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JBR

The Journal of Biomedical Research, 2015, 29(4):308-315

Original Article

Class II transactivator (CIITA) mediates transcriptional repression of *pdk4* gene by interacting with hypermethylated in cancer 1 (HIC1)

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Abstract

Increased accumulation and/or impaired utilization of fatty acid in extra-adipose tissues are implicated in the pathogenesis of insulin resistance and type 2 diabetes. Pyruvate dehydrogenase kinase 4 (Pdk4) is a key enzyme involved in fatty oxidation and energy expenditure, and its expression can be repressed by pro-inflammatory stimuli. Previously, we have shown that class II transactivator (CIITA) mediates the adverse effect of interferon gamma (IFN-γ) in skeletal muscle cells by cooperating with hypermethylated in cancer 1 (HIC1) to repress silent information regulator 1 (SIRT1) transcription. Building upon this finding, we report here that CIITA interacted with HIC1 *via* the GTP-binding domain (GBD) while HIC1 interacted with CIITA *via* the BTB/POZ domain. The GBD domain was required for CIITA to repress SIRT1 transcription probably acting as a bridge for CIITA to bind to HIC1 and consequently to bind to the *SIRT1* promoter. IFN-γ stimulation, CIITA over-expression, or HIC1 over-expression repressed *Pdk4* promoter activity while silencing either CIITA or HIC1 normalized Pdk4 expression in the presence of IFN-γ. An increase in SIRT1 expression or activity partially rescued Pdk4 expression in the presence of CIITA, but SIRT1 inhibition abrogated Pdk4 normalization even in the absence of CIITA. Taken together, our data have identified a HIC1-CIITA-SIRT1 axis that regulates Pdk4 transcription in response to IFN-γ stimulation.

Keywords: CIITA, HIC, SIRT1, IFN gamma, PDK4, transcriptional regulation

Introduction

Changes in lifestyle have contributed to the development of metabolic syndrome in humans over the past several decades[1]. Type 2 diabetes, which has become a global pandemic, is a major form of metabolic syndrome[2]. Type 2 diabetes is characterized by insulin resistance in peripheral organs including the liver, adipose tissues, and skeletal muscles. Chronic, low-magnitude inflammation (dubbed as metabolic inflammation) has been extensively investigated as a primary force to promote insulin resistance[3]. Pro-inflammatory cytokines, including interleukins (ILs), macrophage chemoattractant protein 1

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Received 07 April 2015, Accepted 09 May 2015, Epub 30 June 2015

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CLC number: R589.2, Document code: A

The authors reported no conflict of interests

doi: 10.7555/JBR.29.20150055

(MCP1), tumor necrosis factor (TNF-γ), and interferon gamma (IFN-γ), are considered culprits in metabolic inflammation and insulin resistance[4-6]. How these pro-inflammatory mediators disrupt normal cellular metabolic programs and consequently cause metabolic syndrome is not entirely clear.

Silent information regulator 1 (SIRT1) is the founding member of the mammalian family of class III lysine deacetylases. Numerous studies have demonstrated the beneficial role of SIRT1 in maintaining the homeostasis of cellular metabolism. *SIRT1* gain-of-function transgene or activation of SIRT1 with small-molecule agonists has been shown to antagonize both diet-induced and hereditary obesity and the development of type 2 diabetes in mice[7-8]. In contrast, SIRT1 ablation promotes metabolic inflammation and exacerbates insulin resistance in mice[9-10]. One of the mechanisms by which SIRT1 prevents insulin resistance is to stimulate cellular energy expenditure by re-programming cellular metabolic circuits. For instance, SIRT1 activation in cells increases fatty acid oxidation by deacetylation of PGC1-α, FOXO1, and p53[11].

Previously, we have reported that class II transactivator (CIITA) is activated by IFN-γ in skeletal muscle cells[12]. CIITA interacts with hypermethylated in cancer 1 (HIC1), a sequence-specific transcription factor, to repress SIRT1 transcription. Consequently, genes involved in energy expenditure such as *Mcad*, *Ppargc-1a*, *Cpt1*, and *Cox4* are down-regulated in response to IFN-γ stimulation, leading to a skewed metabolic phenotype mimicking insulin resistance. In the present study, we have addressed several lingering issues: 1) which domains within CIITA and HIC1 mediate their interaction? 2) Do CIITA and HIC1 mediate the repression of Pdk4, a key enzyme involved in fatty acid oxidation, by IFN-γ? 3) Is SIRT1 required for CIITA to mediate the disruptive effect of IFN-γ? Our data re-affirm the HIC1-CIITA-SIRT1 axis in regulating cellular metabolism in skeletal muscle cells.

Materials and methods

Cell culture and treatment

Mouse myotube/myoblast cells (C2C12) and human embryonic kidney cells (HEK293) were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA). Cells were serum starved overnight and then performed with murine recombinant interferon-gamma (IFN-γ, R&D Systems, Abingdon, UK), resveratrol (Sigma, St Louis, MO, USA), or nicotinamide (Sigma) for 24-48 hours before the treatment.

Plasmids, transient transfection, and reporter assay

FLAG-tagged CIITA constructs, GFP-tagged HIC1 constructs, SIRT1 promoter-luciferase construct, Pdk4 promoter-luciferase construct, SIRT1 expression constructs, siRNA sequences for mouse C2ta and Hic1 have been previously described[12-14]. Transient transfections were performed with Lipofectamine 2000 (Invitrogen). Luciferase activities were assayed 24-48 hours after transfection using a luciferase reporter assay system (Promega, Madison, WI, USA). Experiments were routinely performed in triplicate wells and repeated three times.

Protein extraction, immunoprecipitation and Western blotting assay

Whole cell lysates were obtained by re-suspending cell pellets in RIPA buffer (50 mmol/L Tris pH7.4, 150 mmol/L NaCl, and 1% Triton X-100) with freshly added protease inhibitor (Roche, Indianapolis, IN, USA). FLAG-conjugated beads (M2, Sigma) were added to and incubated with lysates overnight. Precipitated immune complex was eluted with 3X FLAG peptide (Sigma). Western blotting assays were performed with anti-FLAG or anti-GFP (Sigma) antibodies.

RNA isolation and real-time PCR

RNA was extracted with the RNeasy RNA isolation kit (Qiagen, Valencia, CA, USA). Reverse transcriptase reactions were performed using a SuperScript First-strand Synthesis System (Invitrogen). Real-time PCR was performed on an ABI Prism 7500 system. Primers and Taqman probes used for real-time reactions were purchased from Applied Biosystems.

Chromatin immunoprecipitation (ChIP)

Chromatin Immunoprecipitation (ChIP) assays were performed essentially as described before[15]. In brief, chromatin in control and treated cells were cross-linked with 1% formaldehyde. Cells were incubated in lysis buffer (150 mmol/L NaCl, 25 mmol/L Tris pH 7.5, 1% Triton X-100, 0.1% SDS, and 0.5% deoxycholate) supplemented with protease inhibitor tablet and phenylmethanesulfonyl fluoride (PMSF). DNA was fragmented into -500 bp fragments using a Branson 250 sonicator. Aliquots of lysates containing 200 μg of protein were used for each immunoprecipitation reaction with anti-FLAG (Sigma). Precipitated genomic DNA was amplified by real-time PCR using previously described primers[12].

Statistical analysis

One-way ANOVA with post-hoc Scheffe analyses were performed using an SPSS package. $P<0.05$ was considered statistically significant.

Results

CIITA is a de novo co-repressor for HIC1

Previously, we have found that CIITA mediated SIRT1 repression as a result of IFN-γ stimulation by interacting with HIC1. We made an attempt to delineate the domains within HIC1 that might mediate the interaction with CIITA. To this end, full-length (FL) HIC1 expression construct or deletion mutant HIC1 constructs, all with a GFP tag, were transfected into HEK293 cells with FLAG-tagged CIITA expression construct. Whole lysates extracted from transfected cells were immunoprecipitated with an anti-FLAG antibody. As shown in ***Fig. 1A***, FL HIC1 interacted with CIITA as expected. A deletion mutant lacking the CtBP domain, which was reported to bridge the HIC1-CtBP interaction, retained the ability to interact with CIITA. On the contrary, a mutant missing the N-terminal BTB/POZ domain failed to interact with CIITA. Similarly, we determined the domain within CIITA necessary for interaction with HIC1. As shown in ***Fig. 1B***, while FL CIITA, an N-terminal deletion CIITA mutant without the acidic domain (AD), acetyltransferase domain (AT), and proline-serine-threonine domain (PST), and a C-terminal deletion CIITA mutant without the leucine-rich

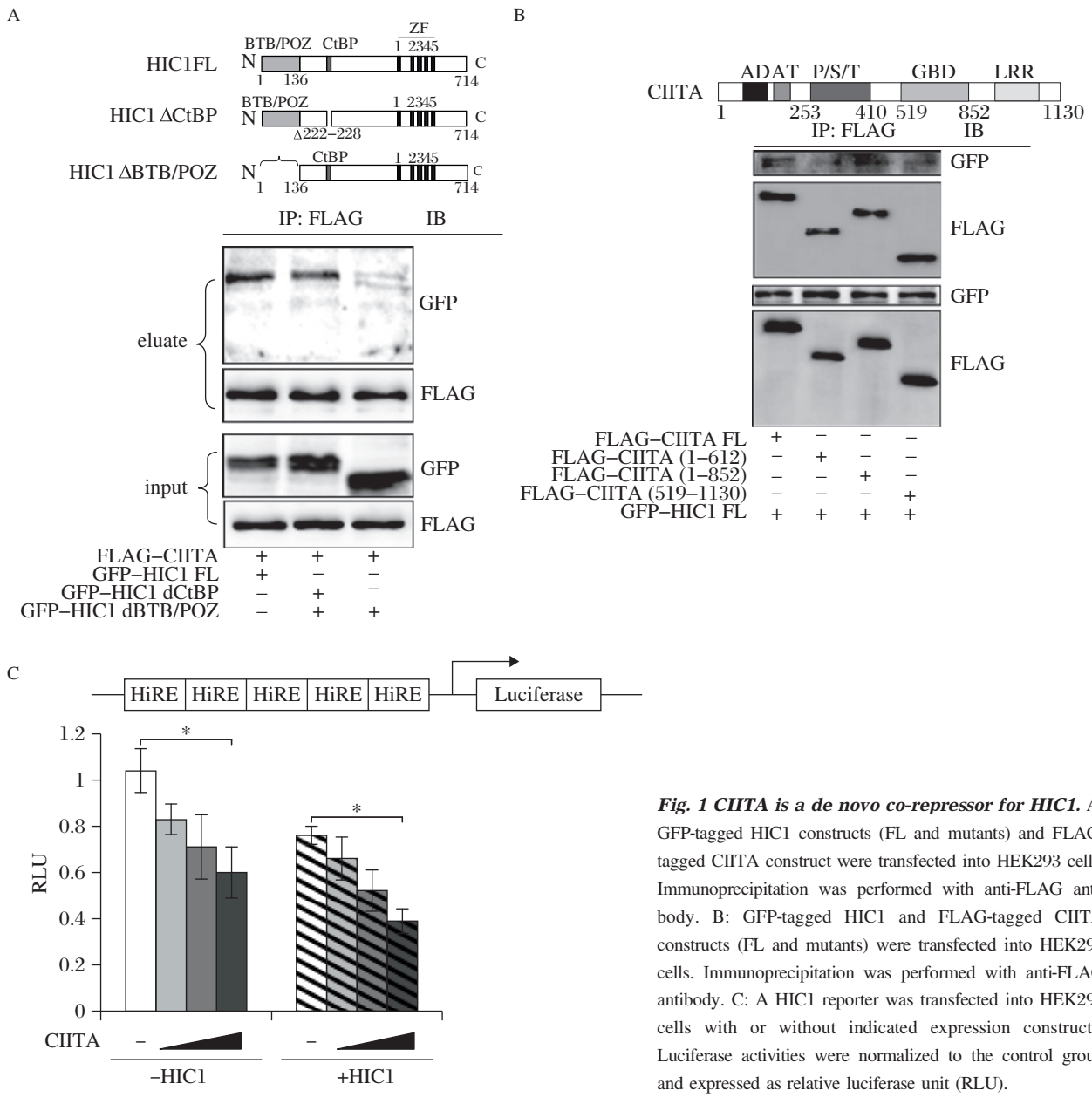

Fig. 1 CIITA is a de novo co-repressor for HIC1. A: GFP-tagged HIC1 constructs (FL and mutants) and FLAG-tagged CIITA construct were transfected into HEK293 cells. Immunoprecipitation was performed with anti-FLAG antibody. B: GFP-tagged HIC1 and FLAG-tagged CIITA constructs (FL and mutants) were transfected into HEK293 cells. Immunoprecipitation was performed with anti-FLAG antibody. C: A HIC1 reporter was transfected into HEK293 cells with or without indicated expression constructs. Luciferase activities were normalized to the control group and expressed as relative luciferase unit (RLU).

repeat domain (LRR) all interacted with HIC1, a more extensive C-terminal deletion CIITA mutant lacking the GTP-binding domain (GBD) exhibited diminished HIC1 interaction. Thus, we concluded that HIC1 may interact with CIITA through its N-terminal BTB/POZ domain while CIITA may interact with HIC1 through its GBD domain in the middle.

Next, we tackled the question whether CIITA could serve as a genuine co-factor for HIC1-dependent transcriptional repression. A reporter, constructed by fusing five copies of HIC1 consensus sequences with the luciferase gene, was transfected into HEK293 cells[16]. CIITA repressed the HIC1 reporter in a dose-dependent manner suggesting that CIITA might indeed facilitate HIC1-dependent transcriptional repression in cells (***Fig. 1C***).

The GBD domain of CIITA is required for SIRT1 repression

Having determined that the GBD domain of CIITA mediates its interaction with HIC1 (***Fig. 1B***), we

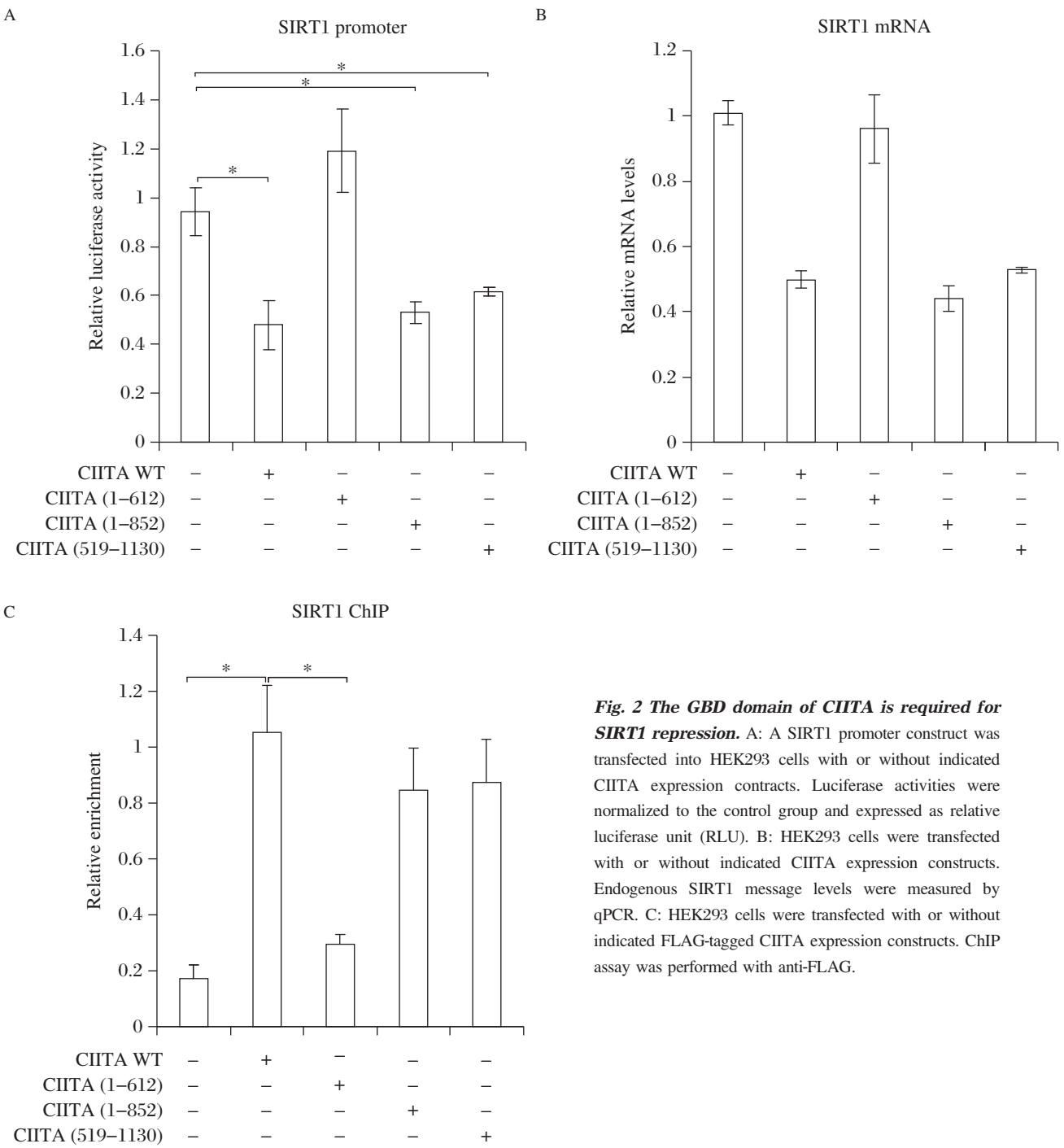

Fig. 2 The GBD domain of CIITA is required for SIRT1 repression. A: A SIRT1 promoter construct was transfected into HEK293 cells with or without indicated CIITA expression contracts. Luciferase activities were normalized to the control group and expressed as relative luciferase unit (RLU). B: HEK293 cells were transfected with or without indicated CIITA expression constructs. Endogenous SIRT1 message levels were measured by qPCR. C: HEK293 cells were transfected with or without indicated FLAG-tagged CIITA expression constructs. ChIP assay was performed with anti-FLAG.

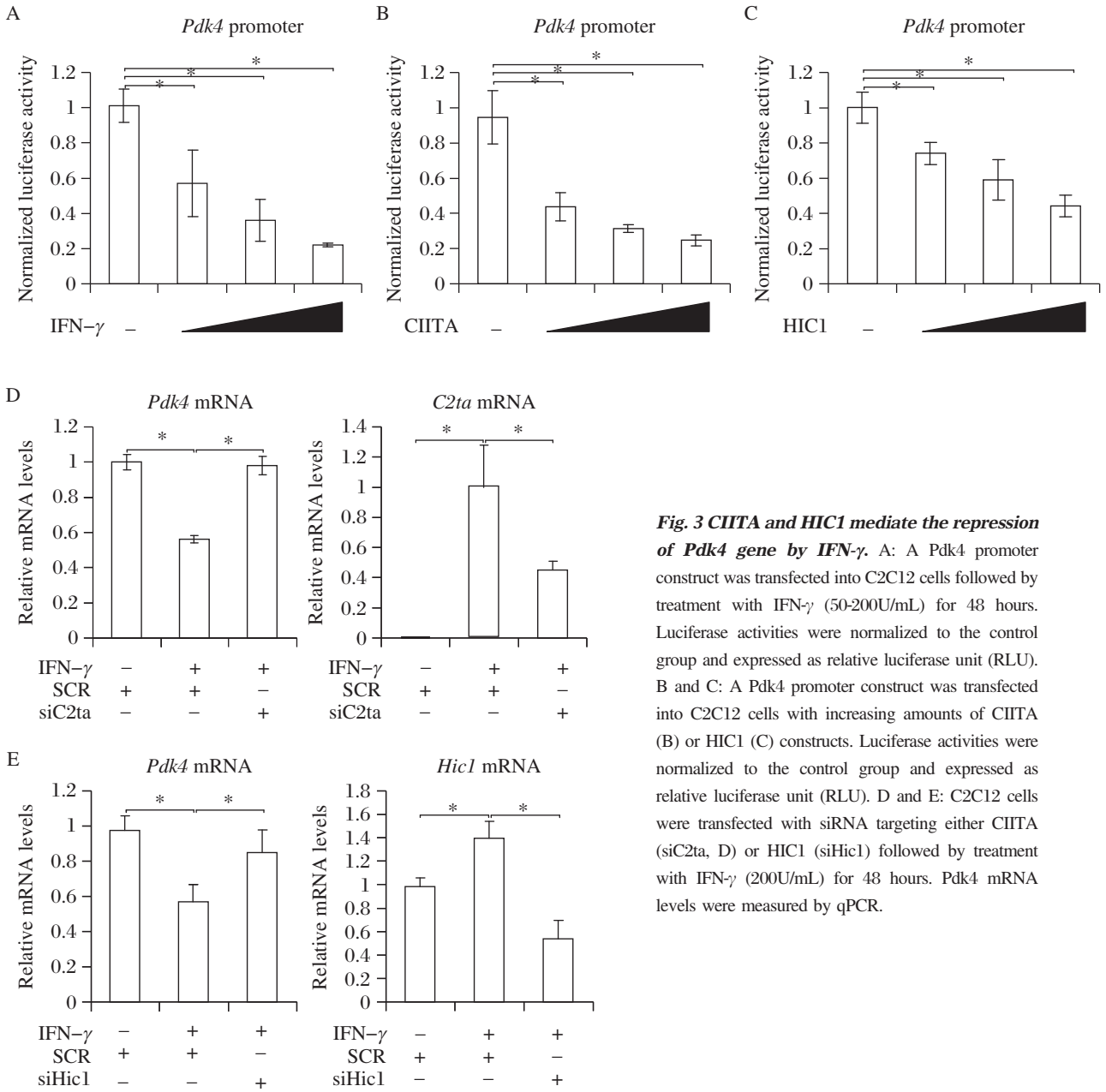

Fig. 3 CIITA and HIC1 mediate the repression of Pdk4 gene by IFN-γ. A: A Pdk4 promoter construct was transfected into C2C12 cells followed by treatment with IFN-γ (50-200U/mL) for 48 hours. Luciferase activities were normalized to the control group and expressed as relative luciferase unit (RLU). B and C: A Pdk4 promoter construct was transfected into C2C12 cells with increasing amounts of CIITA (B) or HIC1 (C) constructs. Luciferase activities were normalized to the control group and expressed as relative luciferase unit (RLU). D and E: C2C12 cells were transfected with siRNA targeting either CIITA (siC2ta, D) or HIC1 (siHic1) followed by treatment with IFN-γ (200U/mL) for 48 hours. Pdk4 mRNA levels were measured by qPCR.

proceeded to examine whether this domain might also be required for SIRT1 repression. Over-expression of either FL CIITA or deletion CIITA mutants that retained the GBD domain (1-852 and 519-1130) were able to, while the CIITA mutant that lacked the GBD domain (1-612) failed to, repress SIRT1 promoter activities in HEK293 cells (***Fig. 2A***). Consistent with this result, endogenous SIRT1 messages were down-regulated by ectopic CIITA constructs with the GBD domain but not by the GBD domain deletion CIITA construct (***Fig. 2B***). Furthermore, ChIP assays showed that FL CIITA and GBD-containing CIITA were recruited to the SIRT1 promoter, but GBD-null CIITA lost the binding to the SIRT1 promoter apparently due to its inability to interact with HIC1 (***Fig. 2C***). In conclusion, these data confirm our previous observations that CIITA relies on HIC1 to bind to the SIRT1 promoter to repress transcription.

CIITA and HIC1 mediate repression of Pdk4 gene by IFN-γ

Pyruvate dehydrogenase kinase 4 (pdk4) is a key enzyme involved in fatty acid oxidation and can be activated by SIRT1 during starvation and/or fasting to maintain metabolic homeostasis[13]. Previously, we have demonstrated that CIITA and HIC1 cooperate to mediate SIRT1 repression by IFN-γ[12]. Therefore, we

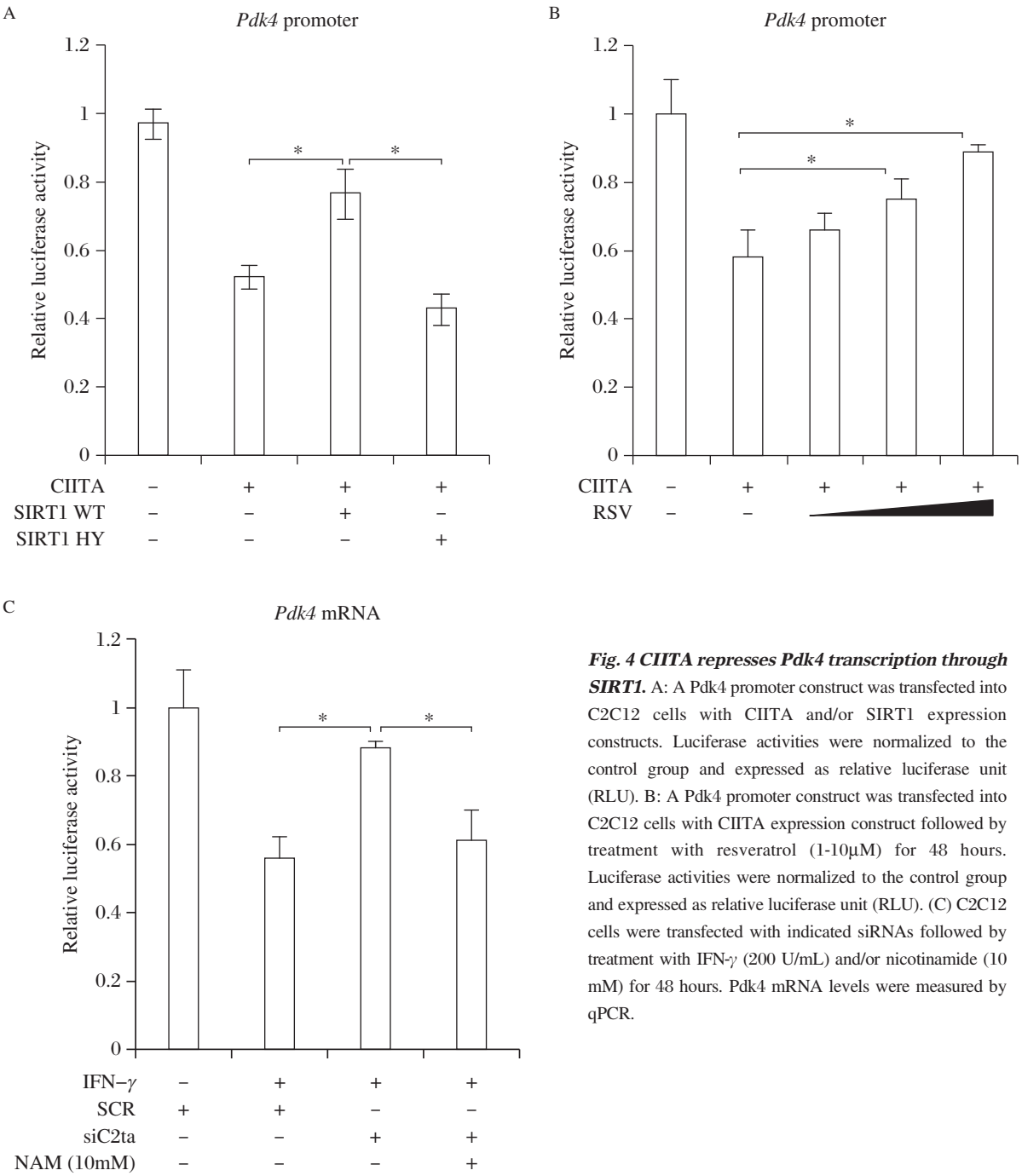

Fig. 4 CIITA represses Pdk4 transcription through SIRT1. A: A Pdk4 promoter construct was transfected into C2C12 cells with CIITA and/or SIRT1 expression constructs. Luciferase activities were normalized to the control group and expressed as relative luciferase unit (RLU). B: A Pdk4 promoter construct was transfected into C2C12 cells with CIITA expression construct followed by treatment with resveratrol (1-10μM) for 48 hours. Luciferase activities were normalized to the control group and expressed as relative luciferase unit (RLU). (C) C2C12 cells were transfected with indicated siRNAs followed by treatment with IFN-γ (200 U/mL) and/or nicotinamide (10 mM) for 48 hours. Pdk4 mRNA levels were measured by qPCR.

examined the possibility that IFN-γ might disrupt Pdk4 transcription via CIITA/HIC1. IFN-γ treatment repressed Pdk4 promoter activity in a dose-dependent manner (***Fig. 3A***). Alternatively, over-expression of CIITA (***Fig. 3B***) or HIC1 (***Fig. 3C***) directly repressed Pdk4 promoter activity in a dose-dependent manner. Finally, knockdown of CIITA or HIC1 using small interfering RNA (siRNA) abrogated the repression of Pdk4 messages by IFN-γ (***Fig. 3D and 3E***). In summary, we propose that CIITA and HIC1 might mediate IFN-γ-induced repression of *Pdk4* gene.

CIITA represses Pdk4 transcription through SIRT1

Finally, we assessed the hypothesis that CIITA might repress Pdk4 transcription indirectly through SIRT1. As shown in ***Fig. 4A***, over-expression of CIITA repressed the Pdk4 promoter activity; co-expression of a wild type (WT) form, but not an enzyme deficient mutant (HY), of SIRT1 partially abrogated the effect of CIITA. In addition, pre-treatment of cells with a SIRT1 agonist resveratrol also alleviated the

repression of Pdk4 promoter activity by CIITA (***Fig. 4B***). In contrast, siRNA-mediated knockdown of CIITA normalized Pdk4 expression in the presence of IFN-γ, which was reversed by the addition of a SIRT1 inhibitor nicotinamide (***Fig. 4C***). Together, these data suggest that CIITA targets SIRT1 to regulate Pdk4 transcription.

Discussion

Metabolic inflammation contributes to insulin resistance in part by disrupting normal cellular metabolism[17]. Our data as summarized in this report echo our previous finding that IFN-γ could modify cellular metabolism by decreasing energy expenditure in skeletal muscle cells[12]. Of note, the detrimental effects of IFN-γ have been observed in other insulin-sensitive organs as well. For instance, McGillicuddy *et al.* have reported that IFN-γ attenuates insulin signaling in human adipocytes by activating the JAK/STAT pathway[18]. In addition, Wensveen *et al.* demonstrated that natural killer cell-derived IFN-γ promotes insulin resistance in visceral fat tissue by promoting M1 macrophage differentiation[19]. Accordingly, mice with deficiency in either *IFN-*γ gene itself or IFN-γ receptor gene (*Ifngr1*) display improved metabolic profile and resistance to obesity upon high-fat diet challenge[20-21]. Therefore, though relying on various mechanisms, these data together argue for a unified role for IFN-γ in the development of insulin resistance and type 2 diabetes, which is consistent with epidemiology studies that show increased serum levels of IFN-γ in obese population[22-25].

CIITA was initially identified as a co-activator of major histocompatibility complex (*MHC II*) genes. Follow-up studies have revealed that CIITA is capable of both activating and repressing gene expression[26]. Previously, we showed that CIITA relies on HIC1 to be brought down to the *SIRT1* promoter to repress transcription. Here, we show that the GTP-binding domain (GBD) is required for CIITA to interact with HIC1. Ting *et al.* have reported that GBD is necessary for the nuclear import of CIITA[27]. This observation, according to our new finding, alludes to the possibility that the CIITA-HIC1 complex is assembled in the nucleus and probably on the chromatin. CIITA has the ability to bridge sequence-specific transcription factors to the epigenetic machinery[28-31]. CIITA might help recruit certain epigenetic factors (e.g., HDAC2) to facilitate SIRT1 repression by HIC1. Alternatively, the GBD domain is shown to mediate CIITA oligomerization and stability of CIITA[32]; binding to HIC1 may promote CIITA self-assembly and/or stability increasing its transcriptional activity. Clearly, the interaction between HIC1 and CIITA needs to be further examined in the context of different pathophysiological processes.

We show here that HIC1 and CIITA repress Pdk4 transcription by directly targeting SIRT1. A reasonable extrapolation would be that targeting HIC1 and/or CIITA may be beneficial in metabolic disorders. Indeed, a recent discovery suggesting that mice with MHC II deficiency develop resistance to diet-induced obesity and type 2 diabetes seems to support this hypothesis[33]. However, systemic inhibition of HIC1 or CIITA may lead to either carcinogenesis or immune insufficiency, rendering the strategy undesirable[34-36]. Since CIITA activation is a pre-requisite for HIC1 to repress SIRT1 transcription in metabolically challenged situations, an alternative strategy would be to modify/temper CIITA activity rather than abolish it. This could be achieved by either post-translational modifications or, as suggested by several recent reports, by modulating the IFN-γ-STAT1 pathway[26,37].

In summary, we re-affirm our previous finding that HIC1-CIITA-SIRT1 axis modulates cellular metabolism in response to pro-inflammatory stress. Future studies should focus on unveiling a genome-wide role for the CIITA-HIC1 complex in this process to provide a more solid basis for the development of interventional strategies against type 2 diabetes.

Acknowledgements

This work was supported, in part, by the National Natural Science Foundation of China (31200645), the Natural Science Foundation of Jiangsu Province (BK20141498), and a grant from Jiangsu Jiankang Vocational University (JK201405). Y.X. is a Fellow at the Collaborative Innovative Center for Cardiovascular Translational Medicine.

References

[1] Magkos F, Yannakoulia M, Chan JL, et al. Management of the metabolic syndrome and type 2 diabetes through[J]. *Annu Rev Nutr,* 2009,29:223-256.

[2] Chan JC, Malik V, Jia W, et al. Diabetes in Asia: epidemiology, risk factors, and pathophysiology[J]. *JAMA,* 2009,301(20):2129-2140.

[3] Hotamisligil GS. Inflammation and metabolic disorders[J]. *Nature,* 2006,444(7121):860-867.

[4] Brenner C, Galluzzi L, Kepp O, et al. Decoding cell death signals in liver inflammation[J]. *J Hepatol,* 2013, 59(3):583-594.

[5] Kleemann R, Zadelaar S, Kooistra T. Cytokines and atherosclerosis: a comprehensive review of studies in mice[J]. *Cardiovasc Res,* 2008,79(3):360-376.

[6] Chow EK, Razani B, Cheng G. Innate immune system regulation of nuclear hormone receptors in metabolic diseases[J]. *J Leukoc Biol,* 2007,82(2):187-195.

[7] Milne JC, Lambert PD, Schenk S, et al. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes[J]. *Nature,* 2007,450(7170):712-716.

[8] Banks AS, Kon N, Knight C, et al. SirT1 gain of function increases energy efficiency and prevents diabetes in mice[J]. *Cell Metab,* 2008,8(4):333-341.

[9] Purushotham A, Schug TT, Xu Q, et al. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation[J]. *Cell Metab,* 2009,9(4):327-338.

[10] Purushotham A, Xu Q, Li X. Systemic SIRT1 insufficiency results in disruption of energy homeostasis and steroid hormone metabolism upon high-fat-diet feeding[J]. *FASEB J,* 2012,26(2):656-667.

[11] Feige JN, Lagouge M, Canto C, et al. Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation[J]. *Cell Metab,* 2008,8(5):347-358.

[12] Li P, Zhao Y, Wu X, et al. Interferon gamma (IFN-gamma) disrupts energy expenditure and metabolic homeostasis by suppressing SIRT1 transcription[J]. *Nucleic Acids Res,* 2012,40(4):1609-1620.

[13] Kwon HS, Huang B, Ho Jeoung N, et al. Retinoic acids and trichostatin A (TSA), a histone deacetylase inhibitor, induce human pyruvate dehydrogenase kinase 4 (PDK4) gene expression[J]. *Biochim Biophys Acta,* 2006,1759(3-4):141-151.

[14] Wu X, Kong X, Chen D, et al. SIRT1 links CIITA deacetylation to MHC II activation[J]. *Nucleic Acids Res,* 2011,39(22):9549-9558.

[15] Fang M, Kong X, Li P, et al. RFXB and its splice variant RFXBSV mediate the antagonism between IFNgamma and TGFbeta on COL1A2 transcription in vascular smooth muscle cells[J]. *Nucleic Acids Res,* 2009,37(13):4393-4406.

[16] Pinte S, Stankovic-Valentin N, Deltour S, et al. The tumor suppressor gene HIC1 (hypermethylated in cancer 1) is a sequence-specific transcriptional repressor: definition of its consensus binding sequence and analysis of its DNA binding and repressive properties[J]. *J Biol Chem,* 2004,279(37):38313-38324.

[17] Brestoff JR, Artis D. Immune Regulation of Metabolic Homeostasis in Health and Disease[J]. *Cell,* 2015,161(1):146-160.

[18] McGillicuddy FC, Chiquoine EH, Hinkle CC, et al. Interferon gamma attenuates insulin signaling, lipid storage, and differentiation in human adipocytes via activation of the JAK/STAT pathway[J]. *J Biol Chem,* 2009,284(46):31936-31944.

[19] Wensveen FM, Jelencic V, Valentic S, et al. NK cells link obesity-induced adipose stress to inflammation and insulin resistance[J]. *Nat Immunol,* 2015,16(4):376-385.

[20] Rocha VZ, Folco EJ, Sukhova G, et al. Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity[J]. *Circ Res,* 2008,103(5):467-476.

[21] Wong N, Fam BC, Cempako GR, et al. Deficiency in interferon-gamma results in reduced body weight and better glucose tolerance in mice[J]. *Endocrinology,* 2011,152(10):3690-3699.

[22] Pacifico L, Di Renzo L, Anania C, et al. Increased T-helper interferon-gamma-secreting cells in obese children[J]. *Eur J Endocrinol,* 2006,154(5):691-697.

[23] Schmidt FM, Weschenfelder J, Sander C, et al. Inflammatory cytokines in general and central obesity and modulating effects of physical activity[J]. *Plos One,* 2015,10(3):e0121971.

[24] Utsal L, Tillmann V, Zilmer M, et al. Elevated serum IL-6, IL-8, MCP-1, CRP, and IFN-gamma levels in 10- to 11-year-old boys with increased BMI[J]. *Horm Res Paediatr,* 2012,78(1):31-39.

[25] Surendar J, Mohan V, Rao MM, et al. Increased levels of both Th1 and Th2 cytokines in subjects with metabolic syndrome (CURES-103) [J]. *Diabetes Technol Ther,* 2011,13(4):477-482.

[26] Wu X, Kong X, Luchsinger L, et al. Regulating the activity of class II transactivator by posttranslational modifications: exploring the possibilities[J]. *Mol Cell Biol,* 2009,29(21):5639-5644.

[27] Harton JA, Cressman DE, Chin KC, et al. GTP binding by class II transactivator: role in nuclear import[J]. *Science,* 1999,285(5432):1402-1405.

[28] Xu Y, Harton JA, Smith BD. CIITA mediates interferon-gamma repression of collagen transcription through phosphorylation dependent interactions with co-repressor molecules[J]. *J Biol Chem,* 2008,283(3):1243-1256.

[29] Zika E, Fauquier L, Vandel L, et al. Interplay among coactivator-associated arginine methyltransferase 1, CBP, and CIITA in IFN-gamma-inducible MHC-II gene expression[J]. *Proc Natl Acad Sci U S A,* 2005,102(45):16321-16326.

[30] Zhou X, Jiang Y, Lu L, et al. MHC class II transactivator represses human IL-4 gene transcription by interruption of promoter binding with CBP/p300, STAT6 and NFAT1 via histone hypoacetylation[J]. *Immunology,* 2007,122(4):476-485.

[31] Kong X, Fang M, Li P, et al. HDAC2 deacetylates class II transactivator and suppresses its activity in macrophages and smooth muscle cells[J]. *J Mol Cell Cardiol,* 2009,46(3):292-299.

[32] Linhoff MW, Harton JA, Cressman DE, et al. Two distinct domains within CIITA mediate self-association: involvement of the GTP-binding and leucine-rich repeat domains[J]. *Mol Cell Biol,* 2001,21(9):3001-3011.

[33] Deng T, Lyon CJ, Minze LJ, et al. Class II major histocompatibility complex plays an essential role in obesity-induced adipose inflammation[J]. *Cell Metab,* 2013,17(3):411-422.

[34] Mohammad HP, Zhang W, Prevas HS, et al. Loss of a single Hic1 allele accelerates polyp formation in Apc(Delta716) mice[J]. *Oncogene,* 2011,30(23):2659-2669.

[35] Pinte S, Guerardel C, Deltour-Balerdi S, et al. Identification of a second G-C-rich promoter conserved in the human, murine and rat tumor suppressor genes HIC1[J]. *Oncogene,* 2004,23(22):4023-4031.

[36] Reith W, Mach B. The bare lymphocyte syndrome and the regulation of MHC expression[J]. *Annu Rev Immunol,* 2001,19:331-373.

[37] Xu Y, Ravid K, Smith BD. Major histocompatibility class II transactivator expression in smooth muscle cells from A2b adenosine receptor knock-out mice: cross-talk between the adenosine and interferon-gamma signaling[J]. *J Biol Chem,* 2008,283(21):14213-14220.