activation. As previously stated [41] this might be achieved possibly through long–range interactions with distal regulatory elements. This notion is in line with the recent observation that BRG1 regulates transcription of *MHC2TA* through many interdependent remote enhancers [42].

In conclusion, we have shown that histone acetylation and methylation modifications are associated with control of *MHC2TA* gene transcription in normal CD4+ T-cells and in malignant T-cells (Fig. 7). Moreover, besides in chromatin of CIITA-PIII, the principal T cell employed promoter, the found histone marks were also noted in chromatin of the other CIITA promoters showing distribution of these marks throughout the 14-kb CIITA multipromoter region. Notably, while the investigated CIITA-PIII and CIITA-PIV promoters in normal unstimulated CD4+ T-cells lacked DNA methylation at CpG residues in the presence of the repressive 3Me-K27-H3 and 3Me-K20-H4 modifications, DNA in the CIITA-deficient leukemic T-cells was found methylated at CpG residues. This supports the notion that the 3Me-K27-H3 modification premarks genes for *de novo* methylation in cancer [39].

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References

[1] Van den Elsen PJ, Holling TM, Kuipers HF, Van der Stoep N. Transcriptional regulation of antigen presentation. Curr Opin Immunol 2004;16:67–75.
[2] Gobin SJ, Peijnenburg A, Keijsers V, Van den Elsen PJ. Site alpha is crucial for two routes of IFN gamma-induced MHC class I transactivation: the ISRE-mediated route and a novel pathway involving CIITA. Immunity 1997;6:601–11.
[3] Martin BK, Chin KC, Olsen JC, Skinner CA, Dey A, Ozato K, et al. Induction of MHC class I expression by the MHC-II transactivator CIITA. Immunity 1997;6:591–600.
[4] Reith W, Mach B. The bare lymphocyte syndrome and the regulation of MHC expression. Annu Rev Immunol 2001;19:331–73.
[5] Gobin SJ, Biesta P, Van den Elsen PJ. Regulation of human beta 2-microglobulin transactivation in hematopoietic cells. Blood 2003;101:3058–64.
[6] Krawczyk M, Reith W. Regulation of MHC-II expression, a unique regulatory system identified by the study of a primary immunodeficiency disease. Tissue Antigens 2006;67:183–97.

[7] Holling TM, Van der Stoep N, Quinten E, Van den Elsen PJ. Activated human T-cells accomplish MHC-II expression through T cell-specific occupation of class II transactivator promoter III. J Immunol 2002;168:763–70.
[8] Wong AW, Ghosh N, McKinnon KP, Reed W, Piskurich JF, Wright KL, et al. Regulation and specificity of MHC2TA promoter usage in human primary T lymphocytes and cell line. J Immunol 2002;169:3112–9.
[9] Muhlethaler-Mottet A, Otten LA, Steimle V, Mach B. Expression of MHC-II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA. EMBO J 1997;16:2851–60.
[10] Muhlethaler-Mottet A, Di Berardino W, Otten LA, Mach B. Activation of the MHC-II transactivator CIITA by interferon-gamma requires cooperative interaction between Stat1 and USF-1. Immunity 1998;8:157–66.
[11] Piskurich JF, Wang Y, Linhoff MW, White LC, Ting JP. Identification of distinct regions of 5′ flanking DNA that mediate constitutive, IFN-gamma, STAT1, and TGF-beta-regulated expression of the class II transactivator gene. J Immunol 1998;160:233–40.
[12] Piskurich JF, Linhoff MW, Wang Y, Ting JP. Two distinct gamma interferon-inducible promoters of the major histocompatibility complex class II transactivator gene are differentially regulated by STAT1, interferon regulatory factor 1, and transforming growth factor beta. Mol Cell Biol 1999;19:431–40.
[13] Van der Stoep N, Quinten E, Van den Elsen PJ. Transcriptional regulation of the MHC-II trans-activator (CIITA) promoter III: identification of a novel regulatory region in the 5′-untranslated region and an important role for cAMP-responsive element binding protein 1 and activating transcription factor-1 in CIITA-promoter III transcriptional activation in B lymphocytes. J Immunol 2002;169:5061–71.
[14] Van der Stoep N, Quinten E, Alblas G, Plancke A, Van Eggermond MCJA, Holling TM, et al. Constitutive and IFNgamma-induced activation of MHC2TA promoter type III in human melanoma cell lines is governed by separate regulatory elements within the PIII upstream regulatory region. Mol Immunol 2007;44:2036–46.
[15] Holling TM, Schooten E, Langerak AW, Van den Elsen PJ. Regulation of MHC-II expression in human T-cell malignancies. Blood 2004;103:1438–44.
[16] Holling TM, Van der Stoep N, Van den Elsen PJ. Epigenetic control of CIITA expression in leukemic T cells. Biochem Pharmacol 2004;68:1209–13.
[17] Morris AC, Spangler WE, Boss JM. Methylation of class II trans-activator promoter IV: a novel mechanism of MHC-II gene control. J Immunol 2000;164:4143–9.
[18] Van den Elsen PJ, Van der Stoep N, Viëtor HE, Wilson L, Van Zutphen M, Gobin SJ. Lack of CIITA expression is central to the absence of antigen presentation functions of trophoblast T-cells and is caused by methylation of the IFN-gamma inducible promoter (PIV) of CIITA. Hum Immunol 2000;61:850–62.
[19] Van der Stoep N, Biesta P, Quinten E, Van den Elsen PJ. Lack of IFN-gamma-mediated induction of the class II transactivator (CIITA) through promoter methylation is predominantly found in developmental tumor cell lines. Int J Cancer 2002;97:501–7.
[20] Croce M, De Ambrosis A, Corrias MV, Pistoia V, Occhino M, Meazza R, et al. Different levels of control prevent interferon-gamma-inducible HLA-class II expression in human neuroblastoma cells. Oncogene 2003;22:7848–57.
[21] Satoh A, Toyota M, Ikeda H, Morimoto Y, Akino K, Mita H, et al. Epigenetic inactivation of class II transactivator (CIITA) is associated with the absence of interferon-gamma-induced HLA-DR expression in colorectal and gastric cancer cells. Oncogene 2004;23:8876–86.
[22] Shi B, Vinyals A, Alia P, Broceño C, Chen F, Adrover M, et al. Differential expression of MHC-II molecules in highly metastatic breast cancer cells is mediated by the regulation of the CIITA transcription Implication of CIITA in tumor and metastasis development. Int J Biochem Cell Biol 2006;38:544–62.
[23] Vaissière T, Sawan C, Herceg Z. Epigenetic interplay between histone modifications and DNA methylation in gene silencing. Mutat Res 2008;659:40–8.
[24] Lachner M, Jenuwein T. The many faces of histone lysine methylation. Curr Opin Cell Biol 2002;14:286–98.
[25] Rice JC, Briggs SD, Ueberheide B, Barber CM, Shabanowitz J, Hunt DF, et al. Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. Mol Cell 2003;12:1591–8.
[26] Berger SL. The complex language of chromatin regulation during transcription. Nature 2007;447:407–12.
[27] Cao R, Zhang Y. The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. Curr Opin Genet Dev 2004;14:155–64.
[28] Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G, et al. A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. Genes Dev 2004;18:1251–62.
[29] Martin C, Zhang Y. The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol 2005;6:838–49.
[30] Marquez VE, Barchi Jr JJ, Kelley JA, Rao KV, Agbaria R, Ben-Kasus T, et al. Zebularine: a unique molecule for an epigenetically based strategy in cancer chemotherapy. The magic of its chemistry and biology. Nucleosides Nucleotides Nucleic Acids 2005;24:305–18.
[31] Miranda TB, Cortez CC, Yoo CB, Liang G, Abe M, Kelly TK, et al. DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. Mol Cancer Ther 2009;8:1579–88.
[32] Saito A, Yamashita T, Mariko Y, Nosaka Y, Tsuchiya K, Ando T. A synthetic inhibitor of histone deacetylase, MS-27-275, with marked in vivo antitumor activity against human tumors. Proc Natl Acad Sci USA 1999;96:4592–7.
[33] Holling TM, Bergevoet MW, Wilson L, Van Eggermond MCJA, Schooten E, Steenbergen RD, et al. A role for EZH2 in silencing of IFN-gamma inducible MHC2TA transcription in uveal melanoma. J Immunol 2007;179:5317–25.
[34] Landmann S, Mühlethaler-Mottet A, Bernasconi L, Suter T, Waldburger JM, Masternak K, et al. Maturation of dendritic cells is accompanied by rapid transcriptional silencing of class II transactivator (CIITA) expression. J Exp Med 2001;194:37991.
[35] Green MR, Yoon H, Boss JM. Epigenetic regulation during B cell differentiation controls CIITA promoter accessibility. J Immunol 2006;177:3865–73.
[36] Viré E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, et al. The Polycomb group protein EZH2 directly controls DNA methylation. Nature 2006;439:871–4.
[37] McGarvey KM, Greene E, Fahrner JA, Jenuwein T, Baylin SB. DNA methylation and complete transcriptional silencing of cancer genes persist after depletion of EZH2. Cancer Res 2007;67:5097–102.
[38] Kondo Y, Shen L, Cheng AS, Ahmed S, Boumber Y, Charo C, et al. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nat Genet 2008;40:741–50.
[39] Schlesinger Y, Straussman R, Keshet I, Farkash S, Hecht M, Zimmerman J, et al. Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. Nat Genet 2007;39:232–6.
[40] Shi B, Liang J, Yang X, Wang Y, Zhao Y, Wu H, et al. Integration of estrogen and Wnt signaling circuits by the polycomb group protein EZH2 in breast cancer cells. Mol Cell Biol 2007;27:5105–19.
[41] Jacob E, Hod-Dvorai R, Schif-Zuck S, Avni O. Unconventional association of the polycomb group proteins with cytokine genes in differentiated T helper cells. J Biol Chem 2008;283:13471–8.
[42] Ni Z, Abou El Hassan M, Xu Z, Yu T, Bremner R. The chromatin-remodeling enzyme BRG1 coordinates CIITA induction through many interdependent distal enhancers. Nat Immunol 2008;9:785–93.
[43] Ghosh N, Piskurich JF, Wright G, Hassani K, Ting JP, Wright KL. A novel element and a TEF-2-like element activate the major histocompatibility complex class II transactivator in B-lymphocytes. J Biol Chem 1999;274:32342–50.