Biochemical and Biophysical Research Communications 440 (2013) 190–195

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Selective modulation of MHC class II chaperons by a novel IFN-γ-inducible class II transactivator variant in lung adenocarcinoma A549 cells

Bau-Lin Chiu, Chia-Hsuan Li, Chien-Chung Chang *

Institute of Molecular and Cellular Biology, National Tsing Hua University, Hsinchu 30013, Taiwan

ARTICLE INFO

Article history:
Received 5 September 2013
Available online 20 September 2013

Keywords:
Human leukocyte antigen
Lung cancer
Class II transactivator
Alternative splicing

ABSTRACT

Class II transactivator (CIITA) plays a critical role in controlling major histocompatibility complex (MHC) class II gene expression. In this study, two novel alternatively spliced variants of human interferon (IFN)-γ-inducible CIITA, one missing exon 7 (CIITAΔE7), the other with TAG inserted at exon 4/5 junction (CIITA-TAG), were identified and characterized. Both variants are naturally occurring since they are present in primary cells. Unlike CIITA-TAG, CIITAΔE7 is expressed more abundantly in lung adenocarcinoma A549 cells than in the non-transformed counterpart BEAS-2B cells following IFN-γ stimulation. Transfection experiments showed that CIITAΔE7 induced a markedly lower level of surface HLA-DR, -DP, -DQ expression than CIITA-TAG in A549 cells but not in BEAS-2B cells, although both variants elicited similar amounts of total DR, DP, and DQ proteins. This differential effect was correlated with, in A549 cells, decreased expression of *Ii* and *HLA-DM* genes, along with increased expression of *HLA-DO* genes. Ii and HLA-DM are chaperons assisting in HLA class II assembly, while HLA-DO functions to inhibit endosomal peptide loading and HLA class II membrane transport. These findings raise the possibility that CIITAΔE7 interacts with unknown cancer-associated factors to selectively modulate genes involved in the assembly and transport of HLA class II molecules.

1. Introduction

Major histocompatibility complex (MHC) class II molecules present exogenous antigens to $CD4^+$ T cells and therefore are pivotal to eliciting antigen-specific adaptive immune responses. In humans, these class II molecules are human leukocyte antigen (HLA)-DR, -DP, and -DQ. Transcription of MHC class II genes are mainly controlled by class II transactivator, or CIITA, which is a 130-kDa, non-DNA-binding nuclear protein [1]. CIITA is recruited to MHC class II gene promoters through a ubiquitous enhanceosome complex interacting with highly conserved responsive elements, such as W/S, X1, X2, and Y-box [2–4]. These elements exist not only in the promoters of genes encoding the DR, DP, and DQ molecules, but also in those encoding Ii, HLA-DM, and HLA-DO, all of which are chaperon proteins involved in the assembly of class II molecules and their transport to the plasma membrane [1–3,5]. CIITA is expressed constitutively through promoter-I (P-I) or P-III in antigen presenting cells [6], and can be induced by IFN-γ through P-IV in almost all cell types [7]. These CIITA molecules differ slightly in their N-termini because of their distinct exon 1 sequence [1].

The mechanism by which CIITA functions has been investigated extensively over the years. A mature CIITA protein has four functional domains: an N-terminal transcriptional acidic activation domain (AAD), a proline/serine/threonine rich (P/S/T) domain, a GTP-binding domain (GBD), and a C-terminal leucine-rich region (LRR) [2–4,8]. AAD interacts with co-activators and effector proteins to promote transcription [9]. P/S/T domain contains a number of potential phosphorylation sites to regulate CIITA functions through post-translational modifications [10]. GBD and LRR are responsible for CIITA self-association and translocation to the nucleus [2–4]. Nevertheless, this wealth of information was derived mostly from the study of the constitutive form of CIITA with a focus on *DR* gene activation. How CIITA regulates the expression of class II chaperons has been poorly understood [5]. In addition, although alternative splicing of CIITA transcripts in conjunction with mutations have been reported in a Burkitt's lymphoma cell line [11], little is known as to whether natural CIITA variants exist and how they function.

The most prominent mutation that disrupts the function of CIITA and T cell responses is represented by group A bare lymphocyte syndrome (BLS), where a 72-bp deletion in *CIITA* gene causes the loss of a.a. 940–963 in the LRR domain of the protein, thus abolishing its nuclear translocation and HLA class II expression

* Corresponding author. Address: Institute of Molecular and Cellular Biology, National Tsing Hua University, No. 101, Section 2, Kuang-Fu Road, Hsinchu 30013, Taiwan. Fax: +886 3 571 5934.
E-mail address: ccchang@life.nthu.edu.tw (C.-C. Chang).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.09.066

[12]. In Burkitt's lymphoma cells Raji, four CIITA variants carrying coding region deletions and/or insertions were isolated, but only one of them displayed loss-of-function because of failure to reverse a HLA class II-negative phenotype [11]. This variant, CIITA-10, contains multiple alterations in different regions of the nucleotide sequence and thus far the critical change to which loss-of-function was attributed has not been determined [11]. More recently, it was found that in primary mediastinal B-cell lymphoma, fusion of the 5′ part of *CIITA* gene with *BX648577* (CIITA-BX648577) causes downregulation of surface HLA class II expression, while upregulates the expression of programmed cell death (PD)-L1 and PD-L2, ligands for PD-1 [13]. This mutation results in a decrease in tumor cell immunogenicity because of HLA class II loss, together with a further impairment of T cell responses because of enhanced PD-1 signaling.

Regardless of these examples, mutations in CIITA have been rare and no naturally occurring variations have been characterized in humans. In the present study, we identified two naturally existing CIITA variants and have tested whether they have lost or retained the canonical function in the context of a lung cancer cell model.

2. Materials and methods

2.1. Cell culture

Human non-transformed bronchial epithelial cell line BEAS-2B (ATCC, Manassas, VA, USA), lung adenocarcinoma cell line A549 (ATCC), B lymphoblastoid cell line LG-2, and monocytic cell line THP-1 (ATCC) were cultured in RPMI-1640 (Hyclon, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco-BRL, Grand Island, NY, USA), 100 U/ml penicillin and 100 U/ml streptomycin (Gibco-BRL). All cell lines were maintained at 37 °C with an atmosphere of 5% CO_2.

2.2. Antibodies and cytokines

HLA-DP, -DQ, -DR-specific monoclonal antibody (mAb) Q5/13 [14], -β chain-specific mAb LGII612.14 [15] and calnexin mAb TO-5 [16], was developed and characterized as described. Anti-Flag mAb was purchased from Sigma–Aldrich (St. Louis, MO, USA). The anti-idiotypic mAb MK2-23, IgG1 used as isotype-matched irrelevant controls [17], was developed and characterized as described. R-phycoerythrin (R-PE)-conjugated F (ab')$_2$ fragments of goat anti-mouse Fc antibodies, horseradish peroxidase (HRP)-conjugated goat anti-mouse Fc antibodies were purchased from Jackson Immunoresearch Laboratories (West Grove, PA, USA). IFN-γ was purchased from PeproTech (Rocky Hill, NJ, USA).

2.3. Plasmid constructs

IFN-γ-inducible full-length CIITA (CIITA-WT), exon 7-deleted CIITA (CIITAΔE7) and TAG-inserted CIITA (CIITA-TAG) cDNA fragments were amplified with gene-specific primers (Supplementary Table 1) from IFN-γ-treated BEAS-2B cells and cloned at *Eco*RV and *Eco*RI sites into pcDNA3.1 (-) vector with an N-terminal Flag sequence. Nucleotide sequencing was performed with 3730x1 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Fragments of *HLA-DRA* promoter (−176 to +76) and *HLA-DOA* promoter (−106 to +124 and −2270 to +124) were amplified from genomic DNA extracted from BEAS-2B and A549 cells and cloned into pGL3-basic vector at *Xho*I and *Hin*dIII sites. The primers used are listed in Supplementary Table 1. All primers used in this study were synthesized by Mission Biotech, Taipei, Taiwan.

2.4. Transfection

Transfection was performed by Lipofectamine 2000 transfection system (Invitrogen, Carlsbad, California, USA) following the manufacturer's instructions. Cells were harvested at indicated time points following transfection.

2.5. Flow cytometry

Cells were harvested, washed by PBS with 1% BSA, incubated with primary antibodies in PBS with 1% BSA at 4 °C for 30 min and then incubated with R-PE-conjugated goat anti-mouse IgG (1:100) at 4 °C for 30 min in the dark and following the same wash steps as previous described. Finally, cells were fixed by PBS with 0.5% paraformaldehyde at RT for 20 min before being analyzed by FASCCalibur (BD Biosciences, San Jose, CA, USA) with the Cellquest software (BD Biosciences).

2.6. Western blot analysis

ell lysates were denatured with sample buffer (Bio-Rad, Hercules, CA, USA) by boiling for 10 min and proteins (50–120 μg) were fractionated on 8% SDS–PAGE under reducing conditions. Subsequently, fractionated proteins were transferred onto PVDF membrane (Millipore, Billerica, MA, USA) and blocked with PBS with 5% BSA at room temperature for 30 min. After the membranes were incubated with primary antibodies at 4 °C for 12–16 h and several washes, they were ncubated with HRP-conjugated goat anti-mouse IgG (1:10000) at RT for 30 min and then the protein blots were detected by the HRP/ECL system (Amersham Biosciences, Piscataway, NJ, USA).

2.7. Primary immune cells from human peripheral blood and mouse spleen

Peripheral venous blood was obtained from healthy donors with informed consent and mononuclear cells were isolated by Ficoll-Paque PLUS (Amersham Biosciences) following the manufacturer's instructions. After an overnight incubation, suspended and adherent fractions of cells were used as lymphocytes and monocytes, respectively. A Balb/c mouse was sacrificed and its spleen was excised, followed by mincing and passing through a 10-μm mesh to isolate splenocytes.

2.8. Reverse transcription (RT)-PCR and Real-time quantitative-PCR (qPCR)

RNA was extracted from cells with TRIZol (Invitrogen) for first-strand cDNA synthesis by reverse transcription was performed with Super Script III reverse transcriptase (Invitrogen) according to the manufacturer's instructions. PCR were performed by Takara Thermo Cycler Dice (Takara Bio, Otsu, Shiga, Japan) with gene-specific primers (Supplementary Table 1). As for qPCR, for one reaction, 0.5 μl of cDNA with 5 μl of Power SYBR Green PCR Master Mix Reagent (Applied Biosystems,), 1 μl of primer pair mix, and 3.5 μl of distilled water were mixed. PCR was performed by ABI StepOnePlus Real-Time systems. Relative mRNA expression was calculated using the difference between cycle thresholds (Ct) values of the interest genes and normalized by housekeeping gene (GAPDH) using the formula ΔCt, then calculated the difference between the ΔCt values of an experimental sample and the control sample. The fold change in mRNA expression is using formula $2^{-\Delta\Delta}$Ct. Primer sequences are listed in Supplementary Table 1.

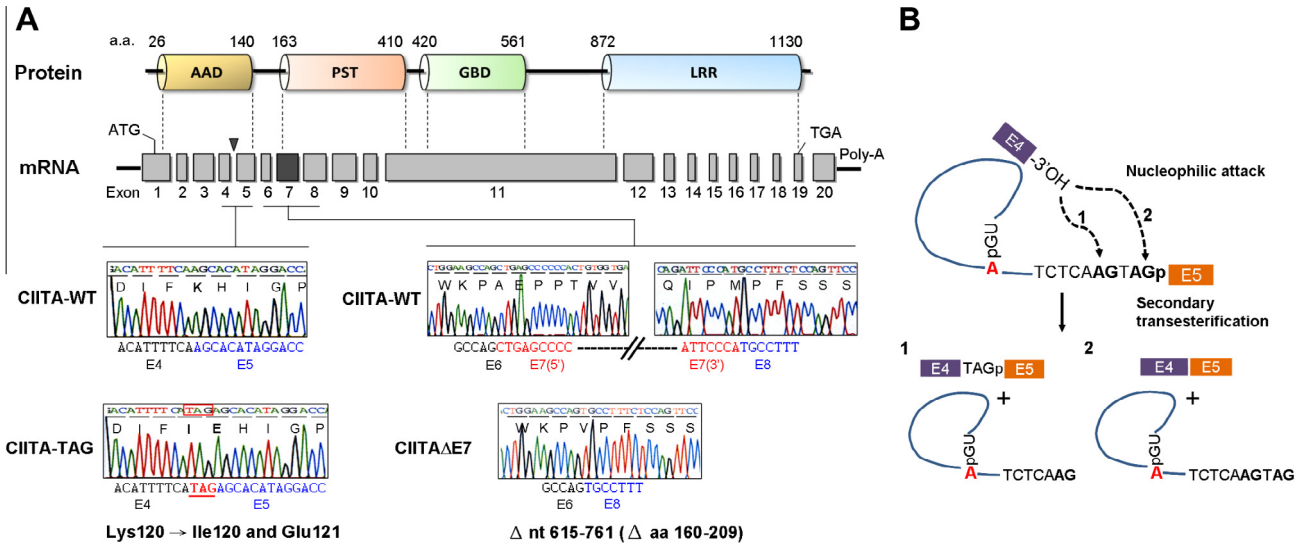

Fig. 1. Identification of two novel CIITA splice variants and their deduced a.a. sequence around the variation sites. (A) Full-length CIITA cDNA was cloned from BEAS-2B cells and sequenced. CIITA-WT, CIITA-TAG, and CIITAΔE7 denote wild-type CIITA, TAG-inserted CIITA, and exon 7-deleted CIITA, respectively. (B) The proposed mechanism of alternative splicing that leads to generation of the CIITA-TAG variant. Bold-type AG denotes the 3′ splice acceptor site, while bold-type A in red shows the critical nucleotide in branch-point sequence. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

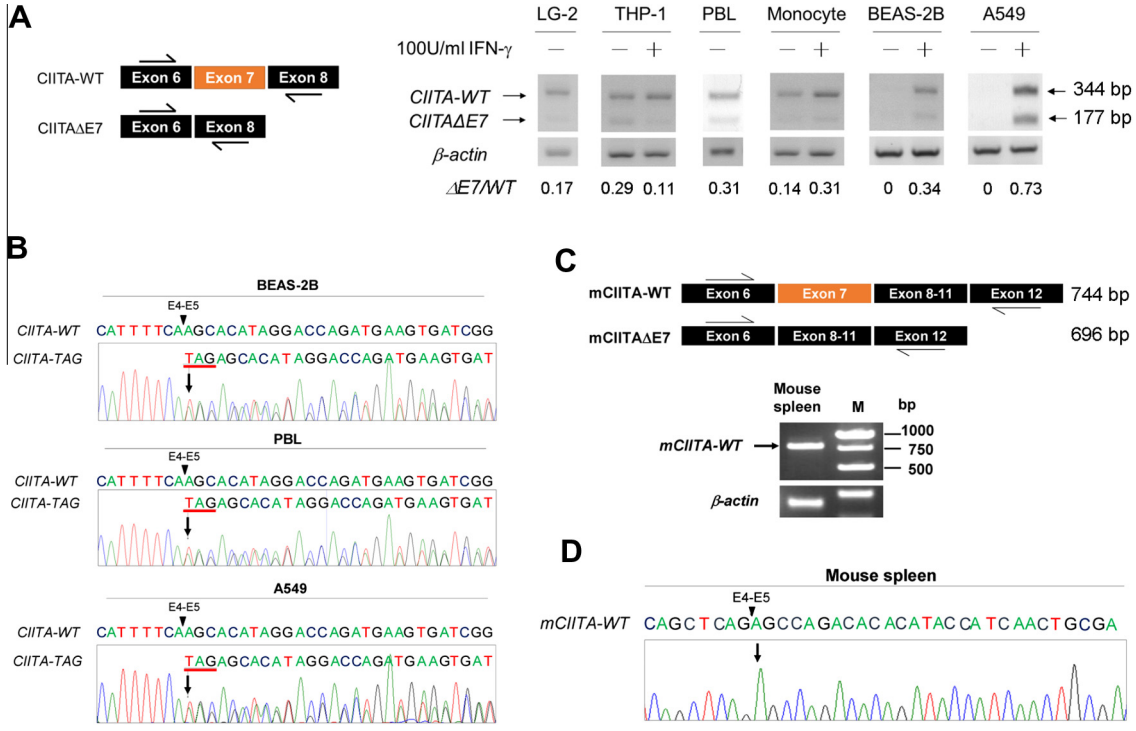

Fig. 2. CIITAΔE7 and CIITA-TAG are expressed in cultured cell lines and primary cells, but do not have their murine counterparts. (A) RT-PCR analysis of CIITAΔE7 and CIITA-WT transcript expression in BEAS-2B and A549 cells, as well as in various indicated immune cell lines and primary cells with primers shown in a diagram (A, left panel). Cells were treated with IFN-γ (100 U/ml) for 24 h. Relative CIITAΔE7/CIITA-WT abundance (ΔE7/WT) was determined by density measurement by Image J. PBL, peripheral blood lymphocytes. (B) Nucleotide sequence analysis of CIITA-TAG in BEAS-2B, A549 cells and PBLs. RT-PCR (C) and nucleotide sequence analysis (D) of the corresponding CIITA variations in mouse splenocytes.

2.9. Luciferase assay

Cells grown in 24-well plate were transfected with CIITA-encoding constructs together with promoter-luciferase reporter plasmid and Renilla luciferase pRL-CMV (Promega, Madison, WI, USA) and then incubated in complete growth medium for 12 or 24 h. Subsequently, cells were lysed and then luciferase activity was measured with a dual luciferase assay system (Promega) following the manufacturer's instructions. Data were recorded using Victor 3 Multi-labeled Microplate Reader.

2.10. Statistical analysis

Data are expressed as mean + S.D. Differences in means between control and experimental groups were evaluated with a

Student's t test. p value less than 0.05 ($p < 0.05$) was considered statistically significant.

3. Results and discussion

3.1. Identification of two novel IFN-γ-inducible CIITA splice variants

In the course of cloning of P-VI-driven CIITA cDNA from lung epithelial BEAS-2B cells, we isolated two clones that were not reported in the literature. One is devoid of the entire exon 7 (Δnt 615∼761, CIITA-ΔE7), and the other contains a TAG-trinucleotide insertion after nt 473 at the exon 4/5 boundary (CIITA-TAG) (Fig. 1A). The loss of exon 7 results in the loss of a.a. 160–209 in the N-terminal part of P/S/T domain of the CIITA protein (Fig. 1A). TAG insertion at the exon 4/5 boundary causes an in-frame insertion that replaces lysine (Lys120) with isoleucine (Ile120) and glutamic acid (Glu121) in the AAD domain (Fig. 1A). While the mechanism leading to exon 7 deletion remains unclear at present, the insertion of TAG can be explained by the existence of an additional AG at the 3′ splice site of intron 4 (TCTCAAGTAGp—Exon5), which provides an inaccuracy in the second step of mRNA splicing (Fig. 1B). Interestingly, although the two variations were jointly present along with an additional mutation in a single constitutive CIITA clone reported before [12], no information is available regarding the characterization of each individual variation.

To investigate a physiological relevance, we tested whether these two CIITA variants were expressed in the lung adenocarcinoma cells A549. Fig. 2A shows that the abundance of CIITA-ΔE7 relative to CIITA-WT was 34% and 73% in IFN-γ-treated BEAS-2B and A549 cells, respectively, implying cancer-associated upregulation of CIITA-ΔE7. These results were corroborated with the results of clonal analysis (data not shown). Furthermore, CIITA-ΔE7 can be readily detected in immortalized B cell line LG2, monocytic cell line THP-1, peripheral blood lymphocytes (PBLs) and monocytes with a relative abundance ranging from 11% to 31% (Fig. 2A). These results indicate that CIITA-ΔE7 is naturally occurring in various cell types. There is also marked CIITA-TAG expression in BEAS-2B cells, A549 cells and PBLs, but with a comparable relative abundance by sequencing analysis (Fig. 2B).

Intriguingly, the described two variations do not have a murine counterpart, since they were not detected in the mouse primary splenocytes (Fig. 2C and D). This finding suggests a phylogenetically non-conservative nature of these CIITA variations.

3.2. Impaired HLA-DR, -DP, -DQ surface expression by CIITAΔE7 in spite of robust DR, -DP, -DQ total protein induction in A549 cells

To test whether CIITAΔE7 and CIITA-TAG have altered functions, BEAS-2B and A549 cells were transfected with plasmids encoding these variants and analyzed for HLA-DR, -DP, -DQ surface expression. Fig. 3A shows that the level of surface expression is

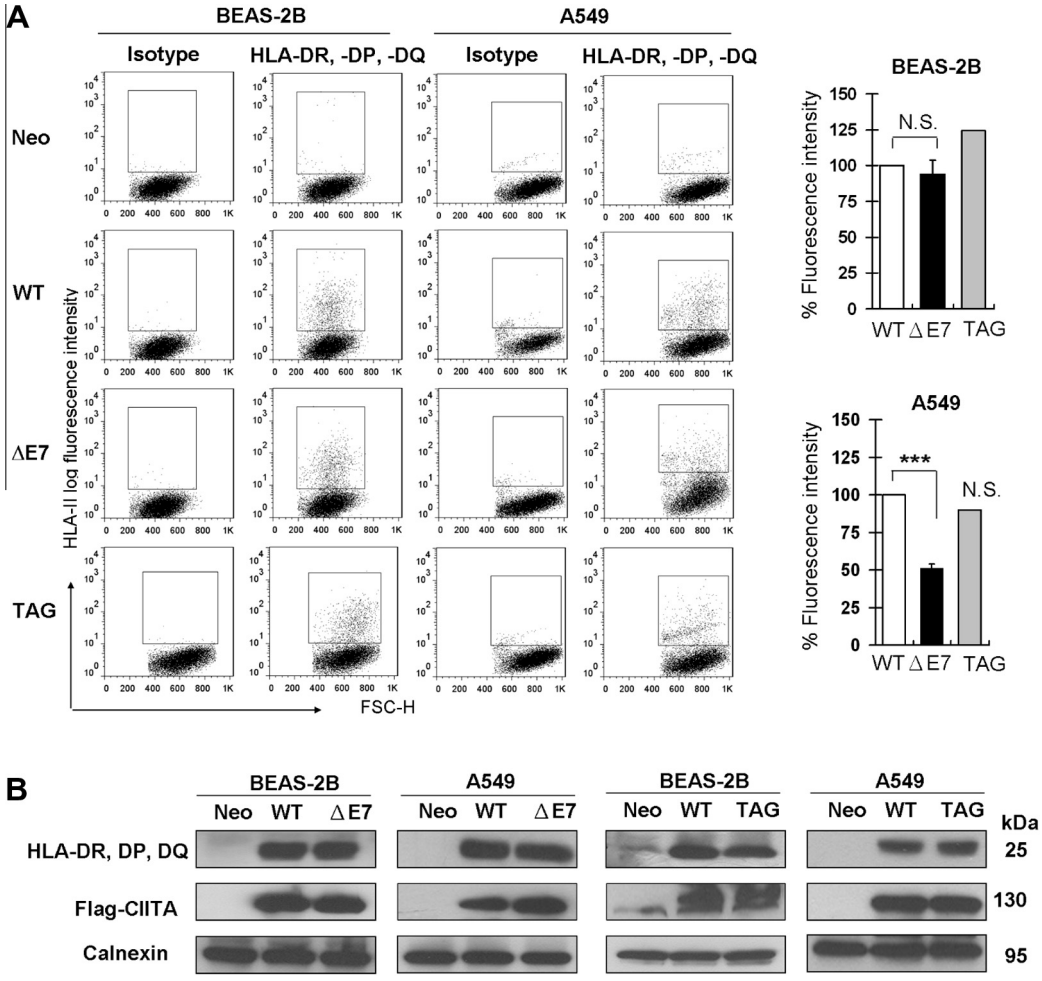

Fig. 3. Differential surface and total HLA-DR, -DP, -DQ protein expression by A549 cells transiently transfected with a CIITAΔE7-encoding plasmid. (A) Surface HLA-DR, -DP, -DQ expression was analyzed by flow cytometry with mAb Q5/13 and positively stained cells were gated and quantified relative to CIITA-WT (A, right panel). Mean ± S.D., $n = 3$, $^{***}p < 0.005$. N.S., non-significant. (B) Total HLA-DR, -DP, -DQ protein expression was analyzed by Western blotting with mAb LGII.612.14, which recognizes denatured DR, -DP, -DQ β chains. CIITA and calnexin expression served as a loading and an internal control, respectively.

~50% lower on A549 cells transfected with CIITAΔE7 compared to those transfected with CIITA-TAG and CIITA-WT. This phenomenon was not observed in BEAS-2B cells, since CIITAΔE7, CIITA-TAG and CIITA-WT induced comparable levels of HLA-DR, -DP, -DQ expression at their surface. However, Western blot analysis revealed that there was no difference in the ability of the two variants and CIITA-WT to induce total DR, -DP, -DQ protein expression in either BEAS-2B or A549 cells (Fig. 3B). These results suggest that the failure of CIITAΔE7 to induce marked HLA class II surface expression is not through lack of activation of *DR*, *-DP*, *-DQ* genes, and this occurs only in A549 cells but not in their normal counterpart. These findings lead to the hypothesis that CIITAΔE7 inhibits HLA class II transport likely through modulating class II chaperons.

3.3. Selective modulation of genes encoding class II chaperons Ii, HLA-DM, and HLA-DO by CIITAΔE7 in A549 cells

To determine whether CIITAΔE7 aberrantly modulate genes involved in HLA class II assembly and transport in A549 cells, we analyzed the steady-state levels of mRNA transcribed from *Ii*, *DMA*, *DOA*, and *DOB* genes, as well as from *DRA*, *DRB*, *DPA*, and *DQA* genes in these cells vs. BEAS-2B cells, 12 and 24 h following transfection with CIITAΔE7 or CIITA-WT. Fig. 4A shows that, compared to CIITA-WT, CIITAΔE7 fails to activate *Ii* and *DMA* genes at high levels, while induces robust *DOA* and *DOB* mRNA expression in A549 cells. This differential effect was not observed in BEAS-2B cells. Furthermore, CIITAΔE7 and CIITA-WT did not differ significantly in the transactivation of *DRA* gene in both cell lines, as determined by a well-documented *DRA* promoter-reporter gene assay [6] (Fig. 4B).

To test whether *DO* mRNA upregulation reflects increased promoter activity, for the first time we constructed two reporter genes incorporating different lengths of *DOA* promoter fragments, one encompassing the conserved W/S-X1-X2-Y module (−106~+124), the other extending further upstream up to −2270. These two reporter genes were tested for activity in BEAS-2B and A549 cells transfected with CIITAΔE7 or CIITA-WT. Only the reporter gene with −2270~+124 DOA promoter fragment displayed statistically significant differential activity by CIITAΔE7 vs. CIITA-WT in A549 cells (Fig. 4C and data not shown). These results suggest that, in A549 cells, CIITAΔE7 does not activate *DOA* merely through the canonical responsive elements, but is assisted by an unknown upstream regulatory sequence. This differential *DOA* promoter activity is especially pronounced in A549 cells treated with IFN-γ (Fig. 4D, left panel). Consistent with this observation, HLA-DR, -DP, DQ expression at the surface of A549 cells was markedly inhibited following IFN-γ treatment, compared to BEAS-2B cells (Fig. 4D, right panel). These findings are in line with the negative role HLA-DO plays in the antigen processing and membrane transport of HLA class II molecules [18–20]. Furthermore, the markedly elevated *DOA* promoter activity in A549 cells following IFN-γ-treatment may also be attributed to its robust CIITAΔE7 induction.

Of the two novel human IFN-γ-inducible CIITA variants characterized in this study, only CIITAΔE7 exhibits altered functions toward genes encoding chaperons regulating HLA class II assembly and transport. Specifically, *Ii* and *HLA-DMA* mRNA is insufficiently induced, while *HLA-DOA* and *-DOB* mRNA was markedly upregu-

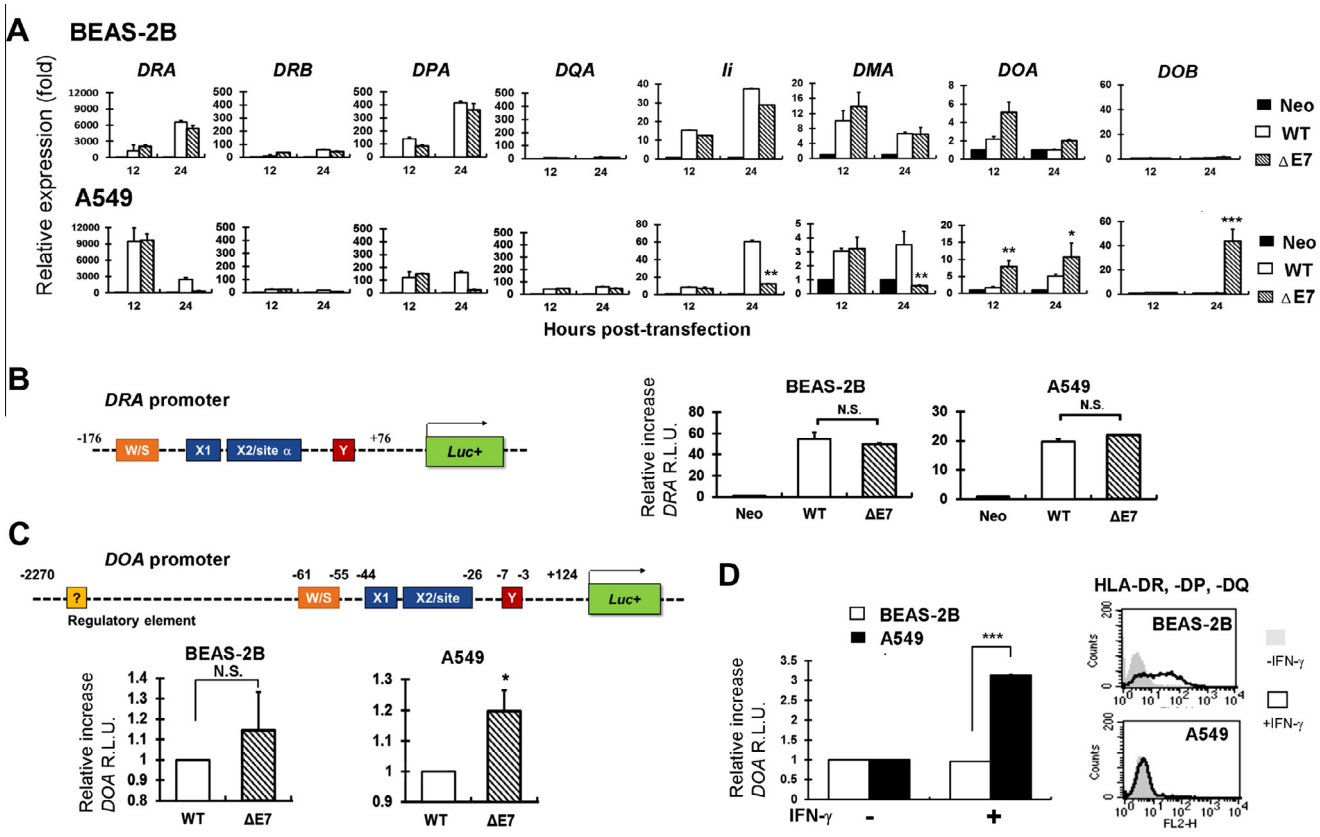

Fig. 4. Selective modulation of *Ii*, *HLA-DMA*, and *HLA-DO* genes by CIITAΔE7 in A549 cells. (A) qRT-PCR analysis of *DRA*, *DRB*, *DPA*, *DQA*, *Ii*, *DMA*, *DOA*, and *DOB* mRNA expression in BEAS-2B and A549 cells and results are expressed as relative fold increase compared to cells transfected with an empty plasmid. Mean + S.D. from three separate experiments $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.005$. Cells were co-transfected with *DRA* (B) or *DOA* (C) promoter-luciferase constructs together with pRL-CMV and analyzed for luciferase activity 24 h post-transfection. Results are expressed as relative fold increase in relative light unit (R.L.U.). R.L.U. was calculated by normalizing firefly luciferase activity with renilla luciferase activity. $^{*}p < 0.05$; N.S., non-significant. (D) BEAS-2B and A549 cells were treated with IFN-γ (100 U/ml) for 24 h and then their *DOA* promoter activity (left panel) and surface HLA-DR, -DP, DQ expression level (right panel) were determined by luciferase assay and flow cytometry, respectively. Relative *DOA* R.L.U.'s are expressed as mean ± S.D. $^{***}p < 0.005$.

lated. In particular, this altered activity is augmented in transformed cells such as A549 cells, raising the possibility that CIITAΔE7 is capable of interacting with cancer-associated factors to exert its function, perhaps directly or indirectly through additional regulatory elements as in the case of *DOA* promoter. Nevertheless, whether this mechanism also applies to *Ii*, *DM* and *DOB* remains to be determined. Changes in HLA class II expression in tumor lesions may have a direct or indirect negative impact on anti-tumor immunity in the host. Although still a debated issue, in some cancer types alterations in HLA class II expression in tumor lesions are associated with an unfavorable clinical course [13,21–23]. Reduced HLA-DR, -DP, -DQ expression has been observed in thymomas in spite of constitutive expression of the IFN-γ-inducible CIITA variant [24]. In neuroblastoma, HLA-DO expression has been found to change the repertoire of peptides presented by HLA class II molecules, leading to impaired $CD4^+$ T cell responses [25]. In the case of lung cancer, future work is warranted to investigate the level of HLA class II, CIITAΔE7 and HLA-DO in the tumor lesions and its functional significance.

Acknowledgments

This work was in part supported by grants NSC 98-2320-B-007-004-MY3 and NTHU 102N2614E1 awarded by the National Science Council and National Tsing Hua University, respectively.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.09.066.

References

[1] W. Reith, S. LeibundGut-Landmann, J.M. Waldburger, Regulation of MHC class II gene expression by the class II transactivator, Nat. Rev. Immunol. 5 (2005) 793–806.
[2] W. Reith, B. Mach, The bare lymphocyte syndrome and the regulation of MHC expression, Annu. Rev. Immunol. 19 (2001) 331–373.
[3] J.P. Ting, J. Trowsdale, Genetic control of MHC class II expression, Cell 109 (2002) S21–S33.
[4] J.M. Boss, P.E. Jensen, Transcriptional regulation of the MHC class II antigen presentation pathway, Curr. Opin. Immunol. 15 (2003) 105–111.
[5] S. LeibundGut-Landmann, J.M. Waldburger, M. Krawczyk, et al., Specificity and expression of CIITA, the master regulator of MHC class II genes, Eur. J. Immunol. 34 (2004) 1513–1525.
[6] A. MuhlethalerMOttet, L.A. Otten, V. Steimle, et al., Expression of MHC class II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA, EMBO J. 16 (1997) 2851–2860.
[7] P.J. van den Elsen, N. van der Stoep, H.E. Vietor, et al., Lack of CIITA expression is central to the absence of antigen presentation functions of trophoblast cells and is caused by methylation of the IFN-γ inducible promoter (PIV) of CIITA, Hum. Immunol. 61 (2000) 850–862.
[8] E. Zika, J.P. Ting, Epigenetic control of MHC class II: interplay between CIITA and histone-modifying enzymes, Curr. Opin. Immunol. 17 (2005) 58–64.
[9] K.P. Bhat, A.D. Truax, J.K. Brooks, S.F. Greer, Association of the 19S proteasomal ATPases with the ATPase-binding domain of CIITA is essential for CIITA stability and MHC class II expression, Immunol. Cell Biol. 88 (2010) 807–816.
[10] K.P. Bhat, A.D. Truax, S.F. Greer, Phosphorylation and ubiquitination of degron proximal residues are essential for class II transactivator (CIITA) transactivation and major histocompatibility class II expression, J. Biol. Chem. 285 (2010) 25893–25903.
[11] J.L. Riley, S.D. Westerheide, J.A. Price, et al., Activation of class-Ii Mhc genes requires both the X-box region and the class-Ii transactivator (Ciita), Immunity 2 (1995) 533–543.
[12] D.E. Cressman, W.J. O'Connor, S.F. Greer, et al., Mechanisms of nuclear import and export that control the subcellular localization of class II transactivator, J. Immunol. 167 (2001) 3626–3634.
[13] C. Steidl, S.P. Shah, B.W. Woolcock, et al., MHC class II transactivator CIITA is a recurrent gene fusion partner in lymphoid cancers, Nature 471 (2011) 377–383.
[14] V. Quaranta, L.E. Walker, M.A. Pellegrino, et al., Purification of immunologically functional subsets of human Ia-like antigens on a monoclonal-antibody (Q5-13) immunoadsorbent, J. Immunol. 125 (1980) 1421–1425.
[15] J. Kaufman, S. Ferrone, M. Flajnik, et al., Mhc-like molecules in some nonmammalian vertebrates can be detected by some cross-reactive monoclonal-antibodies, J. Immunol. 144 (1990) 2273–2280.
[16] T. Ogino, X. Wang, S. Kato, et al., Endoplasmic reticulum chaperone-specific monoclonal antibodies for flow cytometry and immunohistochemical staining, Tissue Antigens 62 (2003) 385–393.
[17] Z.J. Chen, H. Yang, S. Ferrone, Human high-molecular-weight melanoma-associated antigen mimicry by mouse antiidiotypic monoclonal-antibody Mk2-23 – characterization of the immunogenicity in syngeneic hosts, J. Immunol. 147 (1991) 1082–1090.
[18] L.K. Denzin, D.B. Sant'Angelo, C. Hammond, et al., Negative regulation by HLA-DO of MHC class II-restricted antigen processing, Science 278 (1997) 106–109.
[19] A. Bellemare-Pelletier, J. Tremblay, S. Beaulieu, et al., HLA-DO transduced in human monocyte-derived dendritic cells modulates MHC class II antigen processing, J. Leukoc. Biol. 78 (2005) 95–105.
[20] L. Papadimitriou, I. Morianos, V. Michailidou, et al., Characterization of intracellular HLA-DR, DM and DO profile in K562 and HL-60 leukemic cells, Mol. Immunol. 45 (2008) 3965–3973.
[21] M. Campoli, C.C. Chang, S.A. Oldford, et al., HLA antigen changes in malignant tumors of mammary epithelial origin: molecular mechanisms and clinical implications, Breast Dis. 20 (2004) 105–125.
[22] C.C. Chang, M. Campoli, S. Ferrone, Classical and nonclassical HLA class I antigen and NK cell-activating ligand changes in malignant cells: current challenges and future directions, Adv. Cancer Res. 93 (2005) 189–234.
[23] M. Campoli, S. Ferrone, A fresh look at an old story: revisiting HLA class II antigen expression by melanoma cells, Expert Rev. Dermatol. 1 (2006) 737–755.
[24] P. Strobel, W.-Y. Chuang, S. Chuvpilo, et al., Common cellular and diverse genetic basis of thymoma-associated myasthenia gravis: role of MHC class II and AIRE genes and genetic polymorphisms, Ann N Y Acad Sci. 1132 (2008) 143–156.
[25] M.E. Chamuleau, G.J. Ossenkoppele, A.A. van de Loosdrecht, MHC class II molecules in tumour immunology: prognostic marker and target for immune modulation, Immnobiology 211 (2006) 619–625.