PIBB 生物化学与生物物理进展
*Progress in Biochemistry and Biophysics*
2018, 45(1): 79~90
www.pibb.ac.cn

# HDAC3 Maintains Peripheral T Cell Homeostasis by Refraining From AICD*

WANG Shan[1)**], TIAN Feng[2)**], QIAN Ye[1)], LIU Ying[2)], LI Hui-Ting[2)], ZHANG Ai-Hong[3)], HOU Zhi-Hong[1)], LIU Ya-Nan[1)], LI Juan[1)], ZHANG Yan-Shu[4)], ZHAO Yong[5)], ZHENG Quan-Hui[1)***]

([1)] *Hebei Key Laboratory for Chronic Diseases, Tangshan Key Laboratory for Preclinical and Basic Research on Chronic Diseases, School of Basic Medical Sciences, North China University of Science and Technology, Tangshan* 063000, China;
[2)] *Department of Laboratory Animal Science, Pecking University Health Science Center, Beijing* 100191, China;
[3)] *Tangshan Gongren Hospital, Tangshan* 063000, China;
[4)] *School of Public Health, North China University of Science and Technology, Tangshan* 063000, China;
[5)] *State Key Laboratory of Membrane Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing* 100101, China)

**Abstract** To investigate the role of histone deacetylase3 (HDAC3) in T cell homeostasis, we deleted *hdac3* in $CD4^+CD8^+$ double positive(DP) stage of thymocytes using the *cd4-cre* transgene .The CD4Cre-mediated *hdac3* deletion did not impact T cell development in the thymus but resulted in a dramatic loss of peripheral T cells. In addition, peripheral T cells in *hdac3* knock-out mice showed a dominant activation/effector/memory phenotype. Mechanism analysis revealed an increased cell apoptosis which was accompanied by an accelerated cell proliferation in the peripheral T cells of *hdac3* knock-out mice. Moreover, Fas and FasL positive cells and FasL expression increased significantly in the peripheral T cells of *hdac3* knock-out mice. *In vitro* TCR activation did not affect the apoptosis of normal peripheral T cells, but dramatically increased apoptosis of peripheral T cells from *hdac3* knock-out mice. Our results presented here indicate an important role of HDAC3 in maintaining homeostasis of peripheral T cells by refraining them from activation- induced cell death.

**Key words** peripheral T cells, HDAC3, activation-induced cell death, Fas, FasL
**DOI**: 10.16476/j.pibb.2017.0333

T cells that undergo positive selection and negative selection in the thymus become mature T cells, and enter into peripheral lymphoid organs where they play a critical role in mounting adaptive immune responses against pathogens and antigens. T cell numbers in the periphery are almost constant, even though millions of naïve T cells are generated from the thymus daily. This homeostasis likely requires strict regulation. For example, when TCR recognizes cognate antigen presented on major histocompatibility complex (MHC) molecules by an antigen presenting cell, a series of signaling events were triggered, which finally leads to T cell activation, proliferation and effector function. After this process, the activated T cells die through two mechanisms, activation-induced cell death (AICD) and activated T-cell autonomous death [1]. AICD requires antigen re-stimulation of activated effector T cells and is triggered by the extrinsic death pathways regulated by signals of death

*This work was supported by a grant from The National Natural Science Foundation of China (81373111, 81673208).
**These authors contributed equally to this work.
***Corresponding author.
Tel: 86-315-8805516, E-mail: 1078209929@qq.com
Received: August 14, 2017 Accepted: December 14, 2017

receptors (DRs) of the tumor necrosis factor receptor (TNFR) family such as Fas/CD95, FasL/CD95L and type Ⅰ TNFR (TNFR Ⅰ)/DR1 [2]. Activated T-cell autonomous death is mediated by the intrinsic death pathway, which is regulated by the B-cell lymphoma 2 (Bcl-2) family proteins[3].

AICD of T cells can be induced by non-self antigens as well as self antigens [4-5]. Under normal conditions, despite some autoreactive T cells that escape from negative selection exist in peripheral lymph organs, peripheral T cells maintain homeostasis by keeping a naïve state and refraining from AICD. Peripheral tolerance including the expression of molecular "brakes" (e.g., CTLA-4, PD-1) by activated T cells and the suppression of effector T cells in trans by FOXP3-expressing T-regulatory (Treg) cells plays an important role in keeping peripheral homeostasis[6-7], however, the molecular mechanism is still not fully understood.

Histone deacetylases (HDACs) are epigenetic modifiers that are important for regulating gene expression, chromatin structure, and genomic stability. The 11 classical, zinc-dependent HDACs are categorized as class Ⅰ (HDAC1, HDAC2, HDAC3, and HDAC8), class Ⅱa (HDAC4, HDAC5, HDAC7, and HDAC9), class Ⅱb (HDAC6 and HDAC10), and class Ⅲ (HDAC11). HDAC3 belongs to the Class Ⅰ HDAC family, and has 60% amino acid identity with HDAC1 and HDAC2. Research has shown that HDAC3 plays a critical role in T cell development and maturation[8-9]. Whether HDAC3 is involved in the regulation of T cell homeostasis is unclear.

To begin to understand how HDAC3 might contribute to the homeostasis of T cells,we deleted this gene in $CD4^+CD8^+$ double positive (DP) stage of thymocytes using the cd4-cre transgene. The CD4Cre-mediated hdac3 deletion did not impact T cell development in the thymus but resulted in a dramatic loss of peripheral T cells. In addition, peripheral T cells in *hdac3* knock-out (*hdac3*KO) mice showed a dominant activation/effector/memory phenotype. Mechanism analysis showed an increased cell apoptosis which was accompanied by an increased cell cycle S phase accumulation in the peripheral T cells of *hdac3*KO mice. Moreover, Fas and FasL positive cells and FasL expression increased significantly in peripheral T cells of *hdac3*KO mice. *In vitro* TCR activation did not affect the apoptosis of normal peripheral T cells, but dramatically increased apoptosis of peripheral T cells from *hdac3*KO mice. Our results indicate that HDAC3 plays an important role in maintaining the homeostasis of peripheral T cells by refraining them from activation- induced cell death (AICD).

## 1 Materials and methods

### 1.1 Mice

Mice carrying a conditional floxed allele of *hdac3* ($hdac3^{flox}$, Stock No: 024119) were backcrossed onto the C57BL/6 background for 5 generations and then mated to C57BL/6 mice carrying the *cd4* enhancer/promoter/silence cre allele (obtained from The Jackson Laboratory, Stock No:022071) to generate the *hdac3* conditional knockout mice, designated as $hdac3^{fl/fl}cd4^{cre+}$ (*hdac3*KO). All WT mice, unless indicated otherwise, are littermate controls of $hdac3^{fl/fl}cd4^{cre-}$ mice. Experiments were conducted at 4-8 weeks of age, unless otherwise indicated. Mice were housed in a specific pathogen-free barrier unit. Handling of mice and experimental procedures were in accordance with relevant guidelines and regulations of the Animal Care and Use Committee in North China University of Science and Technology.

### 1.2 Genotyping

Offsprings were genotyped using the following PCR primer pairs: for *cd4-cre* mice, 5′ GCATTTCT-GGGGATTGCTTA 3′ and 5′ GTCATCCTTAGCGC-CGTAAA 3′ (product size, 354 bp); and for *hdac3-flox* mice, 5′ GGACACAGTCATGACCCGGTC 3′ and 5′ CTCTGGCTTCTGCTATGTCAAT-G 3′(product sizes: 504 bp from the $hdac3^{flox}$ allele and 464 bp from the wild-type *hdac3* allele); for the cross-breeding mice of *cd4-cre* and $hdac3^{flox/flox}$($hdac3^{fl/fl}cd4^{cre+}$, *hdac3*KO and $hdac3^{fl/fl}cd4^{cre-}$, WT), 5′ CCCAGGTTAGCTTTGAAC-TCT 3′ and 5′ CCACTGGCTTCTCCTAAGTTC 3′. The deletion allele (*hdac3*KO) produced a 211 bp PCR product, while the WT allele resulted in a 935 bp product.

### 1.3 Protein analyses

Thymocyte subsets, splenic B220$^+$ B cell, CD4$^+$ T cells and CD8$^+$ T cells were sorted by BD Aria Ⅱ Cell Sorting System from WT and *hdac3*KO mice. Protein lysates were prepared by lysis in PIPA buffer (containing protease inhibitors) and subjected to 12% SDS-PAGE analysis, 10 μg of lysate were loaded per lane. Anti-HDAC3 rabbit polyclonal antibody (H-99) was obtained from Santa Cruz Biotechnology. Anti-β-actin monoclonal antibody (AC-15) was purchased from Sigma-Aldrich.

### 1.4 FACS analyses

T cell populations from mouse thymus and spleen were analyzed by flow cytometry using the following monoclonal antibodies (mAbs) directly coupled to fluorochromes: CD4 (RM4–5), CD8 (53–6.7), FoxP3 (FJK-16s), CD44 (IM7), CD62L (MEL-14), CD69 (H1.2F3), Fas (15A7), FasL (MFL3), Acetyl-H3 (AH3-120). All the mAbs were purchased from BD Biosciences or eBioscience. FACS data were analyzed using CELLQuest Pro or FlowJo software. Apoptosis assays were carried out by staining with Annexin Ⅴ (BD Biosciences) according to the manufacturer's instructions. For T cell proliferation assay, Bromodeoxyuridine (BrdU, BD Biosciences) was injected at 1mg/mouse (i.p.). After 12 h of BrdU injection, thymocytes and spleen cells were analyzed using FITC BrdU Flow Kit (BD Biosciences) as per manufacturer's protocol.

### 1.5 Activation-induced cell death (AICD)

Splenic T cells ($2\times10^6$/ml) from naïve WT and *hdac3*KO mice were isolated and stimulated with plate-bound anti-CD3(2 mg/L) and anti-CD28(1 mg/L) mAbs in 24-well plates, in a final volume of 1 ml RPMI1640 supplemented with 10 mmol/L HEPES, 100 mg/L streptomycin, 100U/ml penicillin, 0.05 mmol/L 2-ME, 2 mmol/L glutamine (Life Technologies), for 24 h, they were washed twice with phosphate-buffered saline and detected apoptosis by staining with Annexin Ⅴ according to the manufacturer's instructions. For AICD, the *in vitro* activated WT and *hdac3*KO splenic T cells were then re stimulated in the same conditions as the first activation for another 24 h, and detected apoptosis.

### 1.6 Statistics

Statistical significance was determined by a 2-tailed parametric Student's $t$ test (GraphPad Prism software). A $P$ value less than 0.05 were considered significant. Data are shown as the mean ± SEM unless those indicated in the legend. Significance levels were set as following: $^*P < 0.05$; $^{**}P < 0.01$.

## 2 Results

### 2.1 T cell-specific deletion of HDAC3

To determine the physiological function of HDAC3 in T cell homeostasis, we generated a mouse strain bearing a conditional deletion of *hdac3* in the T-cell lineage by cross-breeding *cd4-cre* transgenic mice with $hdac3^{flox/flox}$ mice ($hdac3^{fl/fl}cd4^{cre+}$, *hdac3KO*), littermate $hdac3^{fl/fl}cd4^{cre-}$ mice were used as wild-type control (WT). Double-positive thymocytes (DP), single-positive CD4$^+$ thymocytes (CD4SP), single-positive CD8$^+$ thymocytes (CD8SP), splenic CD4$^+$T cells (CD4$^+$) and CD8$^+$ T cells (CD8$^+$) were sorted by BD Aria Ⅱ Cell Sorting System from WT and *hdac3*KO mice. The effective removal of *hdac3* in DP, CD4SP, CD8SP, splenic CD4$^+$ and CD8$^+$ T cells of *hdac3*KO mice was confirmed at the genomic level (Figure 1a). The reduction of HDAC3 protein was detected by Western-blot. As shown in Figure 1b and Figure 1c, while the level of HDAC3 was similar in the DP thymocytes of WT and *hdac3*KO mice, it reduced significantly in the CD4SP, CD8SP, splenic CD4$^+$ and CD8$^+$ T cells of *hdac3*KO mice compared with that of WT mice, consisting with the previous observations that HDAC3 is stable in DP thymocytes, and it is progressively reduced during positive selection and maturation upon CD4Cre-mediated deletion[8–10].

### 2.2 HDAC3 deficiency causes defective homeostasis of peripheral T cells

The proportions and absolute numbers of DN, DP, CD4SP and CD8SP thymocytes were comparable between WT and *hdac3*KO mice (Figure 2a and 2b), indicating that CD4Cre-mediated *hdac3* deletion did not impact T cell development in the thymus. However, deletion of *hdac3* led to a significant reduction of peripheral CD4$^+$ and CD8$^+$ T cells. About 90% of CD8$^+$ T cells and 75% of CD4$^+$ T cells were

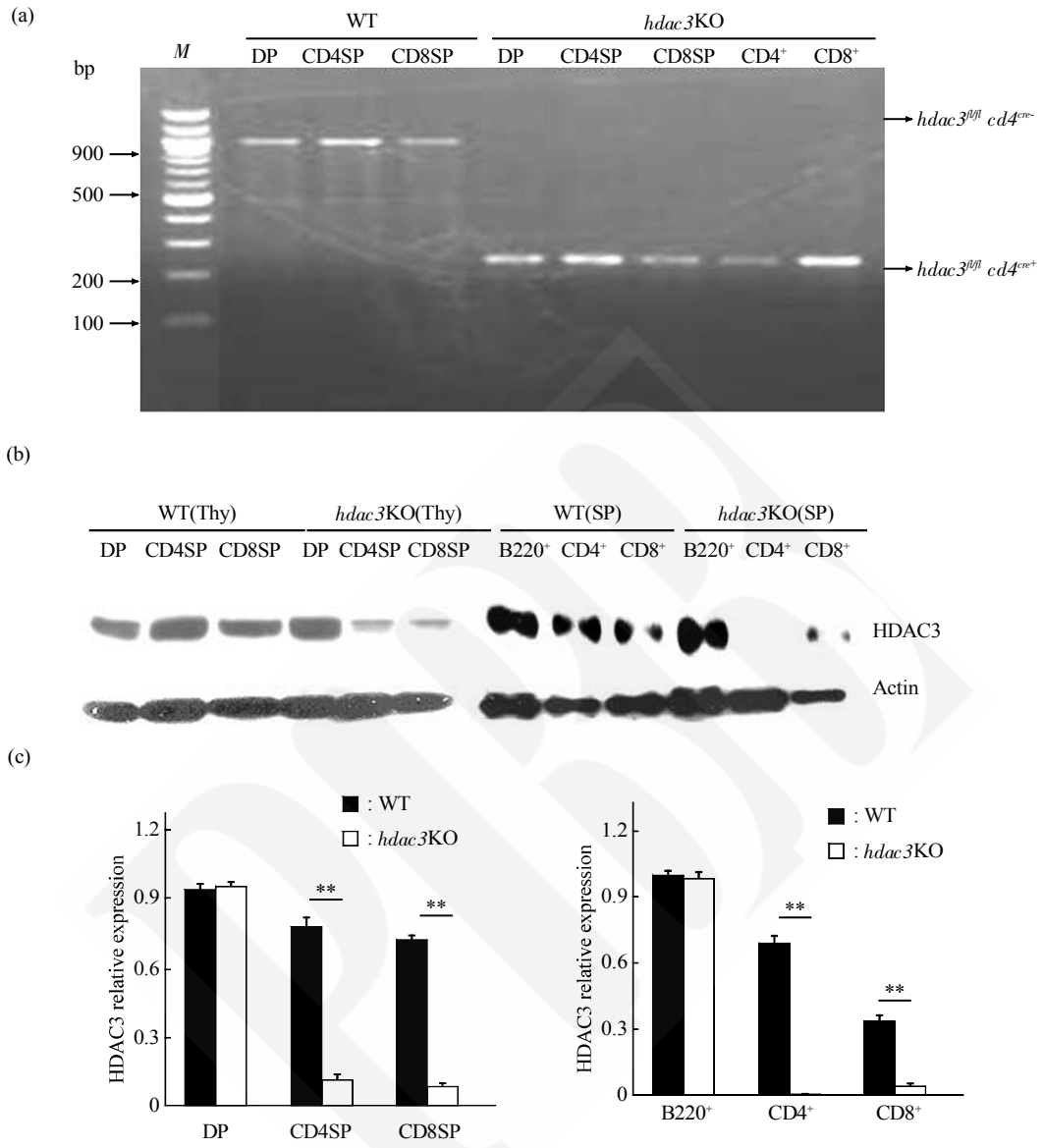


**Fig. 1 Deletion of hdac3 gene by CD4Cre enzyme**

(a) PCR analysis of genomic DNA isolated from subsets of thymocytes and splenic T cells of WT (*hdac3*$^{fl/fl}$ *cd4*$^{cre-}$) and *hdac3*KO (*hdac3*$^{fl/fl}$ *cd4*$^{cre+}$) mice. Primers that flank the floxed region of *hdac3* were used. WT cells produce a 935 bp band and *hdac3*KO cells produce a 211 bp band. WT, wild type control mice; *hdac3*KO, *hdac3* conditional knock-out mice. (b) Western-blot analysis of HDAC3 expression using protein extracted from subsets of thymocytes and splenic T cells of WT and *hdac3*KO mice, the levels of Actin served as the loading control. One representative experiment out of three performed with two or three mice of per genotype in each experiment. (c) Relative HDAC3 protein expression in DP, CD4SP, CD8SP thymocytes and splenic B cells, CD4$^+$ and CD8$^+$ T cells of WT and *hdac3*KO mice were quantified using ImageJ software and plotted. *$P < 0.05$;**$P < 0.01$.

lost in the spleen of *hdac3*KO mice compared with that of WT mice (Figure 2c and 2d). Analysis of naïve and effector/memory T cells in the spleen of *hdac3*KO mice revealed decreased ratio and number of naïve T cells (CD4$^+$/CD44$^-$/CD62L$^+$ and CD8$^+$/CD44$^-$/CD62L$^+$) compared with that of WT mice. Conversely, the ratio of effector memory T cells (CD44$^+$/CD62L$^-$) increased significantly in CD4$^+$ and CD8$^+$ T cells of *hdac3*KO mice, and the ratio of central memory T cells (CD44$^+$/CD62L$^+$) increased as well in CD8$^+$ T cells of *hdac3*KO mice. However, the number of effector/control memory T cells reduced significantly in *hdac3*KO mice compared with that of WT mice (Figure 2e and 2f). These results demonstrated that HDAC3 plays a critical role in maintaining peripheral T-cell homeostasis.

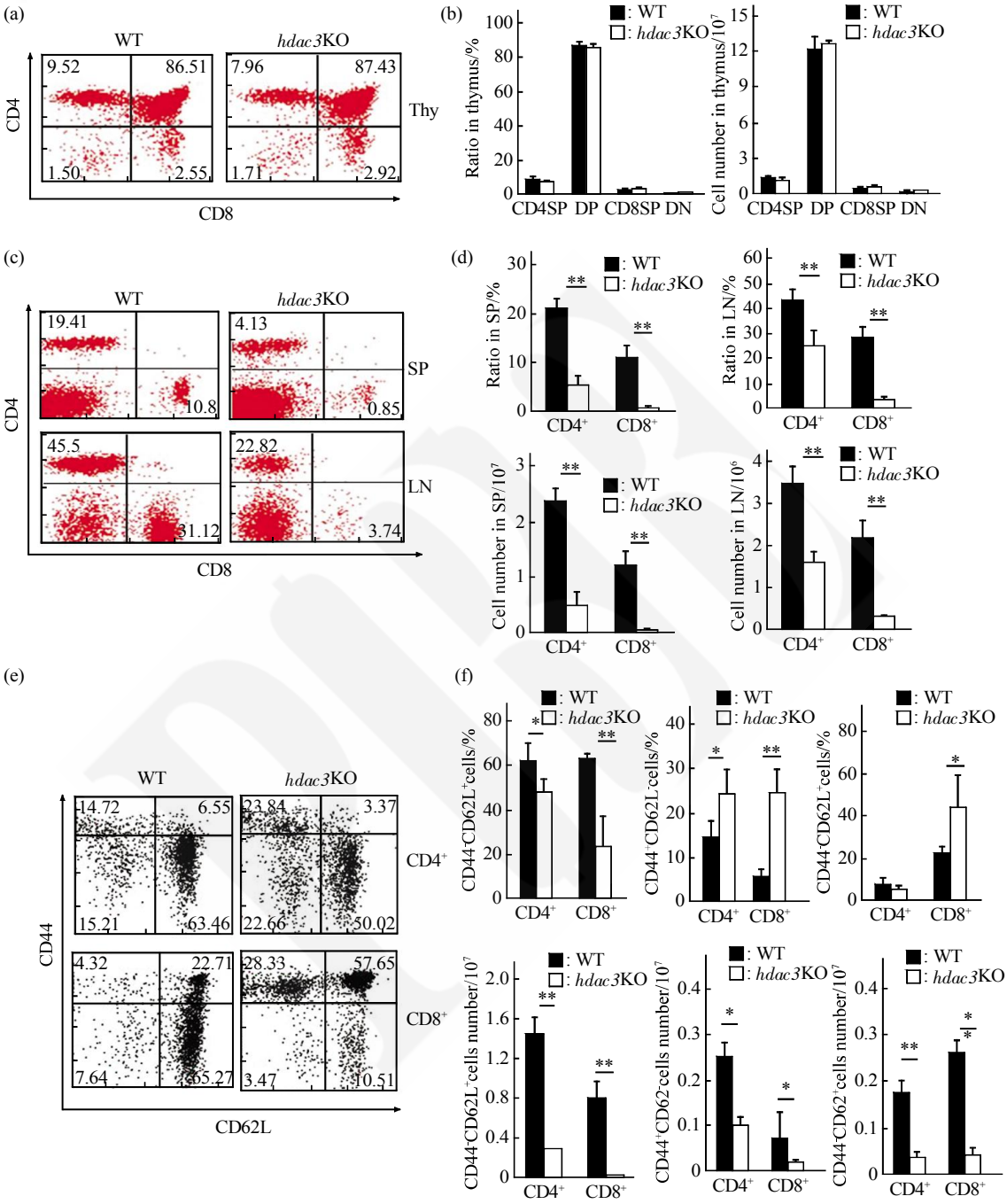


**Fig. 2　HDAC3 deficiency causes defective homeostasis of peripheral T cells**

(a) FACS analysis of thymocyte subsets using anti-CD4 and anti-CD8 antibodies in WT and *hdac3*KO mice. Representative dot plots are from one out of five independent experiments with three to four mice per genotype in each experiment. Numbers in the dot plot indicate the percentage of cells in the respective quadrants. (b) Diagrams showing the summary of the percentage and cell number of CD4SP, DP, CD8SP and DN thymocytes in WT and *hdac3*KO mice. (c) FACS analysis of peripheral $CD4^+$ and $CD8^+$T cells in the spleen of WT and *hdac3*KO mice. Representative dot plots are from one out of five independent experiments with three to four mice per genotype in each experiment. Thy: thymus, SP: spleen, LN: lymph nodes. (d) Summary of the percentage and number of $CD4^+$ and $CD8^+$ T cells in the spleen of WT and *hdac3*KO mice. (e) Naïve and effector /memory T cell analysis in the peripheral T cells of WT and *hdac3*KO mice. (f) Diagrams showing the summary of the percentage and number of naïve and effector /memory T cell in peripheral T cells of WT and *hdac3*KO mice.

### 2.3 HDAC3 deficiency leads to increased apoptosis and proliferation of peripheral T cells

The reduction of peripheral T cells could originate from either increased cell death or impaired cell proliferation, or both. Flow cytometry analysis revealed that $CD4^+$ and $CD8^+$ T cells in *hdac3*KO mice were more susceptible to apoptosis than that of WT mice (Figure 3a and 3b). Interestingly, *in vivo* BrdU labeling revealed accelerated proliferation of $CD4^+$ and $CD8^+$ T cells in *hdac3*KO mice compared with that of WT mice (Figure 3c and 3d). Moreover, the ratio of CD69-positive $CD4^+$ and $CD8^+$ T cells increased, though their number decreased in *hdac3*KO mice compared with that of WT mice (Figure 3e and 3f). As sensitization towards activation-induced cell death (AICD) is accompanied by T cell proliferation[11], these results indicated that the loss of peripheral T cell in *hdac3*KO mice may result from AICD.

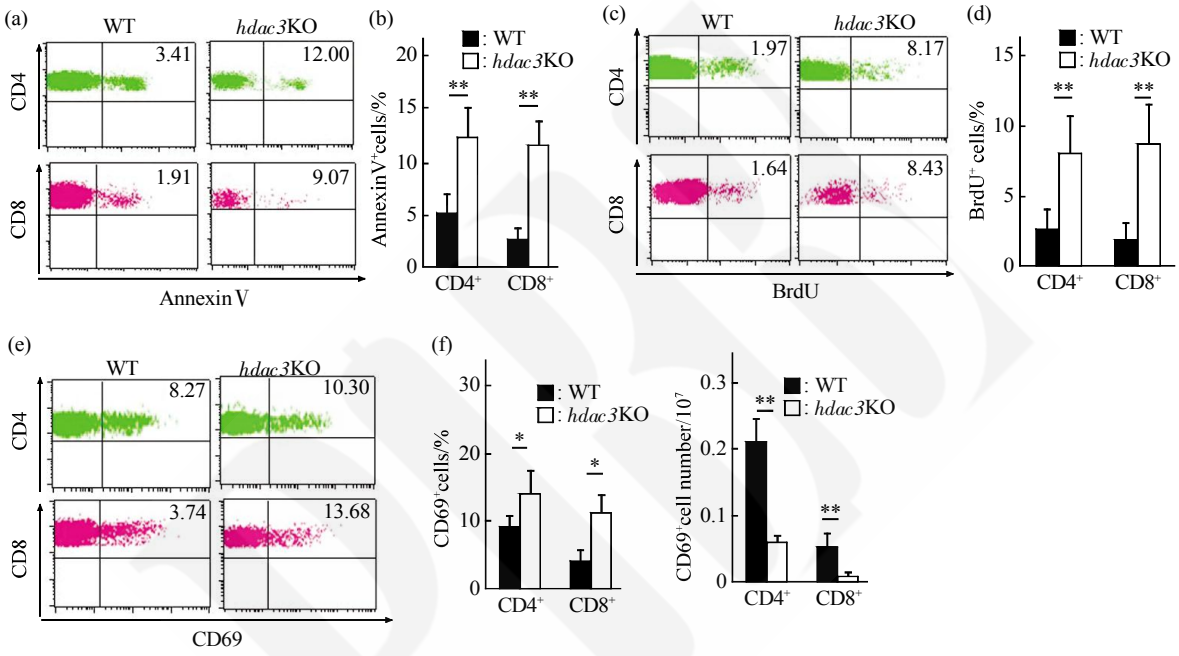


**Fig. 3 Increased apoptosis and proliferation of peripheral T cells in hdac3KO mice**

(a) FACS analysis of Annexin V positive cells in peripheral $CD4^+$(top) and $CD8^+$ (bottom)T cells of WT and *hdac3*KO mice. Representative dot plots are from one out of four independent experiments with three to four mice per genotype in each experiment. (b) Diagrams showing summary of the percentage of AnnexinV positive cells in peripheral $CD4^+$ and $CD8^+$ T cells of WT and *hdac3*KO mice. (c) FACS analysis of BrdU incorporation in peripheral $CD4^+$ and $CD8^+$ T cells of WT and *hdac3*KO mice. Representative dot plots are from one out of four independent experiments with three to four mice per genotype in each experiment.(d) Diagrams showing summary of the percentage of BrdU positive cells in peripheral $CD4^+$ and $CD8^+$T cells of WT and *hdac3*KO mice. (e) Plots showing CD69 positive cells in peripheral $CD4^+$ and $CD8^+$T cells of WT and *hdac3*KO mice. (f) Summary of the percentage and number of CD69 positive cells in peripheral $CD4^+$ and $CD8^+$T cells of WT and *hdac3*KO mice.

### 2.4 HDAC3 deficiency leads to increased Fas-and FasL-expressing cells

To explore the mechanism of apoptosis of peripheral T cells in *hdac3*KO mice, we detected the expression of apoptosis markers by flow cytometry. As shown in Figure 4a and 4b, the ratio of Fas- and FasL-positive cells increased significantly in the peripheral T cells of *hdac3*KO mice compared with that of WT mice, while the ratio of p53-, p21- and Bcl-2- positive T cells are comparable between *hdac3*KO and WT mice. The expression level of FasL, which was represented by the MFI (mean fluorescence intensity), was higher in $CD4^+$ and $CD8^+$ T cells of *hdac3*KO mice than that of WT mice, while the expression level of Fas, p53 and p21 were only slightly increased in $CD4^+$ and $CD8^+$ T cells of *hdac3*KO mice. The expression level of Bcl-2 did not change in $CD4^+$ T cells of *hdac3*KO mice, however, it increased significantly in $CD8^+$ T cells of *hdac3*KO mice (Figure 4c). These results indicated a Fas/FasL-dependent pathway for the apoptosis of peripheral T cells in *hdac3*KO mice.

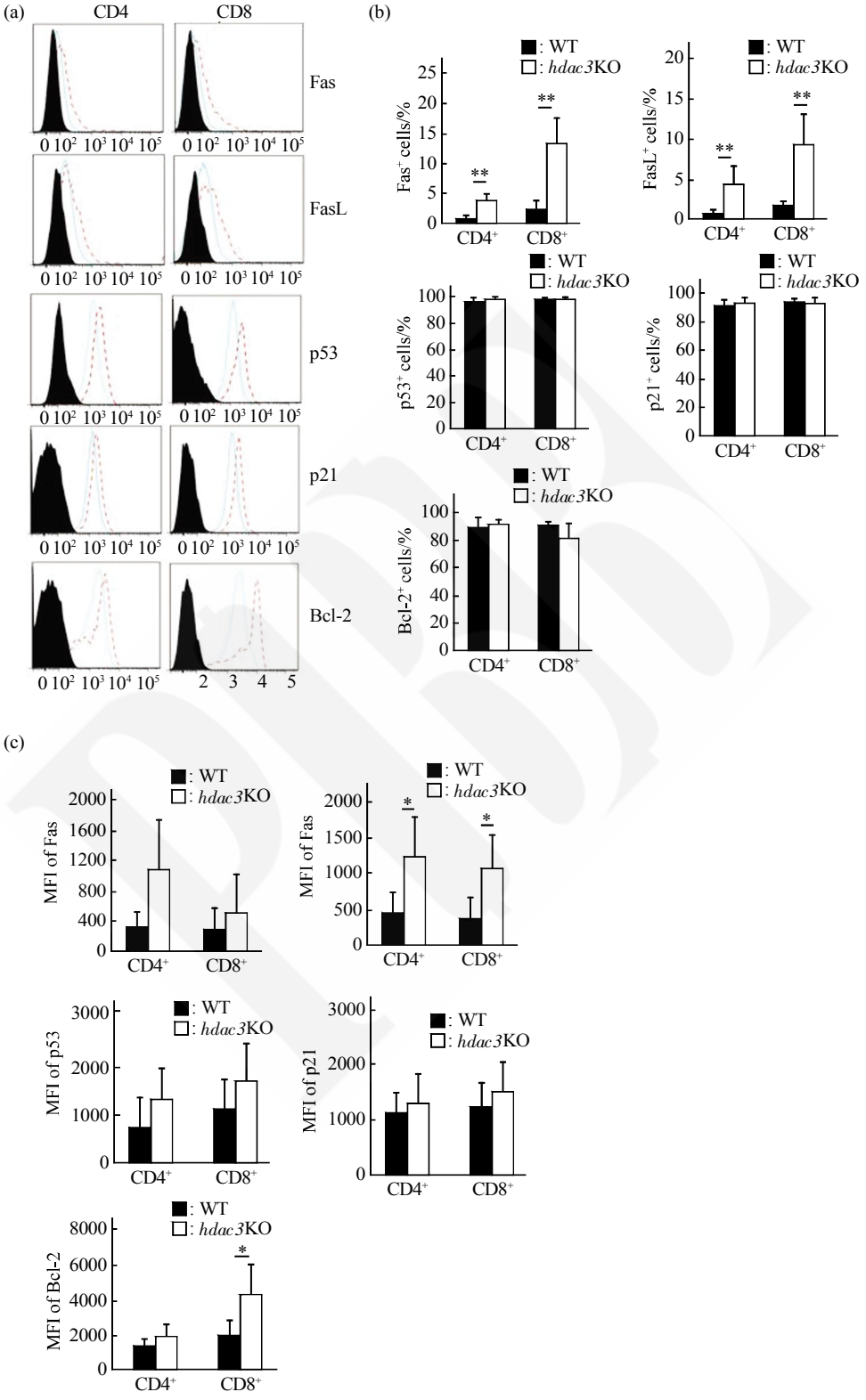


**Fig. 4　Expression of apoptosis markers in the peripheral T cells of hdac3KO mice**

(a)Histograms depict Fas, FasL, p53, p21 and Bcl-2 expression in peripheral $CD4^+$ and $CD8^+$T cells of WT and *hdac3*KO mice. Shaded histograms represent isotype control, solid lines represent WT mice, dotted lines represent *hdac3*KO mice. Representative histogram plots are from one out of three independent experiments with two to three mice per genotype in each experiment. (b) Diagrams showing the summary of the percentage of Fas, FasL, p53, p21 and Bcl-2 positive cells in peripheral $CD4^+$ and $CD8^+$ T cells of WT and *hdac3*KO mice.(c) Diagrams showing the summary of Fas, FasL, p53, p21 and Bcl-2 expression in peripheral $CD4^+$ and $CD8^+$ T cells of WT and *hdac3*KO mice.

### 2.5 HDAC3 deficiency promotes AICD of peripheral T cells

As Fas and FasL are commonly regarded as critical mediators of AICD, and AICD comes from re-activation of pre-activated T cells. The freshly isolated CD4$^+$ and CD8$^+$ T cells from *hdac3*KO and WT mice were stimulated *in vitro* with anti-CD3 plus anti-CD28 Abs. After the first 24 h of activation, CD4$^+$ and CD8$^+$ T cells from WT mice showed no apoptosis compared with their freshly isolated counterparts, and re-activation for another 24 h increased their apoptosis significantly (Figure 5a and 5b *vs*. Figure 3a and 3b).

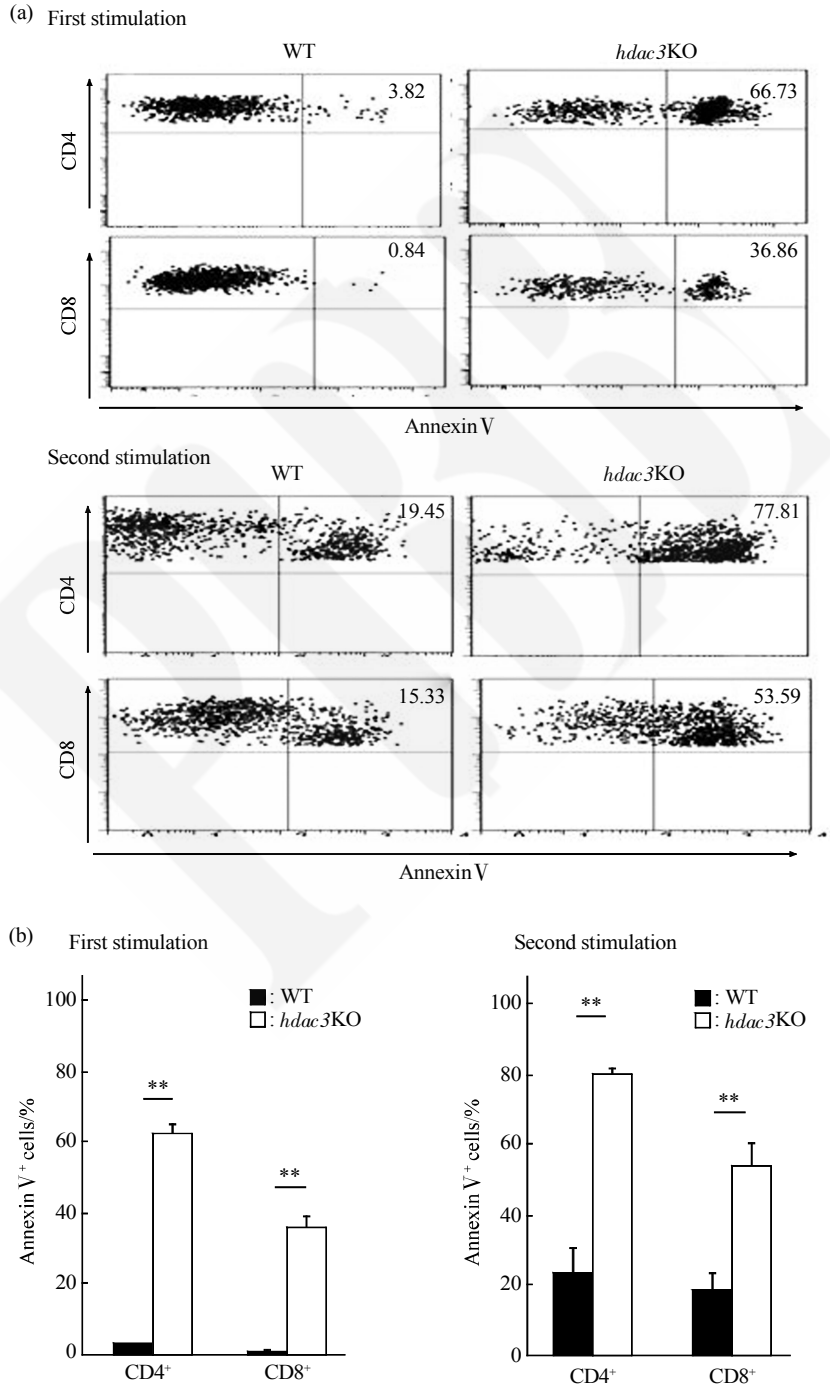


**Fig. 5 In vitro activation promotes the apoptosis of peripheral T cells of hdac3KO mice**

(a) Freshly isolated splenic T cells from WT and *hdac3*KO mice were first activated with anti-CD3 and anti-CD28 antibodies for 24 h, cells were collected for Annexin V staining to detect apoptosis. The activated cells were then washed with PBS to remove dead cells and reactivated for another 24 h, and cell apoptosis were detected. Representative dot plots are from one out of three independent experiments with two to three mice per genotype in each experiment. (b) Diagrams showing the apoptosis of peripheral CD4$^+$ and CD8$^+$ T cells from WT and *hdac3*KO mice after *in vitro* stimulation with CD3/CD28 antibodies for the first and second time.

However, $CD4^+$ and $CD8^+$ T cells from *hdac3*KO mice showed about a 4-fold increase of apoptosis compared to their freshly isolated counterparts after the first activation, and re-activation further increased their apoptosis significantly(Figure 5a and 5b *vs*. Figure 3a and 3b). These data demonstrated that peripheral T cells in *hdac3*KO mice were already in an activation state, and continuous activation promoted their AICD.

### 2.6 Histone acetylation increases in the peripheral T cells of hdac3KO mice

Histone deacetylases function to remove acetyl groups from lysine residues on the histones [12]. To explore the effect of HDAC3 deletion on the acetylation level of peripheral T cells，we examined the acetylation status of histone H3 *in vivo* by intracellular staining. As shown in Figure 6a and 6b, the acetylation level of H3 which was represented by the MFI of H3 staining increased significantly in both $CD4^+$ and $CD8^+$ T cells of *hdac3*KO mice compared with that of WT mice, indicating HDAC3 may function by altering histone acetylation in peripheral T cells.

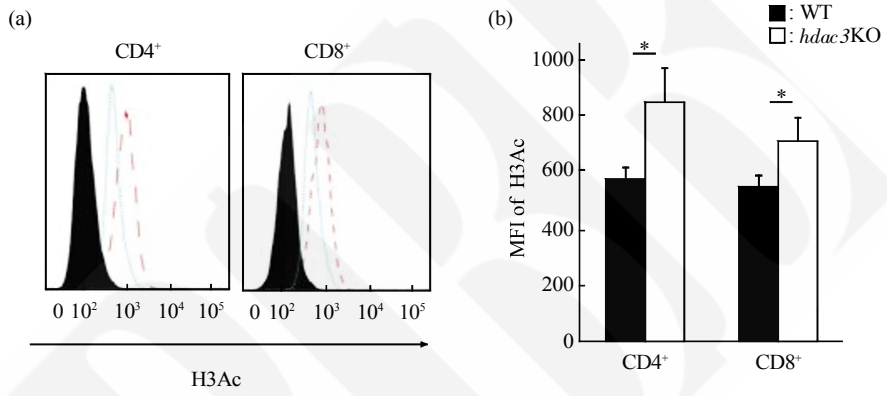


**Fig. 6 Increased histone acetylation in peripheral T cells of hdac3KO mice**

(a) Histograms showed the acetylation level of histone H3 in peripheral $CD4^+$ and $CD8^+$T cells of WT and *hdac3*KO mice. Mouse monoclonal anti-histone H3 (acetyl K9) antibody [AH3-120] and FITC-conjugated rabbit anti-mouse antibody were used to detect acetyl-Histone3, mouse monoclonal IgG1 was used as an isotype control. Shaded histograms represented isotype control, solid lines represented WT mice, dotted lines represented *hdac3*KO mice. Data shown are representative of three independent experiments with three to four mice per genotype in each experiment. (b) Diagrams showing the summary of the acetylation level of histone H3 in peripheral $CD4^+$ and $CD8^+$ T cells of WT and *hdac3*KO mice.

## 3 Discussion

Recent studies of HDAC3 in T cells have revealed that HDAC3 plays a critical role in T cell development and maturation. Conditional deletion of *hdac3* using *lck-cre* transgene impaired T cell development at the CD8SP and DP stage, while fully rearranged T cell receptor αβ transgene compensates these defects, indicating HDAC3 involves in the T cell development by regulating TCR signaling pathway [8]. Using *cd4-cre* transgene, Hsu FC *et al* demonstrated that HDAC3 deficiency led to a block of T cell maturation, and HDAC3-deficient peripheral T cells display a complement -mediated cell loss [9]. In this study, we further examined the role of HDAC3 in T-cell homeostasis by using *cd4-cre* transgene. Though CD4Cre is activated from early DP stage on, thymocyte subpopulations in *hdac3*KO mice are similar to that of WT mice. This could be resulted from the stability of HDAC3 protein, but might also because the depletion was too modest in the thymus to elicit a phenotypic improvement, these results are similar to a comparable lack of obvious phenotype in other studies using CD4Cre-mediated *hdac3* deletion. However, the number of peripheral T cells decreased significantly in *hdac3*KO mice. In addition, peripheral T cells in *hdac3*KO mice displayed active phenotypes, increased cell apoptosis and accelerated cell

proliferation simultaneously. Moreover, peripheral T cells from *hdac3*KO mice underwent obvious apoptosis once they were activated *in vitro*. Therefore, our results suggested that HDAC3 plays an important role in the homeostasis of peripheral T cells by keeping them from activation and activation-induced cell death (AICD).

The increased ratio but decreased number of $CD44^+/CD62L^-$ and $CD69^+$ T cells in *hdac3*KO mice indicated that HDAC3 deficiency resulted in higher activation and death of peripheral T cells than that of WT mice [13]. The mechanism that leads to the activation of peripheral T cells in *hdac3*KO mice is not clear at present. It likely results from both the exogenous and endogenous pathways. The exogenous pathway is the loss of suppression by $CD4^+$ $FOXP3^+$ regulatory T cells (Treg), as reported by Wang *et al*, HDAC3 deletion compromises Treg suppressive function, and thus peripheral tolerance [10]. The endogenous pathway results from the loss of DAF (CD55) expression on the HDAC3-deficient T cells, as CD55 has been shown to play an indirect role in suppressing T cell activation by limiting the generation of C3 cleavage products, such as C3a, and subsequently diminished C5 convertase activity[14].

We considered that the aberrant T-cell activation may be responsible for the loss of peripheral T cell in *hdac3*KO mice. AICD is a contraction phase of the expanded T cells only after their re-stimulation, and it is mainly mediated by the Fas-FasL signal pathway[1, 15]. Consistent with their reports, we found that both the ratio of Fas and FasL positive cells and the expression of FasL increased significantly in the peripheral T cells of *hdac3*KO mice compared with that of WT mice. In addition, unlike normal peripheral T cells which underwent significant apoptosis only after the second stimulation, HDAC3-dificient peripheral T cells underwent significant apoptosis after the first stimulation, and further increased apoptosis after the second stimulation.Therefore, HDAC3 deficiency results in the aberrant AICD and dramatic loss of peripheral T cell.

Several studies have demonstrated that HDAC3 deficiency results in defects in DNA replication and triggers DNA damage induced cell apoptosis, which is often accompanied by the activation of p53/p21 pathway [16–17]. The slightly increased p53 and p21 expression in HDAC3-deficient $CD4^+$ and $CD8^+$ T cells indicated that cell apoptosis induced by DNA damage may play a minor role in the impaired homeostasis of peripheral T cell in *hdac3*KO mice. Lipid rafts constitute a dynamic signaling platform by providing initiation, spatial regulation, and sustenance of TCR-dependent signal transduction. Much of the focus in death receptor signaling has been on the importance of lipid rafts as platforms for Fas receptor signaling[18–19]. In addition, it has been reported that the HDAC1/3-specific inhibitor, MS-275, sensitizes FasL-induced cell death by up-regulating Fas expression and its accumulation in the membrane lipid rafts [20]. Therefore, the FasL-induced cell death of peripheral T cell in *hdac3*KO mice may be resulted from the Fas accumulation in the membrane lipid rafts, and how HDAC3 affects TCR signal transduction through lipid rafts will be an very interesting subject to be further studied.

Bcl-2 expression is similar in $CD4^+$ T cells of WT and *hdac3*KO mice, while it increased significantly in $CD8^+$ T cells of *hdac3*KO mice compared with that of WT mice. It is well known that increased Bcl-2 expression usually promotes cell proliferation and resists apoptosis, however, Bcl-2 expression did not play an important role in AICD [21]. In addition, we found that the ratio of Bcl-2 positive $CD8^+$T cells showed a reduced trend in *hdac3*KO mice compared with that of WT mice. Therefore, we concluded that the increased expression of Bcl-2 in $CD8^+$T cells of *hdac3*KO mice resulted from the remaining high frequency of memory phenotype after a robust of AICD of the activated cells [22–23]. The enhanced acetylation level of histone may be account for the increased expression of FasL and Bcl-2 in *hdac3*KO T cells as well. However, since HDAC3 modulates numerous other proteins such as class Ⅱ HDACs (4, 5, 7, and 10) and nonhistone substrates, the exact mechanism need to be further studied[24].

In summary, our results presented here support a brake role of HDAC3 in keeping peripheral T cells

from aberrant AICD. Knockdown of HDAC3 promoted global histone acetylation, TCR signaling activation and FasL expression, indicating an epigenetic regulation of TCR signaling and FasL expression by HDAC3 in peripheral T cells. In addition, it seems that $CD8^+$ cells are more sensitive to HDAC3-deficiency induced AICD than $CD4^+$ cells. The detailed mechanism associated with the HDAC3 deficiency-induced FasL expression and peripheral T cell apoptosis need to be further investigated.

## References


[1] Saint Fleur S, Hoshino A, Kondo K, *et al*. Regulation of Fas-mediated immune homeostasis by an activation-induced protein. Cyclon Blood, 2009, **114**(7): 1355−1365

[2] Brenner D, Krammer P H, Arnold R. Concepts of activated T cell death. Crit Rev Oncol Hematol, 2008, **66**(1): 52−64

[3] Marsden V S, Strasser A. Control of apoptosis in the immune system: Bcl-2, BH3-only proteins and more. Annu Rev Immunol, 2003, **21**: 71−105

[4] De Panfilis G, Caruso A, Sansoni P, *et al*. Identification of Fas-L-expressing apoptotic T lymphocytes in normal human peripheral blood: *in vivo* suicide. Am J Pathol, 2001, **158**(2): 387−391

[5] Fisher G H, Rosenberg F J, Straus S E, *et al*. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell, 1995, **81**(6): 935−946

[6] Bour-Jordan H, Esensten J H, Martinez-Llordella M, *et al*. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/B7 family. Immunol Rev, 2011, **241** (1): 180−205

[7] Xing Y, Hogquist K A. T-cell tolerance: central and peripheral. Cold Spring Harb Perspect Biol, 2012, **4**(6): pii: a006957

[8] Stengel K R, Zhao Y, Klus N J, *et al*. Histone deacetylase 3 is required for efficient T cell development. Mol Cell Biol. 2015, **35**(22): 3854−3865

[9] Hsu F C, Belmonte P J, Constans M M 1, *et al*. Histone deacetylase 3 is required for T cell maturation. J Immunol, 2015, **195** (4): 1578−1590

[10] Wang L, Liu Y, Han R, *et al*. FOXP3 (+) regulatory T cell development and function require histone/protein deacetylase 3. J Clin Invest, 2015, **125**(3): 1111−1123

[11] Radvanyi L G, Shi Y, Mills G B, *et al*. Cell cycle progression out of G1 sensitizes primary-cultured nontransformed T cells to TCR-mediated apoptosis. Cell Immunol, 1996, **170**(2): 260−273

[12] Gray S G, Ekström T J. The human histone deacetylase family. Exp Cell Res, 2001, **262**(2): 75−83

[13] Radulovic K, Rossini V, Manta C, *et al*. The early activation marker CD69 regulates the expression of chemokines and CD4 T cell accumulation in intestine. PLoS One, 2013, **8**(6): e65413

[14] Fang C, Miwa T, Song W C, *et al*. Decay-accelerating factor regulates T-cell immunity in the context of inflammation by influencing costimulatory molecule expression on antigen-presenting cells. Blood, 2011, **118**(4): 1008−1014

[15] Dhein J, Walczak H, Bäumler C, *et al*. Autocrine T-cell suicide mediated by APO-1/ (Fas/CD95). Nature, 1995, **373** (6513): 438−441

[16] Summers A R, Fischer M A, Stengel K R, *et al*. HDAC3 is essential for DNA replication in hematopoietic progenitor cells. J Clin Invest, 2013, **123**(7): 3112−3123

[17] Bhaskara S, Knutson S K, Jiang G, *et al*. Hdac3 is essential for the maintenance of chromatin structure and genome stability. Cancer Cell, 2010, **18**(5): 436−447

[18] Chaigne-Delalande B, Moreau J F, Legembre P. Rewinding the DISC. Arch Immunol Ther Exp (Warsz), 2008, **56**(1): 9−14

[19] Bionda C, Athias A, Poncet D, *et al*. Differential regulation of cell death in head and neck cell carcinoma through alteration of cholesterol levels in lipid rafts microdomains. Biochem Pharmacol, 2008, **75**(3): 761−772

[20] Rao-Bindal K, Zhou Z, Kleinerman E S. MS-275 sensitizes osteosarcoma cells to Fas ligandinduced cell death by increasing the localization of Fas in membrane lipid rafts. Cell Death Dis, 2012, **3**: e369

[21] Guerrero A D, Welschhans R L, Chen M, *et al*. Cleavage of anti-apoptotic Bcl-2 family members after TCR stimulation contributes to the decision between T cell activation and apoptosis. J Immunol, 2013, **190**(1): 168−173

[22] Grayson J M, Zajac A J, Altman J D, *et al*. Cutting edge: increased expression of Bcl-2 in antigen-specific memory $CD8^+$ T cells. J Immunol, 2000, **164**(8): 3950−3954

[23] Dunkle A, Dzhagalov I, Gordy C, *et al*. Transfer of $CD8^+$ T cell memory using Bcl-2 as a marker. J Immunol, 2013, **190** (3): 940−947

[24] McQuown S C, Wood M A. HDAC3 and the molecular brake pad hypothesis. Neurobiol Learn Mem, 2011, **96**(1): 27−34

# 组蛋白去乙酰化酶 3 通过抑制 AICD 维持 T 细胞自稳 *

王 姗[1)**] 田 枫[2)**] 千 晔[1)] 刘 颖[2)] 李会婷[2)] 张爱红[3)]
侯志宏[1)] 刘亚楠[1)] 李 娟[1)] 张艳淑[4)] 赵 勇[5)] 郑全辉[1)***]

([1)] 华北理工大学基础医学院，河北省慢性疾病重点实验室，唐山市慢性病临床基础研究重点实验室，唐山 063000；
[2)] 北京大学医学部实验动物部，北京 100191；[3)] 唐山市工人医院，唐山 063000；[4)] 华北理工大学公共卫生学院，唐山 063000；
[5)] 中国科学院动物研究所膜生物国家重点实验室，北京 100101)

**摘要** 为探讨组蛋白去乙酰化酶 3(HDAC3)在 T 细胞自稳中的作用，采用 *LoxP-cd4cre* 酶系统在胸腺 $CD4^+CD8^+$ 双阳性 T 细胞(DP)中敲除 *hdac3* 基因．*hdac3* 基因敲除小鼠不影响 T 细胞在胸腺中的发育，但导致外周 T 细胞显著降低，而且，*hdac3* 基因敲除的外周 T 细胞主要以活化 / 效应 / 记忆表型为主．机制分析表明，*hdac3* 基因敲除的外周 T 细胞凋亡增加并伴随细胞增殖加速，同时，Fas 和 Fas 配体阳性细胞比率以及 Fas 配体的表达显著增加．体外 TCR 活化不影响正常外周 T 细胞的凋亡，但导致 *hdac3* 基因敲除的外周 T 细胞凋亡显著增加．实验结果表明，HDAC3 通过抑制活化诱导的细胞凋亡维持外周 T 细胞自稳．

**关键词** 外周 T 细胞， 组蛋白去乙酰化酶 3， 活化诱导的细胞凋亡，Fas， Fas 配体
**学科分类号** R392.11 **DOI**: 10.16476/j.pibb.2017.0333

* 国家自然科学基金面上项目 (81373111, 81673208)资助.
** 并列第一作者.
*** 通讯联系人.
Tel: 0315-8805516, E-mail: 1078209929@qq.com
收稿日期：2017-08-14，接受日期：2017-12-14