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Histone Deacetylation, But Not Hypermethylation, Modifies Class II Transactivator and MHC Class II Gene Expression in Squamous Cell Carcinomas

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In this study, we first categorized nine squamous cell carcinoma (SCC) cell lines into two groups in terms of the expression of HLA-DR, -DP, and -DQ molecules. Subsequently, the expression of class II transactivator (CIITA) was studied in these cell lines, because it is widely accepted that the expression of MHC class II molecules is regulated by different types of CIITA transcripts that are initiated by distinct promoters. The majority of the SCC cell lines (six of nine) expressed HLA-DR molecules and CIITA promoter IV (pIV) transcripts in the presence of IFN-γ. In contrast, three of the nine SCC cell lines were completely negative for class II molecules and all types of CIITA, suggesting epigenetic changes in the promoter region in these cells. Previously, methylation of CIITA pIV was reported to silence CIITA gene expression. We extensively studied the methylation status of CIITA pIV using a panel of 22 SCC cell lines. Remarkably, none of the SCC cell lines demonstrated hypermethylation at the site. In contrast, treatment with a histone deacetylation inhibitor in combination with IFN-γ clearly restored the expression of the CIITA type IV gene in the HLA-DR-negative SCC cell lines, and the acetylation status of histone H3 examined by chromatin immunoprecipitation analysis was closely associated with the gene expression. Moreover, stable transfection of the CIITA gene into an HLA-DR-negative cell line restored constitutive expression of MHC class II molecules. Therefore, histone deacetylation, but not hypermethylation, modifies CIITA DNA and class II gene expression in SCC. The Journal of Immunology, 2003, 170: 4980–4985.

Clinical trials for cancer immunotherapy have recently progressed and have achieved objective responses in melanomas and some malignancies (1–3). CD8+ T cells play a major role in target killing, and MHC class I-restricted tumor Ags are critically important. However, recent reports indicate that tumor-specific reactive CD4+ T cells are also important to enhance the efficiency of tumor immunotherapy (4–6). CD4+ T cells help in the proliferation and maintenance of tumor-specific killer T cells and also directly lyse target cells (7, 8). However, a very few MHC class II-restricted tumor Ags have been reported (9, 10). This is probably because MHC class II and class II-restricted Ag presentation may be much more complex than that of MHC class I (11). Most solid tumor cell lines hardly express MHC class II Ags on their surfaces, because some tumor cells may lose expression of MHC class I and/or class II molecules for several reasons (12). Loss of MHC molecules is one of the major mechanisms for tumor cells to escape from immune surveillance. Squamous cells are located near the surface of human body and may constitute the first-line defense against many external Ags. We have found that cell lines derived from squamous cell carcinomas (SCC) express MHC class II molecules; however, the expression decreases in vitro (13), and their mechanisms are not clarified yet. MHC class II expression is constitutive in professional APCs such as dendritic cells and B lymphocytes. The general mechanisms of MHC class II expression have been reported (14). Three MHC class II molecules, HLA-DR, -DP, and -DQ, are all regulated by cis-acting promoter elements, W, X1, X2, Y boxes, and their interactions with transcriptional factors such as members of the regulatory factor X family, NF-Y, and CREB have been reported (15–17). In addition to these binding factors, MHC class II transactivator (CIITA) is also required for efficient promotion of MHC class II genes (18, 19). CIITA is a strong transactivator that does not bind to DNA directly, but to these promoter-binding factors, and stabilizes their interactions (20–23). Cells without CIITA gene expression cannot induce MHC class II molecules sufficiently. Four types of CIITA transcripts with different first exons initiated by four distinct promoters have been reported (24). Each promoter activity is regulated in vivo according to the cell type. CIITA type I expression is observed in dendritic cells; the function of type II is not clear at this time, and type III expression is mainly shown by B cells. Both type I and type III transcripts are observed constitutively in each type of cell, but the type IV, and to some lesser extent, type III expression is induced by IFN-γ stimulation. The CIITA promoter IV sequence includes IFN-γ activation site, E Box, and IFN regulatory factor-1 (IRF-1) sites that interact with transcriptional factors located in the IFN-γ signal pathway such as STAT1 and IRF-1 (25).

We have found that SCC cell lines have unstable expression of class II molecules (13). In this work, we studied the expression of class II molecules and CIITA in SCC cell lines by FACS and
RT-PCR. Because epigenetic alterations were suggested to silence the CIITA gene, hypermethylation and the histone deacetylation status were extensively studied by combined bisulfite restriction analysis (COBRA) and chromatin immunoprecipitation (ChIP) analysis. Remarkably, histone deacetylation, but not hypermethylation, modified CIITA gene expression in SCC.

Materials and Methods

Cell lines and culture

SCC cell lines, KE4, TE11 (kindly provided by K. Ito, Kurume University Kurume, Japan), KKK- TT, KKK-KO, HC-MA (kindly provided by J. Yoda, Wakayama Medical University, Wakayama, Japan), KUMA-1 (kindly provided by M. Eura, Kumamoto University, Kumamoto, Japan) OSC20, OSC40, and STK-1 (established in our laboratory), and other cell lines, KK-EBV (EBV-transfected B lymphocytes from a healthy donor established in our laboratory) and Jar (choriocarcinoma cell line; American Type Culture Collection (ATCC), Manassas, VA) were all cultured at 37°C in the presence of 5% CO2 in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) with 10% heat-inactivated FBS (Filtron, Brooklyn, New South Wales, Australia). To enhance the expression of MHC class II Ags, IFN-γ, kindly provided by Shionogi Pharmaceutical (Osaka, Japan), was added at 100 U/ml for 48 h. The 5-Aza-2' deoxycytidine (Sigma-Aldrich) was added at 1 μM for 72 h in methylation-relevant experiments. For acetylation-related experiments, trichostatin A (TSA; Sigma-Aldrich) was added at 300 nM for 24 h.

DNA construction and stable transfection

A pIRE5-CIITA stable transfectant named KE4/CIITA was established by transfecting KE-4 cells with the pIREs-CIITA using the Lipofect AMINE 2000 method, according to the manufacturer’s instructions (Life Technologies, Rockville, MD). Mock cDNA, which had only the pIREsPuro plasmid, was used for the transfection of a negative control, named KE4/ pIREs. First, 0.5 μg/ml puromycin was added to the culture medium for the selection, and cells were screened for MHC class II expression by flow cytometry. pIRE5-CIITA contained an insert with the CIITA sequence, which was created by double digestion with PmeI and EcoRI of HACIITA/pcDNA3.1+, and was cloned into EcoRV and EcoRI sites of the original pIREsPuro vector (Clontech Laboratories, Palo Alto, CA). HACIITA/ pcDNA3.1+ was kindly provided by J. M. Boss (Emory University School of Medicine, Atlanta, GA).

Flow cytometry

Cells were incubated with the following first Abs: anti-HLA-DR mAb L243 (ATCC), anti-HLA-DP mAb anti-HLA-DP-pure (BD Biosciences, Mountain View, CA), anti-HLA-DQ mAb-purified anti-human HLA-DQ (BD Biosciences), and anti-HLA-ABC mAb W6/32 (ATCC). Cells were washed and incubated with affinity-purified Abs fluorescein-labeled goat anti-mouse IgG + IgM (Kirkegaard & Perry Laboratories, Gaithersburg, MD) as a secondary Ab (60 min, 4°C) and analyzed in a FACScan flow cytometer (BD Biosciences). At least 30,000 viable cells were analyzed per condition. The data were analyzed using CellQuest software (BD Biosciences).

Reverse transcription and RT-PCR

Total RNA was prepared from KE4, KKK-TT, KKK-KO, TE11, OSC20, OSC40, STK-1, HC-MA, KUMA-1, KK-EBV, Jar, and human DCs treated with or without IFN-γ, 5-Aza-2’-deoxycytidine, and TSA using ISOGEN (Nippon Gene, Tokyo, Japan). Reverse transcription was performed with 1 μg of total RNA, 1 μg of oligo(dt) (Life Technologies Invitrogen, Carlsbad, CA), and 1 μl of Super Script II (Life Technologies), according to the manufacturer’s instructions. First, 0.5 μl of reverse-transcriptase reaction product was used as a template for PCR with 1.25 U of ExTaq HS polymerase (TaKaRa Biotechnology, Otsu, Japan). Each PCR product was electrophoresed in 2% agarose gel and stained with ethidium bromide. The primers for CIITA type I (expected length, 368 bp) were forward 5’-act tccagcgactgctg-3’ and reverse 5’-tgaagcagagagctggc-3’; for CIITA type III (248 bp) they were 5’-ttcctacacaatgcgttgcc-3’ and 5’-ttgctgaact gtcgagctggagg-3’; for CIITA type IV (166 bp) 5’-agacctggcgccgac gag-3’ and 5’-ttgacagcagagctggc-3’; for HLADR (87 bp) 5’-gc caacgtgtaaatcagca-3’ and 5’-aggctgctgagccac-3’; and for GAPDH (203 bp) 5’-cgac ccagtgaatcacaga-3’ and 5’-aggggtagtcagggg-3’.

COBRA

The methods were essentially as described (26). Briefly, 2 μg of genomic DNA was denatured by 0.2 M NaOH for 10 min at 37°C. Thirty microliters of 10 mM hydroquinone (Sigma-Aldrich) and 520 μl of 3 M Na-bisulfite (Sigma-Aldrich) were added at pH 5.0, and the mixture was incubated at 50°C for 16 h. Treated DNA was purified with a Wizard Plus DNA Purification System (Promega, Madison, WI) and ethanol precipitation. Bisulfite PCR was performed using 2 μl of the aliquot as a template. The primers for CIITA promoter IV methylation analysis were 5’-gaggtg ggagatttttatttgaag-3’ and 5’-tcttccctcercacaact-3’. These primers were designed for the presence of 10 positions of CG contents in the PCR product, and both methylated and unmethylated DNA were expected to be amplified yielding 195 bp. The PCR products were then digested with BsrUI, which digests the CGCG sequence that indicates the methylated positions. The primer set was run on 2.5% NuSieve GT agarose (BioWhittaker Molecular Applications, Rockland, ME), providing 196 bp (if methylated) in addition to 195 bp (if not methylated). HSC-2, HSC-3, HSC-4, Ca9-22, OSC-19, OSC-30, OSC-70, SAS, KOSK-3, Ho-1-u-1, Ho-1-N-1, SCC-4, HOC119, HOC621, MoN2, MoT, and OM-1 cells were described elsewhere (27).

ChIP assay

The methods were essentially as described (28). Briefly, DNA was cross-linked with chromatin by incubation of 1 × 106 cells for 10 min in formaldehyde at 37°C. The cells were scraped and washed with ice-cold PBS containing protease inhibitors (1 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml pepstatin A). Pelleted cells were resuspended within 200 μl of SDS lysis buffer (Upstate Biotechnology, Lake Placid, NY) and broken to 200- to 1000-bp DNA fragments by sonication. Samples were immunoprecipitated for 16 h at 4°C using anti-acetylated histone H3 (Upstate Biotechnology) as a probe, and chromatin solution for a no-Ab control (DNA input) was saved. The immune complexes were collected with protein A agarose. Uncross-linked DNA with chromatin was recovered by phenol/chloroform extraction and ethanol precipitation. Samples containing 1/100 of the immunoprecipitated DNA were used for PCR. DNA input was used for the control to be quantitative. The intensity of PCR products was measured by densitometry using a Lane and Spot Analyzer 6.0 (Atto, Tokyo, Japan).

Results

MHC class II expression status in SCC cell lines

IFN-γ stimulation enhances MHC class II expression in some SCC cell lines. We first analyzed the cell surface expression of HLA-DR, -DP, and -DQ molecules in nine SCC cell lines by flow cytometry with or without IFN-γ stimulation. Examples of the positive (STK-1) and negative (KKK-TT) expression of HLA-DR are shown in Fig. 1A. No SCC cell lines had MHC class II expression on the cell surface without IFN-γ (Fig. 1B). However, IFN-γ induced HLA-DR expression in six of the nine cell lines (67%) and HLA-DP expression in two of the nine cell lines. Both HLADP-positive cell lines STK-1 and HC-MA also had HLA-DR expression. In contrast, three of the nine cell lines (33%), KE-4, KKK-TT, and KKK-KO, had no HLA-DR and no HLA-DP expression even with IFN-γ. Expression of HLA-DQ was not observed in any cell line (Fig. 1B). We examined expression of MHC class I molecules, and all nine cell lines showed almost the same level of MHC class I without IFN-γ (data not shown).

CIITA distinguished MHC class II expression status

We divided the nine SCC cell lines in two major groups based on the expression of HLA-DR with IFN-γ, DR positive and DR negative. To examine the mechanism by which these two groups were distinguished, we analyzed mRNA expression of CIITA, master coactivator of class II molecules, by RT-PCR in all nine SCC cell lines that were cultured either with or without IFN-γ. CIITA has three unique transcriptional subtypes, I, III, and IV, that have different 5’ ends of each mRNA initiated by three distinct promoters. We designed specific primers to distinguish each CIITA subtype. Without IFN-γ, no expression of CIITA could be detected in any SCC cell line (Fig. 2). However, the cell lines that were HLA-DR
positive in flow cytometry showed CIITA mRNA expression, especially in type IV, and to some lesser extent, in type III. No significant expression of CIITA mRNA type I was observed in the presence of IFN-γ/H9253. In contrast, there was no expression or very weak expression (KKK-KO cells) of CIITA mRNA type IV in the DR-negative group even with IFN-γ/H9253 (Fig. 2). To confirm whether CIITA was essential for the MHC class II expression on the cell surface, we transfected the CIITA gene to KE4, one of the HLA-DR-negative cell lines, and established a stable transfectant, KE4/CIITA. We analyzed the cell surface expression of HLA-DR, -DP, and -DQ molecules by flow cytometry. KE4/CIITA clearly showed expression of all three MHC class II molecules compared with the mock transfectant KE4/pIRES (Fig. 3).

CIITA promoter IV DNA methylation was not found in SCC cell lines

DNA hypermethylation is widely accepted as one of the chief mechanisms for silencing gene expression. The promoter region sequence of CIITA type IV includes a typical CpG island, and DNA methylation at this site was identified in developmental tumor cell lines (29), in early stage B cell lines (30), and in fetal trophoblasts (31–33). We found two groups of SCC cell lines according to HLA-DR cell surface expression, and the expression of the CIITA type IV gene was thought to possibly correlate with HLA-DR expression in these two groups. Thus, we further examined whether DNA methylation at the promoter site of CIITA type IV could explain the difference of MHC class II expression status in the SCC cell lines. First, we treated several SCC cell lines with IFN-γ in combination with 5-Aza-2'-deoxycytidine; however, no strong induction of class II expression was observed, suggesting that apparently CIITA type IV was not heavily methylated in the SCC cell lines (data not shown). Next, we set up bisulfite PCR analysis at the promoter site and we analyzed its methylation status by digesting the products with BstUI. Hypermethylated DNA was expected to show smaller size digested bands (169 bp), as shown in Jar cells; however, only 195-bp band, but not 169 bp, was found in any of the nine SCC cell lines, suggesting no evidence for DNA hypermethylation at the promoter region of CIITA type IV (Fig. 4A). We further analyzed the methylation status at this site by direct sequencing of bisulfited products of STK-1 cells or STK-1 cells treated with 5-Aza-2'-deoxycytidine. Jar cells clearly showed CGCG sequence at BstUI site; however, both STK-1 cells and STK-1 treated with 5-Aza-2'-deoxycytidine showed only TGTG sequence at this site, confirming no hypermethylation (data not shown). To certify that DNA at this site was not hypermethylated in SCC, the samples derived from another 17 SCC cell lines were also analyzed by the same COBRA method, even though the expression of class II with or without IFN-γ was not determined. No sample was found to have hypermethylation at this site, as for the previously described nine SCC cell lines (Fig. 4B).

Histone deacetylation is a regulatory mechanism for CIITA type IV mRNA gene expression

To explore the mechanism silencing MHC class II cell surface expression, especially that of HLA-DR molecules, we further examined the histone acetylation status. Histone acetylation at the
HLA-DRA promoter region has been reported (34), and we hypothesized that a similar mechanism was essential for CIITA type IV gene expression. We first analyzed mRNA expression of CIITA types I, III, and IV treated with only IFN-γ/H9253 or with IFN-γ/H9253 in combination with a histone deacetylase inhibitor, TSA. TSA alone did not induce any HLA-DR expression both in DR-positive and DR-negative cell lines (data not shown). SCC cell lines belonging to the HLA-DR-negative group could not express CIITA type IV with only IFN-γ/H9253. Interestingly, IFN-γ/H9253 in combination with TSA restored their mRNA expression in all three cell lines (KE4, KKK-TT, and KKK-KO) (Fig. 5). These HLA-DR-negative cell lines could express CIITA type IV only under the conditions with IFN-γ/H9253 and TSA in our experiments. The combination of IFN-γ/H9253 and TSA treatment also restored the expression of CIITA type III, but not that of CIITA type I in DR-positive cell lines (Fig. 5). Thus, CIITA types III and IV were suggested to be epigenetically modified in SCC cell lines. Because we were particularly interested in the mechanisms of HLA-DR-negative cell lines, we focused on CIITA type IV for additional experiments.

Next, we examined the status of histone acetylation in the CIITA type IV promoter region by acetyl-histone H3 immunoprecipitation (ChIP) assay. We used KKK-TT and STK-1 from the DR-negative and DR-positive group, respectively. The ChIP assay was done with IFN-γ and TSA (+/+), with only IFN-γ (−/+), and with no treatment (−/−). After sonication, DNA was fragmented into 200- to 500-bp segments (data not shown). PCR was performed using DNA precipitated with anti-acetylated histone H3. STK-1, an HLA-DR-inducible cell line, showed acetylated histone at the CIITA type IV promoter site without IFN-γ treatment, and this acetylation status was clearly enhanced by IFN-γ treatment (Fig. 6A). In contrast, KKK-TT, an HLA-DR-negative cell line, showed little histone acetylation in mock and IFN-γ-treated cells. Remarkably, TSA treatment in combination with IFN-γ greatly restored histone acetylation of CIITA promoter type IV in KKK-TT (Fig. 6A). The amounts of PCR products after compensation by DNA input were quantified by densitometry, indicating a comparable degree of histone acetylation status at the CIITA

**FIGURE 4.** A, Analysis of CIITA promoter IV methylation in SCC cell lines. A 195-bp fragment around CpG islands in CIITA promoter IV was amplified by bisulfite PCR and digested by BstUI. Jar is a positive control for methylation in this site, and shorter band in Jar control cells represents methylated alleles (169 bp). All five cell lines, including both HLA-DR-positive and HLA-DR-negative cell lines, had no methylation at this site. B, Methylation analysis was done in another 17 SCC cell lines. No methylation was detected in any of the 17 SCC cell lines.

**FIGURE 5.** Modification of CIITA mRNA expression by a histone deacetylase inhibitor. SCC cell lines were treated with IFN-γ or in combination with 300 nM TSA, a histone deacetylase inhibitor. CIITA type I expression was not influenced by addition of TSA. However, CIITA type III expression was enhanced in DR-positive cells, and type IV expression in HLA-DR-negative cell lines.

**FIGURE 6.** A, Acetylation status of histone H3 in SCC cell lines examined by ChIP. We compared the CIITA type IV acetylation status around the CIITA promoter region (−308 to −125) in KKK-TT for HLA-DR-negative and STK-1 for HLA-DR-positive cells. Abbreviations mean treatments with −/−, none; +/+, 100 U/ml IFN-γ; and +/−, 100 U/ml IFN-γ and 300 nM TSA. B, Quantified acetylation status of CIITA type IV. Signal intensities of each ChIP-PCR band in A were quantified using densitometry after compensation by DNA input. Bar represents the CIITA type IV/DNA input ratio. C, Expression of HLA-DRA by RT-PCR. We compared the expression of HLA-DRA in KKK-TT for HLA-DR-negative cell. Abbreviations are the same as in A. EBV-transformed B cells were used for positive control (no treatment).
type IV promoter site (Fig. 6B). Under the same experimental condition, mRNA level of HLA-DRA gene in KKK-TT was analyzed by RT-PCR. No treatment or treatment of IFN-γ alone did not show significant HLA-DR expression; however, treatment of TSA in combination with IFN-γ restored the HLA-DRA expression (Fig. 6C). This is comparable to the ChIP experiment. Taken together, histone acetylation of CIITA gene may influence both CIITA and HLA-DR expression.

Discussion

MHC class II molecules are expressed constitutively in APCs such as dendritic cells, B, or activated CD4+ T lymphocytes and macrophages, but their expression is not constant in most nonhemopoietic cells. In some human epithelial cell lines, their MHC class II expression is enhanced by IFN-γ stimulation, but not in all. We tried to induce tumor-specific CD4+ T lymphocytes restricted by MHC class II molecules; however, some SCC cell lines tended to lose expression of class II molecules (13). The loss of MHC molecules is one of the mechanisms by which cancer cells escape from host immunity (12). Recent reports suggest that epigenetic changes may silence MHC class I genes in esophageal SCC (35), but such changes are still not precisely clarified, especially for MHC class II. We first examined MHC class II expression patterns in nine SCC cell lines by FACS analysis. Six of the nine SCC cell lines (67%) had up-regulation of HLA-DR molecules by IFN-γ. No MHC class II molecules were expressed without IFN-γ stimulation (Fig. 1B). B lymphocytes showed constitutive HLA-DQ expression in addition to that of HLA-DR and HLA-DP, but none of the SCC lines could express HLA-DQ. We classified SCC cell lines into two groups based on their HLA-DR expression patterns, HLA-DR-negative and HLA-DR-positive induction groups by IFN-γ.

Recent studies have revealed CIITA and its crucial roles for MHC class II expression. There are three subtypes of CIITA transcripts, namely, types I, III, and IV, in different cell tissues (the role of type II is unclear). We made specific primers for these three types and compared CIITA mRNA expression by RT-PCR with the results of FACS analysis for MHC class II cell surface expression. We confirmed in this experiment that the expression pattern of type IV correlated with HLA-DR (Fig. 2). This result suggests that CIITA type IV mRNA expression plays an important role in HLA-DR induction through IFN-γ stimulation in SCC. CIITA type IV transcripts are initiated by CIITA promoter IV, and IFN-γ-responsive elements such as GAS and IRF-1 binding site are located around this promoter region (25). We also confirmed that a CIITA stable transfectant of KE4, which had no expression of MHC class II molecules, changed to show constitutive expression of HLA-DR, -DP, and -DQ (Fig. 3).

We found two types of HLA-DR expression according to CIITA type IV in SCC cell lines. We next analyzed the key factor that distinguished these two groups. Morris et al. (31) reported that MHC class II down-regulation in trophoblast cells was caused by methylation of the CIITA promoter IV region, so methylation might be one of the important epigenetic changes around CIITA promoter IV. We selected representative cell lines from HLA-DR-negative and HLA-DR-positive cell lines, and examined their methylation status in terms of CIITA promoter IV. Methylation analysis was done using bisulfite PCR and methylation site-specific digestion by BsuRI. DNA samples were also directly sequenced after the bisulfite treatments (data not shown). All SCC samples, including the HLA-DR-negative group, revealed no methylation at this site (Fig. 4A). To generalize methylation status of CIITA type IV in SCC, we further examined another 17 SCC cell lines. Remarkably, no methylation was found in a total of 22 SCC cell lines (Fig. 4B). Thus, we concluded that, unlike previously reported, methylation of CIITA promoter IV did not contribute to MHC class II down-regulation in SCC.

Recent studies indicate that deacetylation of histone also plays a role in gene silencing through remodeling of the chromatin structure (36). In fact, Landmann et al. (37) reported that loss of MHC class II mRNA synthesis in dendritic cells was mediated by a mechanism implicating histone deacetylation. To determine the role of histone deacetylation in gene silencing of CIITA in SCC, we examined the effects of TSA, a histone deacetylase inhibitor, on the expression of CIITA types I, III, and IV. Although expression of CIITA types I and III was not induced by IFN-γ in HLA-DR-negative cell lines, expression of CIITA type IV was clearly induced by treatment with IFN-γ and TSA (Fig. 5). These data strongly suggest that the absence of HLA-DR in SCC is associated with the loss of CIITA due to histone deacetylation of the promoter region. To clarify the acetylation status of histone in cell lines with or without CIITA expression, we performed ChIP assay using an anti-acetylated histone H3 Ab in two SCC cell lines, KKK-TT (HLA-DR negative) and STK-1 (HLA-DR positive). KKK-TT showed little histone acetylation after IFN-γ treatment. However, the acetylation level was significantly enhanced by treatment with TSA, suggesting the role of histone deacetylation rather than loss of transcription factors in gene silencing (Fig. 6A). Indeed, HLA-DRA mRNA was induced under the same experimental condition assessed by RT-PCR (Fig. 6C), although histone acetylation status of HLA-DRA gene may be somehow affected in the experiment, because multiple histone acetyltransferase activities are associated with MHC class II expression (34). It would be interesting to know whether TSA in combination with IFN-γ can induce HLA-DR on the cell surface. However, TSA treatment was quite toxic to SCC cells, and no convincing FACS data were obtained. Recently, we set up the gene transfection experiment of only CIITA coding sequences downstream of promoter III or IV into DR-negative cells, however, under control of CMV promoter in these cases, with the results that expression of HLA-DR and other class II molecules was clearly restored without IFN-γ (Y. Takamura et al., unpublished results). This suggests that the silencing of relevant CIITA promoters may be responsible for the amount of CIITA molecules, and subsequently for the expression of class II molecules in these cells.

In conclusion, our data demonstrate that there are two types of SCC in terms of MHC class II expression. CIITA type IV may be responsible for the class II expression in an IFN-γ-dependent manner. CIITA type IV expression in SCC is dependent on the acetylation status of its promoter region, not on methylation. We conclude that histone deacetylation in CIITA promoter IV may contribute to silencing of MHC class II genes in SCC.

It is reasonable to hypothesize that the silencing mechanism of MHC class II may be responsible for tumor escape from CD4+ T cells in vivo. Our results may have an important implication for cancer immunotherapy. If the absence of MHC class II molecules is one of the major causes of tumor escape for CD4+ T cells, it may be feasible to induce tumor-specific CD4+ T cells by treating cancer cells with histone deacetylase inhibitors. Obviously, further study is necessary to clarify whether re-expression of CIITA induces tumor-specific CD4+ T cells through induction of MHC class II molecules.

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