Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 2022 23(5):392-406
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Research Article

https://doi.org/10.1631/jzus.B2100780

Romidepsin (FK228) improves the survival of allogeneic skin grafts through downregulating the production of donor-specific antibody via suppressing the IRE1α-XBP1 pathway

Yuliang GUO[1,2], Siyu SONG[1,2], Xiaoxiao DU[3], Li TIAN[1,2], Man ZHANG[1,2], Hongmin ZHOU[4], Zhonghua Klaus CHEN[1,2], Sheng CHANG[1,2]✉

[1]*Institute of Organ Transplantation, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*
[2]*Key Laboratory of Organ Transplantation, Ministry of Education, NHC Key Laboratory of Organ Transplantation, Key Laboratory of Organ Transplantation, Chinese Academy of Medical Sciences, Wuhan 430030, China*
[3]*Henan Key Laboratory of Digestive Organ Transplantation, Open and Key Laboratory of Hepatobiliary & Pancreatic Surgery and Digestive Organ Transplantation at Henan Universities, Zhengzhou Key Laboratory of Hepatobiliary & Pancreatic Diseases and Organ Transplantation, Department of Hepatobiliary and Pancreatic Surgery, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China*
[4]*Department of Cardiothoracic and Vascular Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*

Abstract: Antibody-mediated rejection (AMR) is one of the major causes of graft loss after transplantation. Recently, the regulation of B cell differentiation and the prevention of donor-specific antibody (DSA) production have gained increased attention in transplant research. Herein, we established a secondary allogeneic in vivo skin transplant model to study the effects of romidepsin (FK228) on DSA. The survival of grafted skins was monitored daily. The serum levels of DSA and the number of relevant immunocytes in the recipient spleens were evaluated by flow cytometry. Then, we isolated and purified B cells from B6 mouse spleens in vitro by magnetic bead sorting. The B cells were cultured with interleukin-4 (IL-4) and anti-clusters of differentiation 40 (CD40) antibody with or without FK228 treatment. The immunoglobulin G1 (IgG1) and IgM levels in the supernatant were evaluated by enzyme-linked immunosorbent assay (ELISA). Quantitative reverse transcription-polymerase chain reaction (RT-qPCR) and western blotting were conducted to determine the corresponding levels of messenger RNA (mRNA) and protein expression in cultured cells and the recipient spleens. The results showed that FK228 significantly improved the survival of allogeneic skin grafts. Moreover, FK228 inhibited DSA production in the serum along with the suppression of histone deacetylase 1 (HADC1) and HDAC2 and the upregulation of the acetylation of histones H2A and H3. It also inhibited the differentiation of B cells to plasma cells, decreased the transcription of positive regulatory domain-containing 1 (*Prdm*1) and X-box-binding protein 1 (*Xbp1*), and decreased the expression of phosphorylated inositol-requiring enzyme 1 α (p-IRE1α), XBP1, and B lymphocyte-induced maturation protein-1 (Blimp-1). In conclusion, FK228 could decrease the production of antibodies by B cells via inhibition of the IRE1α-XBP1 signaling pathway. Thus, FK228 is considered as a promising therapeutic agent for the clinical treatment of AMR.

Key words: Histone acetylation; Romidepsin (FK228); Skin transplantation; Donor-specific antibody; Unfolded protein response

1 Introduction

Histone deacetylases (HDACs) are vital members of the proteinase family involved in epigenetic regulation, which can alternate the acetylation status of chromatin histones in the nucleus and act in the balance of acetylation/deacetylation (Rafehi et al., 2017). Depending on the differences in molecular structure, HDACs are divided into four classes: class I (HDACs 1–3 and 8), class II (HDACs 4–7, 9, and 10), class III (Sirtuins (SIRTs) 1–7), and class IV (HDAC 11) (Bezu et al., 2019; Gujral et al., 2020). Structurally contributing to the huge multi-subunit complexes, HDACs have thousands of histone targets that correlate with the transcription and expression of various genes,

✉ Sheng CHANG, changsheng2000@126.com
Sheng CHANG, https://orcid.org/0000-0002-0820-0934

Received Sept. 11, 2021; Revision accepted Dec. 29, 2021; Crosschecked May 11, 2022

exert specific effects on a variety of cellular biological activities, and play certain roles in human diseases. In cancer and inflammation research, HDACs have been reported to be involved in regulating several critical cellular processes, including DNA damage repair, cell cycle arrest, metabolism, and oncogene activation (Peng et al., 2019).

With the continuous improvement of immunosuppressive regimens in transplantation, the incidence of T cell-mediated acute rejection has been dramatically reduced, and the short-term survival of grafts has been significantly prolonged. However, long-term graft survival must be urgently improved (Loupy and Lefaucheur, 2018). Antibody-mediated rejection (AMR) has been identified as the primary cause of end-stage graft loss (Li et al., 2019; Clotet-Freixas et al., 2020), for which only limited therapeutic regimens are available, including plasma exchange, large doses of intravenous immunoglobulin (Ig), rituximab, and bortezomib, which can remove pathogenic antibodies. However, the efficacy of these treatments is limited and they can result in irreversible complications in patients (Chong, 2019). The production of donor-specific antibodies (DSAs) in transplant recipients, including pre-existing DSA and de novo DSA, could hamper the functionality and long-term survival of grafts. Accordingly, preventing the generation of de novo DSA and reducing the level of preformed DSAs are vital to the clinical treatment of AMR (McCaughan and Tinckam, 2018).

DSAs are produced by plasma cells previously exposed to major histocompatibility complex (MHC) antigens by organ perfusion, transfusion, transplantation, pregnancy, or de novo post-transplantation (Clatworthy et al., 2010), each of which can generate thousands of antibodies per second. The expansions of endoplasmic reticulum (ER) and Golgi complex are prerequisites for plasma cells to meet the metabolic demands of antibody production, and the ultrahigh rate of antibody synthesis must be efficiently regulated by the expression of several genes, protein modifications, and signal pathways to match the Ig folding and secretion capacity. Moreover, inositol-requiring enzyme 1 α (IRE1α) is the only ER unfolded protein response (UPR) molecule required to produce antibodies (Aragon et al., 2012; Jurkin et al., 2014).

Within the HDAC family, class I HDACs exist in various tissues and play important roles in regulating cell metabolism and proliferation. HDAC1 and HDAC2 are both required for B cell development; the deletion of genes encoding these HDACs could block naive B cell development and hamper their proliferation and differentiation, but not the activity of mature resting B cells in HDAC1/2$^{-/-}$ mouse models (Yamaguchi et al., 2010). This suggests that the inhibition of HDACs might be effective for alleviating AMR (Suliman et al., 2012). Although pan-histone deacetylase inhibitors (pan-HDACis), such as suberoylanilide hydroxamic acid and valproic acid, have been reported to alleviate AMR to a certain extent, the specific HDAC targets have not been identified, and the underlying mechanism remains unclear (Zhang et al., 2013; Ye et al., 2016). HDACis are a class of enzymatic compounds that can potently interfere with HDAC-mediated deacetylation, upregulating the acetylation of histones and modifying the expression of related epigenetic genes.

Romidepsin (FK228), a selective class I HDACi that targets HDAC1 and HDAC2, is a bicyclic dipeptide antibiotic isolated from *Chromobacterium violaceum*, which has been reported to have antitumor activity (Bondarev et al., 2021). Following uptake by cells, such as tumor cells, FK228 could specifically inactivate and inhibit HDAC1 and HDAC2, thereby leading to the modification of gene expression, suppression of cell differentiation, and promotion of cell cycle arrest and apoptosis. Moreover, FK228 has been approved for treating certain hematologic tumors by the US Food and Drug Administration (FDA). Nonetheless, the effects of FK228 on allogeneic immune response, especially the immunity, proliferation, differentiation, and function of B cells, remain unclear and warrant further investigation.

This study aimed to evaluate the effects of FK228 on antibody production using cultured B cells in vitro and DSA in vivo. In addition, to elucidate the mechanism of FK228 in antibody production, we investigated the effects of acetylated histones on the IRE1α-X-box-binding protein 1 (XBP1) pathway.

2 Materials and methods

2.1 Reagents

FK228, purchased from Selleckchem (Houston, TX, USA), was produced by *C. violaceum* and diluted with phosphate-buffered saline (PBS). The mice were intraperitoneally injected at 0.5 mg/(kg·d) FK228.

2.2 Animals

Male BALB/c (H-2d) and C57BL/6 (H-2b) specific pathogen-free (SPF) mice (8–12 weeks, weight 19–24 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All mice were raised in SPF-level animal room.

2.3 Skin transplant

The skin transplant model was created by transferring tail skin from the donor to the back of the recipient as previously reported (Buelow et al., 1995). The first skin transplant from BALB/c to C57BL/6 mice was operated for sensitization on Day −14. The secondary skin graft was transplanted to the same recipient on Day 0. The recipient mice were divided into three groups: the syngeneic group, the allogeneic group, and the FK228-treated allogeneic group, with each group comprising at least 6 recipients. In the syngeneic group, the skin grafts were transplanted from C57BL/6 to C57BL/6 mice. In the allogeneic and FK228 groups, the skin grafts were transplanted from BALB/c to C57BL/6 mice. The FK228 was administered at 0.5 mg/(kg·d) by intraperitoneal injection from Day 0 until the endpoint of the observation. After the operation, the survival of the skin grafts was monitored every day. The rejection of a skin graft was established when the area of skin necrosis of >90%.

2.4 Cell isolation and culture

The splenocytes were isolated from C57BL/6 (H-2b) mice. Then, B cells were purified using Mouse CD19 Nanobeads (BioLegend, San Diego, CA, USA) according to the manufacturer's protocol. Purified B cells were seeded in Roswell Park Memorial Institute (RPMI) 1640 (Gibco, Dublin, Ireland) containing 10% (volume fraction) fetal calf serum (FCS) and 100 U/mL penicillin. In the presence of interleukin-4 (IL-4) and anti-clusters of differentiation 40 (CD40) antibody with FK228 or the vehicle, cells were cultured in 24-well plates at a density of 3×10^5 cells/well at 37 °C under a saturated 5% CO_2 incubator for 6 d. At the end of this step, the cells and supernatants were collected for further detection. Cell counting kit-8 (CCK-8) assays and Annexin V-fluoresceine isothiocyanate (FITC) Apoptosis Kit (BD Pharmingen, San Diego, CA, USA) were used for the determination of cell proliferation rates and apoptosis, respectively (the details are shown in File S1).

2.5 Flow cytometry

Murine splenocytes were freshly isolated and stained with antibodies, then washed and detected by flow cytometry. The antibodies used were FITC/phycoerythrin (PE)/allophycocyanin (APC)-CD3, PE/PE/Cyanine7 (Cy7)-CD4, peridinin-chlorophyll-protein (PerCP)/Cy5.5-CD25, Alexa Fluor® 647/Brilliant Violet 421™ FoxP3, APC-CXCR5, Brilliant Violet 510-PD-1, PE-CD138 (Syndecan-1), PerCP-CD45R/B220, and APC-CD19 (all obtained from BioLegend).

2.6 Flow cytometric cross-matching assay

The donor spleen cells were incubated with the serum of the recipient, and then stained with anti-IgG and IgM antibodies for flow cytometry (the details are shown in File S1).

2.7 Histopathological analysis

The collected skin grafts were fixed in 4% (volume fraction) paraformaldehyde, embedded in paraffin, and sliced. The slices were stained with hematoxylin and eosin (H&E) for histological analysis. The histological changes were assessed in five discontinuous fields at 100× magnification under an optical microscope (Olympus, Tokyo, Japan).

2.8 ELISA

The levels of IgG1 (R&D Systems Minneapolis, MN, USA) and IgM (eBioscience, San Diego, CA, USA) in the B-cell culture supernatants were analyzed by enzyme-linked immunosorbent assay (ELISA) kits. The ELISA reader (Spectra Max M3) was manufactured by Molecular Devices (USA).

2.9 RNA isolation and RT-qPCR analysis

The RNA was extracted from the recipient mouse spleens and cell pellets in B cell culture using TRIzol (Invitrogen, Carlsbad, CA, USA). With glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference, quantitative reverse transcription-polymerase chain reaction (RT-qPCR) was carried out using the SYBR Green Master Mix (Toyobo, Tokyo, Japan). The RT-qPCR cycles were run in a StepOne System (Life Technologies, Carlsbad, CA, USA) for *GAPDH*, positive regulatory domain-containing 1 (*Prdm1*), interferon regulatory factor 4 (*Irf4*), *Xbp1*, and activation-induced cytidine deaminase (*Aicda*) (the primer sequences are shown in Table S1).

2.10 Western blot analysis

The total and nuclear proteins were extracted from the spleen tissues and cultured cells using a Nuclear/Plasma Protein Extraction kit (Beyotime Biotechnology, Shanghai, China). The protein concentrations were measured using the Protein BCA Assay Kit (BioTek, Vermont, USA). The protein samples were pretreated with loading buffer in boiling water at 95–100 °C for 5 min. The protein was electrophoresed, transferred to the membrane, and exposed to light, and the gray value of the band was calculated (the specific details are shown in File S1).

2.11 Statistical analysis

GraphPad Prism 7 software was used for all statistical analyses. The data were expressed as mean± standard deviation (SD). Student's *t*-test or analysis of variance (ANOVA) was conducted to compare the means. The differences in graft survival between the syngeneic transplant, allogeneic transplant, and FK228 groups were compared by the Kaplan-Meier method. A *P*-value of less than 0.05 was considered as statistically significant.

3 Results

3.1 Effects of FK228 on the DSA levels in mice with allogeneic skin grafts and the survival of skin grafts

In order to investigate the effects of FK228 on the level of DSA, we established a secondary skin transplantation mouse model. As shown in Fig. 1a, the median survival time (MST) of the grafts after the first and secondary allogeneic skin transplantations in the allogeneic group was 12 and 11 d, respectively. With the administration of FK228 after the secondary allogeneic skin transplantation, the survival of skin grafts was obviously prolonged (MST=25 d, $P<0.0001$; Fig. 1a). We then examined the level of DSA in recipient sera by flow cytometry, which revealed that the mean fluorescence intensities (MFIs) of IgG and IgM in the allogeneic group were increased after the secondary skin transplantation compared with those in the syngeneic group. Furthermore, with the administration of FK228, the production of IgG and IgM was significantly reduced on Days 7 and 14 (both $P<0.01$; Fig. 1b). The overall graphs in the three groups on Day 10 displayed that skin grafts were ruddy soft with new hair in the syngeneic group, were hardened with spots and black crusts in the allogeneic group, and were slightly inflamed at the boundary in the FK228-treated group. The H&E staining of skin grafts on Day 10 revealed that the hair follicles and epidermis in the allogeneic group were significantly damaged, along with the inhibition of keratinization and extensive infiltration of inflammatory cells, as compared with those in the syngeneic grafts. Notably, the condition of grafts in the FK228-treated group was improved (Figs. 1c and 1d). Overall, the administration of FK228 prolonged the survival of secondary skin grafts and decreased the DSA level.

3.2 Effects of FK228 on the differentiation of TFH and plasma cells

The spleen is a key lymphoid organ for cellular differentiation and inducing B-lymphocyte (B cell)-mediated humoral immunity. To explore the effects of FK228 on mouse immune cells, flow cytometry was conducted on the recipient spleen cells collected on Day 10 after secondary skin transplantation. We found that the ratios of $CD4^+PD\text{-}1^+CXCR5^+$ T follicular helper (TFH) cells and $B220^{lo}CD138^+$ plasma cells were significantly suppressed (both $P<0.01$; Fig. 2).

3.3 Effects of FK228 on IRE1α-XBP1 pathway activity in the ER UPR and class switch recombination (CSR) in vivo

We further tested the effects of FK228 on the expression of genes related to plasma cell differentiation in mice. Specifically, we determined the relative messenger RNA (mRNA) levels of plasma cell-related genes *Irf4*, *Prdm1*, and *Xbp1* in each group. After FK228 treatment, the relative mRNA levels of *Prdm1* and *Xbp1* were significantly decreased ($P<0.05$; Fig. 3a). The results showed that, in the IRE1α-XBP1 pathway, FK228 inhibited the expression of XBP1 and B lymphocyte-induced maturation protein-1 (Blimp-1), as well as the phosphorylation of IRE1α ($P<0.01$; Fig. 3b). Furthermore, FK228 treatment downregulated the transcription of *Aicda* and the expression of AID, which is encoded by *Aicda* ($P<0.05$; Fig. 3b).

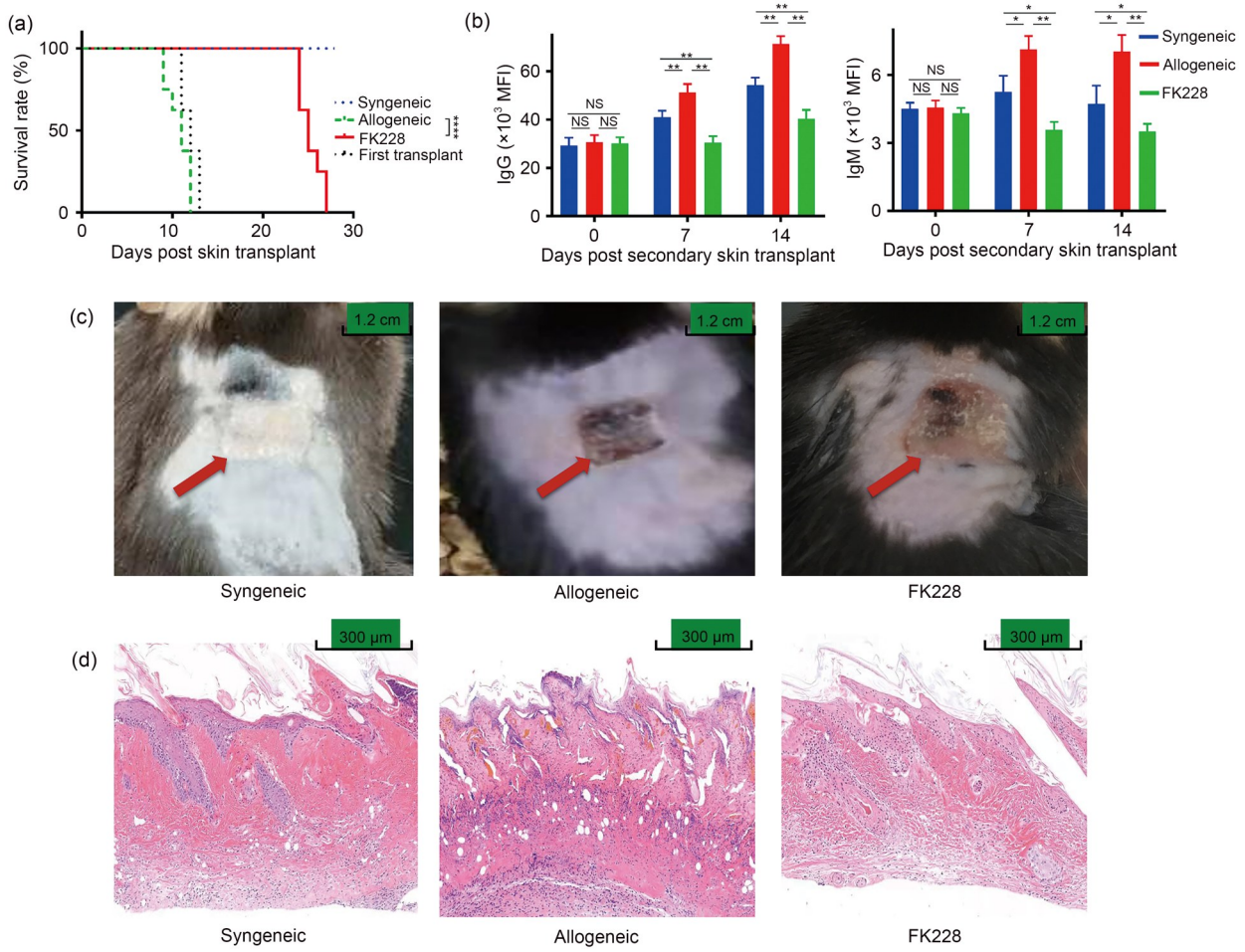

Fig. 1 Effects of FK228 on the production of DSA in vivo. (a) The survival of primary skin grafts (MST=12 d), and the survival of secondary skin grafts from the establishment of skin transplantation models in the syngeneic group (MST> 28 d), allogeneic group (MST=11 d), and FK228-treated group (MST=25 d) (n=8 per group, P<0.0001). (b) The levels of DSAs including IgM and IgG in the serum of the syngeneic, allogeneic, and FK228-treated groups on Days 0, 7, and 14 after secondary skin transplantation, as detected by flow cytometric cross-matching assay (n=6 per group, * P<0.05, ** P< 0.01). Data are shown as mean±standard deviation (SD). (c) The representative photographs of grafted skins in the syngeneic, allogeneic, and FK228-treated groups on Day 10 after secondary skin transplantation. The data are representative of six separate experiments. (d) The representative hematoxylin and eosin (H&E) staining of skin grafts in the syngeneic, allogeneic, and FK228-treated groups on Day 10 after secondary skin transplantation. The data are representative of six separate experiments. DSA: donor-specific antibody; MST: median survival time; NS: not significant; Ig: immunoglobulin; MFI: mean fluorescence intensity.

3.4 Effects of FK228 on the acetylation levels of histones H2A and H3 in the spleens of skin-transplanted mice

The direct effects of FK228 include the inhibition of HDAC1 and HDAC2 expression and the alteration of histone acetylation levels. The results confirmed the inhibitory effects of FK228 on HDAC1 and HDAC2 expression in vivo (P<0.01; Fig. 4a). The histones H2A and H3 reportedly have important immunomodulatory roles (Cobaleda et al., 2007; Wu et al., 2019). We extracted nucleocapsid proteins from recipient mouse spleens and detected the acetylation levels of H2A and H3. The results showed that FK228 significantly upregulated the acetylation levels of both H2A (Lys4 and Lys5) and H3 (Lys9, Lys27, and Lys56) (P<0.05; Figs. 4b and 4c).

3.5 Effects of FK228 on the proliferation and apoptosis of B cells in vitro

To observe the effects of FK228 on B cells in vitro, we isolated $CD19^{+}$ B cells by magnetic-activated

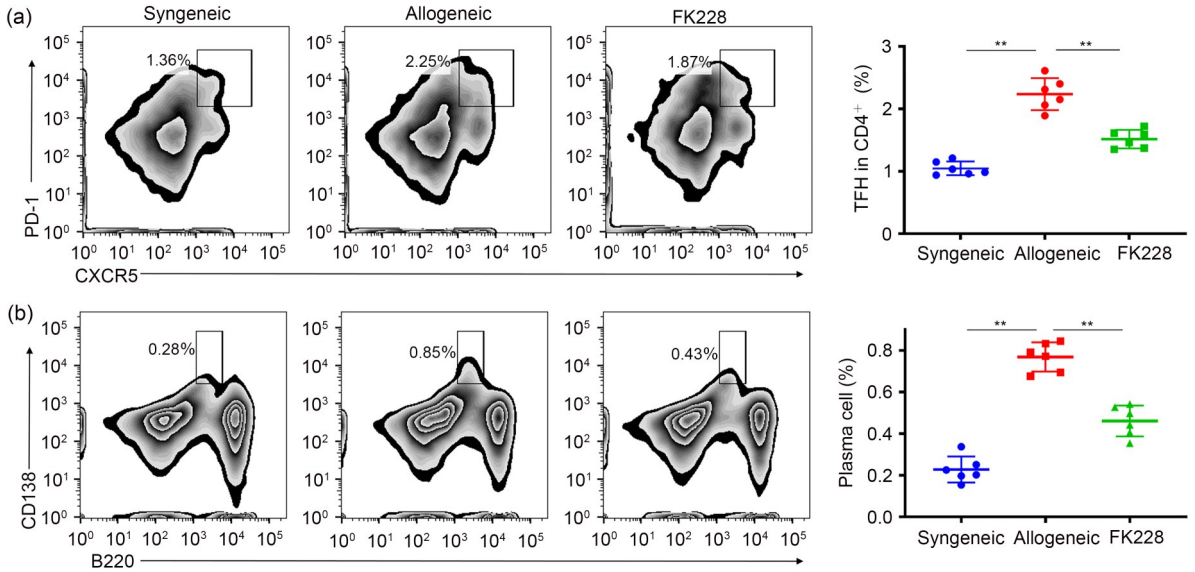

Fig. 2 Effects of FK228 on the percentages of T follicular helper (TFH) and plasma cells in splenocytes in vivo. (a) The percentages of TFH ($CD4^+PD\text{-}1^+CXCR5^+$) cells in $CD4^+$ T cells from splenocytes were compared between the syngeneic, allogeneic, and the FK228-treated groups (n=6, ** P<0.01). (b) The representative cytometry figures of $B220^{lo}CD138^+$ plasma cells in the spleen from the syngeneic, allogeneic, and FK228-treated groups, and the statistical analysis of the percentages among the three groups (n=6, ** P<0.01). All data are shown as mean±standard deviation (SD).

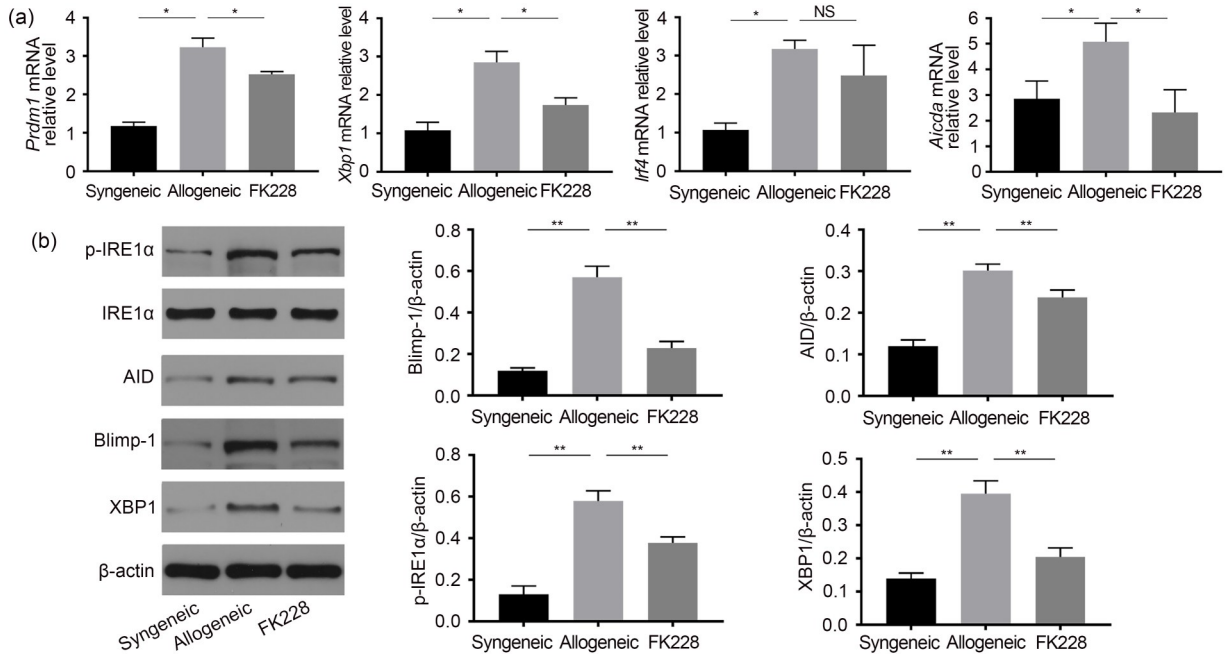

Fig. 3 Evaluation of mRNA and protein levels of genes involved in antibody production in the spleens with FK228 treatment in vivo. (a) The mRNA levels of *Prdm1*, *Xbp1*, *Irf4*, and *Aicda*, in the spleens of skin-transplanted mice (syngeneic, allogeneic, and FK228-treated groups) were determined by RT-qPCR, and the target genes were normalized to the corresponding GAPDH. (b) The western blot analyses of AID, Blimp-1, IRE1α, p-IRE1α, and XBP1 in the spleens of skin-transplanted mice (syngeneic, allogeneic, and FK228-treated groups), with β-actin as a loading control. All data are shown as mean±standard deviation (SD), and are representative of three separate experiments. * P<0.05, ** P<0.01. mRNA: messenger RNA; *Aicda*: activation-induced cytidine deaminase; *Xbp1*: X-box-binding protein 1; *Prdm1*: positive regulatory domain-containing 1; *Irf4*: interferon regulatory factor 4; RT-qPCR: quantitative reverse transcription-polymerase chain reaction; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; AID: protein encoded by *Aicda*; Blimp-1: B lymphocyte-induced maturation protein 1; IRE1α: inositol-requiring enzyme 1 α; p-IRE1α: phosphorylated IRE1α; NS: not significant.

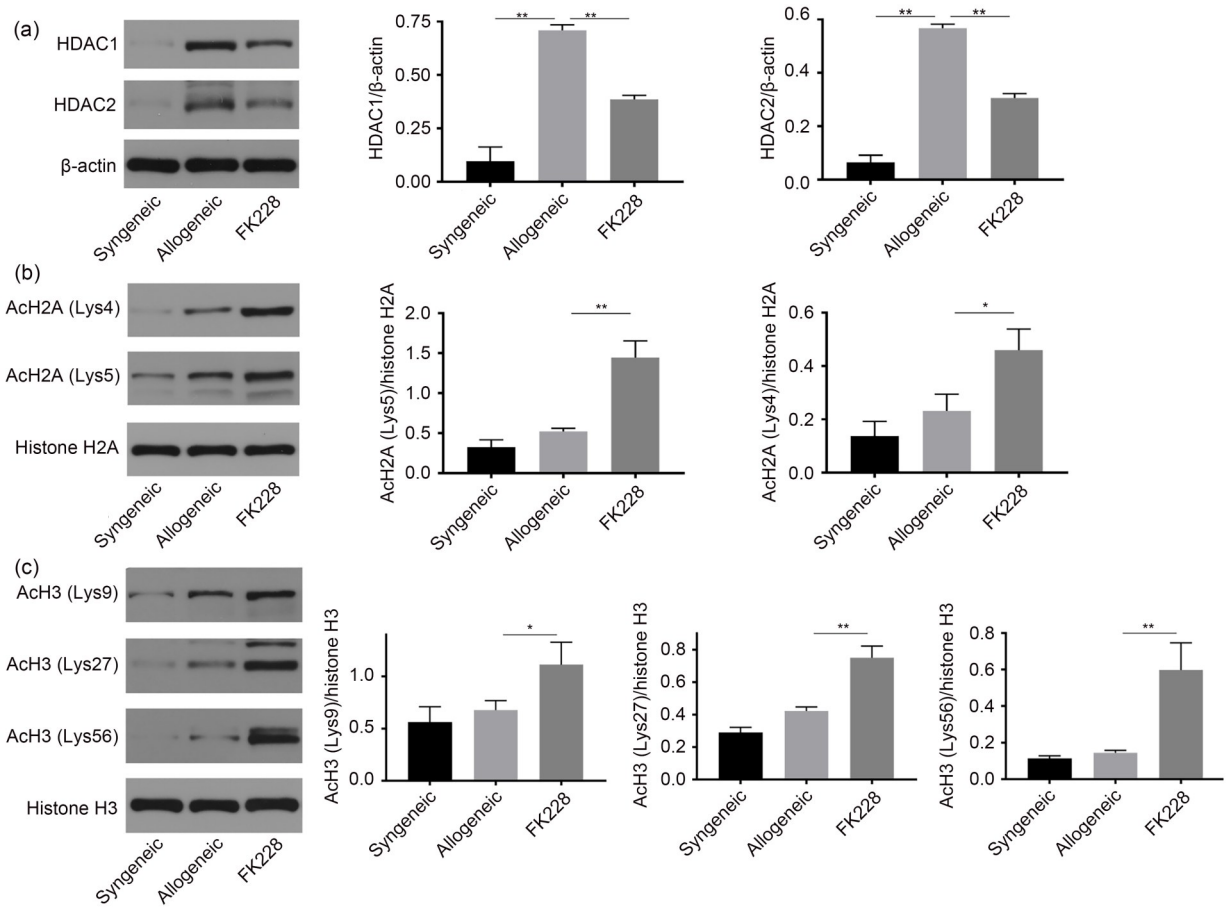

Fig. 4 Regulation of histone deacetylases (HDACs) and acetylated histones by FK228 in vivo. (a) The western blot analyses of HDAC1 and HDAC2 in spleens from three groups of skin-transplanted mice (syngeneic, allogeneic, and FK228-treated groups) with β-actin as the control. It is a common control with Fig. 3b. (b, c) The western blot analyses of acetylated histones H2A (Lys4 and Lys5) and H3 (Lys9, Lys27, and Lys56) separately in spleens from the three groups of skin-transplanted mice, with histones H2A and H3 as the corresponding control. All data are shown as mean± standard deviation (SD), and are representative of three separate experiments. * $P<0.05$, ** $P<0.01$.

cell sorting (MACS) from mouse spleens and performed flow cytometry analysis, which showed a purity of above 90% (Fig. 5a). We used IL-4 and anti-CD40 antibody to stimulate the B cells, and then detected B cell proliferation and apoptosis using the CCK8 and Annexin V kit.

Based on the cytotoxicity test in a previous study, we found that FK228 could exert a certain cytotoxicity effect on primary B cells at concentrations above 10 nmol/L (data not shown). Thus, we selected 5 and 8 nmol/L FK228 to explore the effects on B cells in vitro. The results showed that the proliferation of B cells was significantly inhibited by FK228 on Day 5 as compared with that in the control group ($P<0.05$; Fig. 5b). Consistently, the apoptosis rate of B cells was significantly increased ($P<0.01$; Fig. 5c).

3.6 Effects of FK228 on plasma cell differentiation and IgG1 and IgM levels in vitro

IL-4 and anti-CD40 antibody can co-stimulate B cells in vitro, promoting the proliferation, differentiation, and secretion of IgG1 via CSR. Our data showed that the levels of IgG1 and IgM in the supernatant were decreased dose-independently in the FK228-treated group ($P<0.01$; Fig. 6a). The expression levels of the B-cell co-stimulatory molecules, CD80 and MHC-II, were downregulated in the FK228-treated group, indicating that FK228 inhibited B cell activation in vitro (Fig. 6b). Moreover, the significant decrease detected in the proportion of $B220^{lo}CD138^{+}$ plasma cells in FK228-treated cells ($P<0.01$; Fig. 6c) demonstrated that the differentiation of plasma cells was also inhibited by FK228. Therefore, FK228 inhibited

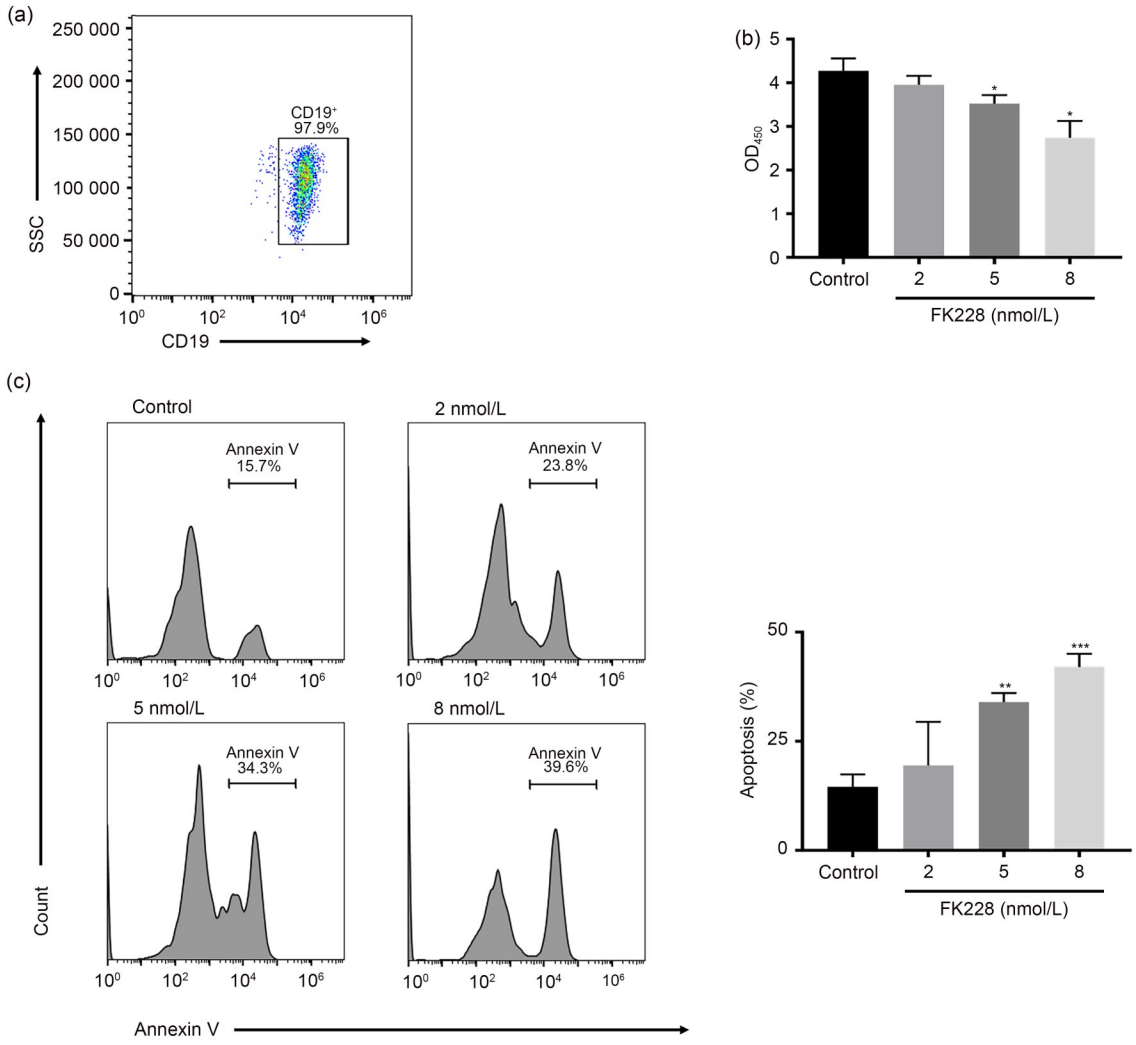

Fig. 5 Effects of FK228 on the proliferation and apoptosis of B cells in vitro. (a) The percentage of isolated and purified splenic CD19$^+$ B cells via magnetic-activated cell sorting performed through flow cytometry. The data are representative of three separate experiments. (b) The cellular viability analysis of B cells cultured with different concentrations of FK228 (2, 5, and 8 nmol/L) after 48 h performed by a CCK8 essay kit in vitro (n=4). (c) The apoptosis analysis of the effect of different concentrations of FK228 on B cells after 48 h, as detected by flow cytometry. The data are expressed as mean±standard deviation (SD), n=4. * P<0.05, ** P<0.01, *** P<0.001, compared with the control group. OD_{450}: optical density at 450 nm.

B cell activation and differentiation as well as the production of antibodies in vitro.

3.7 Effects of FK228 on AID and the IRE1α-XBP1 pathway in the ER UPR in vitro

During the differentiation of B cells into plasma cells, *Prdm1*, *Irf4*, and *Xbp1* are the key transcription factors. AID, encoded by *Aicda*, can promote CSR of B cells (Stavnezer et al., 2008). To explore the molecular mechanism of FK228, we evaluated the mRNA expression of *Aicda*, *Prdm1*, *Xbp1*, and *Irf4*. The transcription of *Aicda*, *Xbp1*, and *Prdm1* was dramatically inhibited by FK228 (P<0.05), whereas *Irf4* was not significantly affected (Fig. 7a). Consistently, FK228 significantly inhibited Blimp-1, XBP1, and AID expression at the protein level (P<0.01; Figs. 7b and 7c). These results indicate that the inhibition of CSR and plasma cell differentiation by FK228 are related to the transcriptional downregulation of *Aicda*, *Prdm1*, and *Xbp1*.

The IRE1α-XBP1 pathway in the ER UPR is the most important signaling route by which B cells produce Igs, providing a complete connection among XBP1, UPR, and plasma cell differentiation. Therefore,

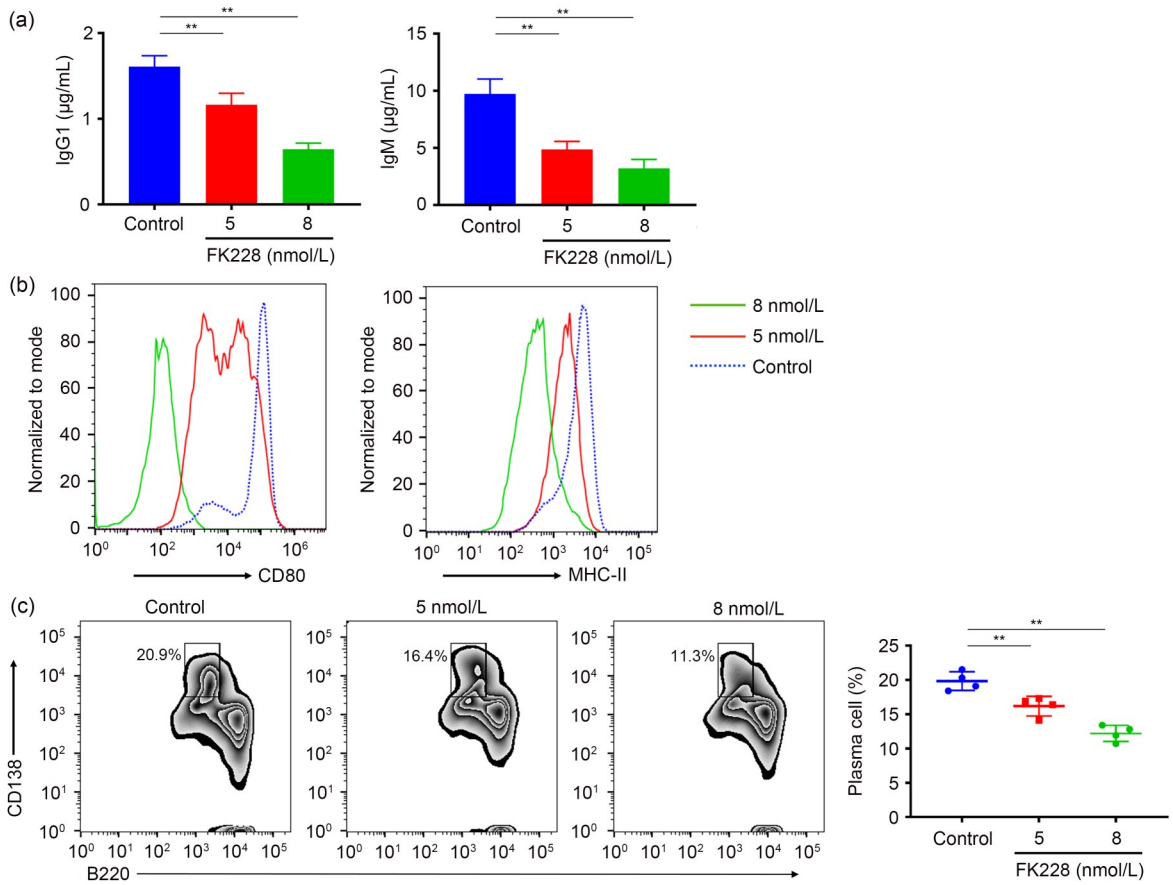

Fig. 6 Effects of FK228 on the production of IgG1 and IgM and the differentiation of plasma cells in vitro. (a) The levels of IgG1 and IgM in the supernatant of B cells cultured with 5 and 8 nmol/L FK228 and the vehicle control measured with ELISA kits (n=4, ** P<0.01). (b, c) The percentages of B220^{lo}CD138^{+} plasma cells and mean fluorescence intensity (MFI) of CD80 and MHC-II on B cells cultured with FK228 (5 and 8 nmol/L) or vehicle (n=4, ** P<0.01). The data are expressed as mean±standard deviation (SD); Ig: immunoglobulin; ELISA: enzyme-linked immunosorbent assay; MHC-II: major histocompatibility complex class II.

to verify whether FK228 inhibits antibody production by alleviating UPR, western blotting was conducted to detect the expression of IRE1α. As shown in Fig. 7c, the phosphorylation levels of IRE1α in the FK228-treated group were significantly lower than those in the soluble in dimethyl sulfoxide (DMSO) group (as control) (P<0.001). These findings indicate that FK228 treatment inhibited the IRE1α-XBP1 pathway.

3.8 Effects of FK228 on histone acetylation in B cells in vitro

We performed western blot analysis on the cultured B cells to confirm the HDAC inhibitory activity of FK228. FK228 treatment resulted in a significant decrease in the expression levels of HDAC1 and HDAC2 (P<0.01; Fig. 8a) and an obvious increase in the expression levels of acetylated histones H2A (Lys4 and Lys5) and H3 (Lys9, Lys27, and Lys56) with increasing concentration (P<0.01; Figs. 8b and 8c).

4 Discussion

As mediators of epigenetic regulation, HDACs are known to play vital roles in various diseases, including malignancies, autoimmune diseases, inflammation, and fibrosis (Lyu et al., 2019; Bondarev et al., 2021; Tordera and Cortés-Erice, 2021). FK228 has been characterized as a natural class I HDACi, which has proved to exert clinically therapeutic effects in myeloid leukemia, T cell lymphoma, multiple myeloma, and human immunodeficiency virus (HIV), as well as in mouse models of biliary tract cancer, breast cancer, and bladder cancer cell lines and kidney fibrosis

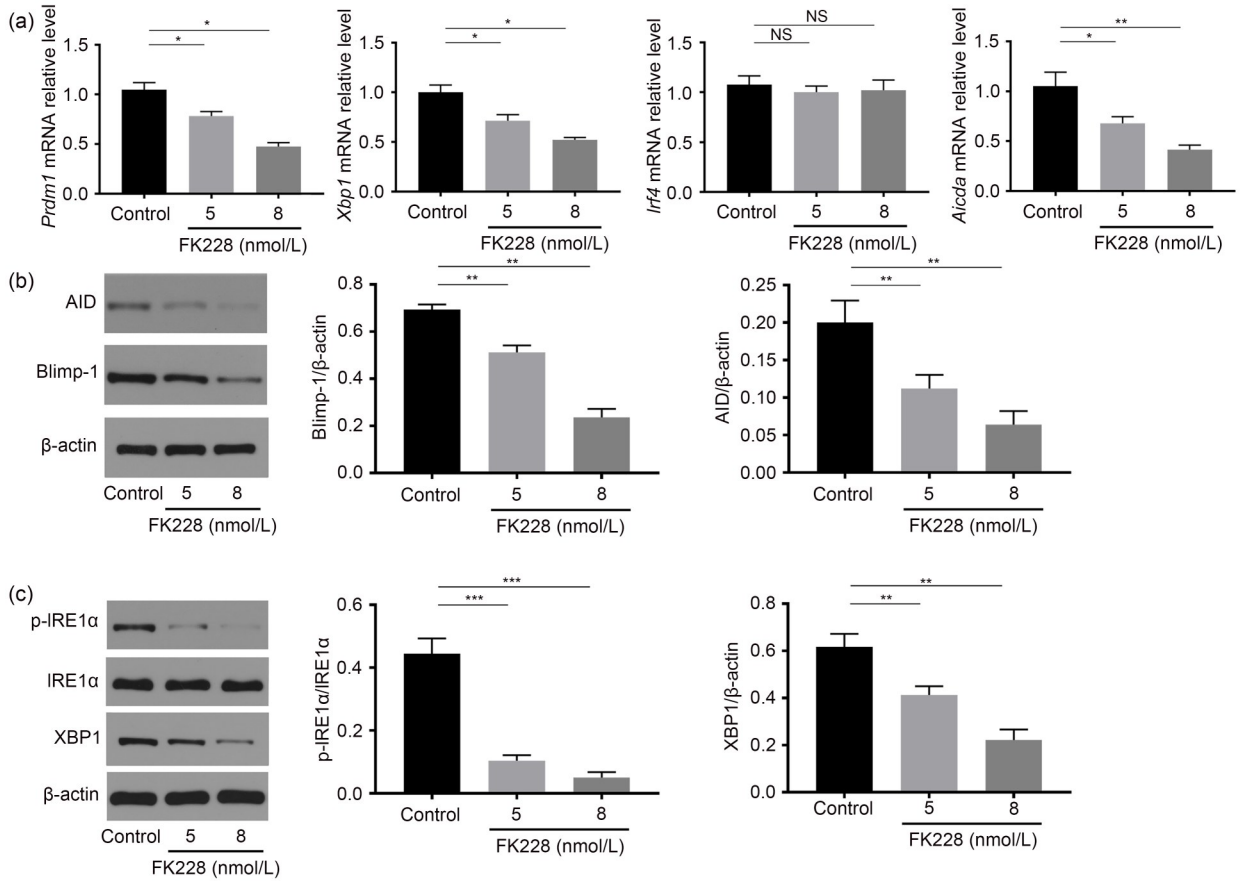

Fig. 7 Levels of mRNAs and proteins involved in the IRE1α-XBP1 pathway of B cells treated with FK228. (a) The RT-qPCR analyses of *Prdm1*, *Xbp1*, *Irf4*, and *Aicda*, genes in cultured B cells treated with FK228 or vehicle, where the target genes were normalized to the corresponding GAPDH level. (b, c) Western blot analyses of AID, Blimp-1, IRE1α, p-IRE1α, and XBP1 in cultured B cells treated with FK228 or vehicle, where β-actin served as a loading control. The data are expressed as mean±standard deviation (SD), and are representative of three separate experiments. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. mRNA: messenger RNA; IRE1α: inositol-requiring enzyme 1 α; XBP1: X-box-binding protein 1; RT-qPCR: quantitative reverse transcription-polymerase chain reaction; *Aicda*: activation-induced cytidine deaminase; *Prdm1*: positive regulatory domain-containing 1; *Irf4*: interferon regulatory factor 4; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; AID: protein encoded by *Aicda*; Blimp-1: B lymphocyte-induced maturation protein 1; p-IRE1α: phosphorylated IRE1α; NS: not significant.

(Yang et al., 2019; Pojani and Barlocco, 2021). However, the effects of FK228 on B cell biology and function, and related immune diseases have been rarely explored. Our research group previously found that FK228 could attenuate renal fibrosis by inhibiting the small mothers against decapentaplegic (Smad) and non-Smad pathways in a mouse UUO model (Yang et al., 2019). Here, we focused on exploring its effects on B lymphocyte immunobiology in vitro and in vivo. We established a mouse model of secondary allogeneic skin transplantation to study the effects of FK228 on DSA production and the related mechanism. The response of B cells could be activated by the first skin graft, and DSA was subsequently produced in the recipients. We first reported that FK228 could significantly improve the survival of secondary skin grafts and downregulate the production of DSAs. Mechanistically, FK228 was found to upregulate H2A expression and H3 acetylation by specifically inhibiting HDAC1/2 and blocking the endoplasmic reticulum IRE1α-XBP1 pathway in B cells.

After allogeneic organ transplantation, B cells and plasma cells are responsible for the production of DSAs. During the overall complex programs of B cell activation, proliferation, differentiation, and the secretion of antibodies, epigenetic modifications are involved in almost every process, and the expression profiles of the related genes could be significantly

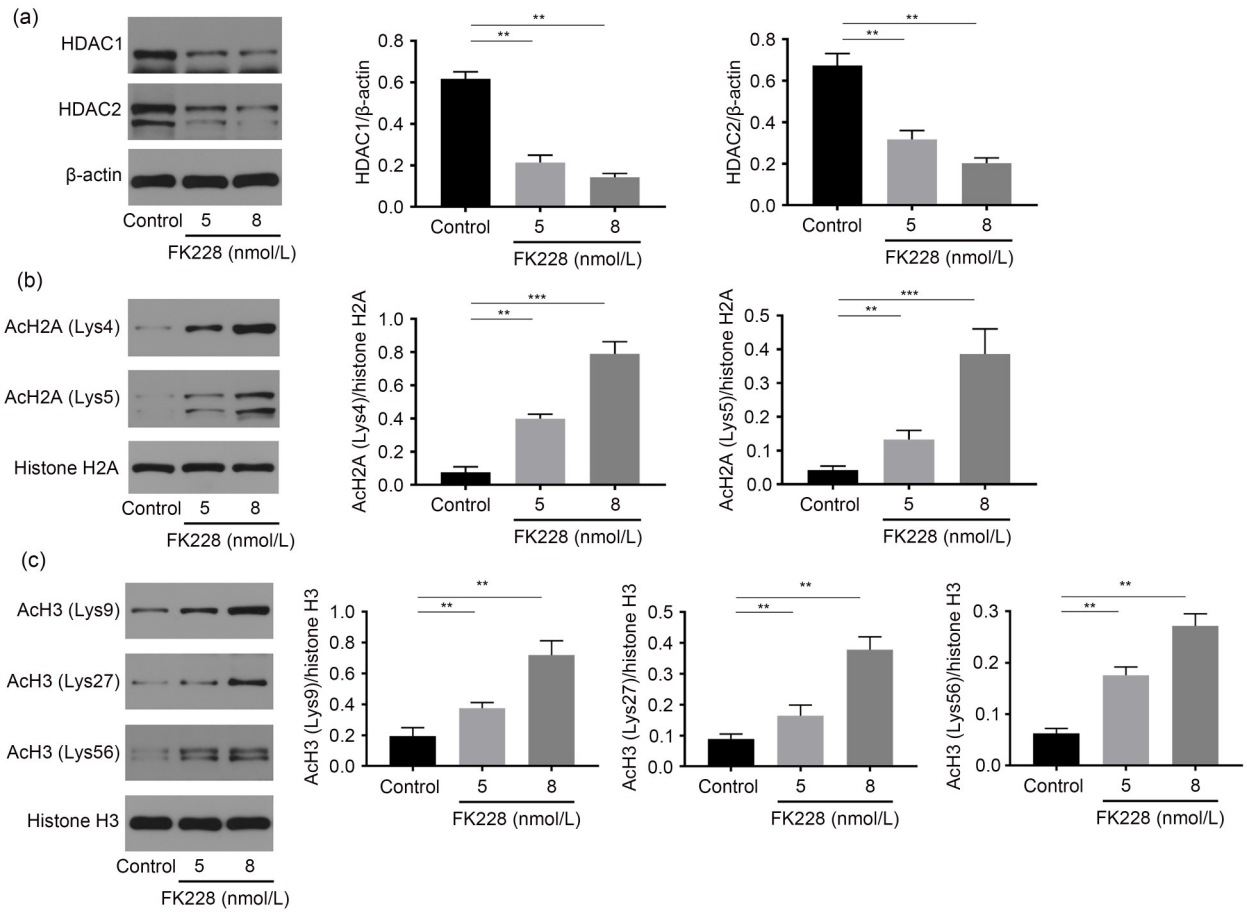

Fig. 8 Protein analyses of histone deacetylases (HDACs) and the acetylated (Ac) histones of B cells cocultured with FK228. (a) The western blot analyses of HDAC1 and HDAC2 in B cells treated with FK228 or vehicle, where β-actin served as a loading control. (b, c) The western blot analyses of acetylated histones H2A (Lys4 and Lys5) and H3 (Lys9, Lys27, and Lys56) in cultured B cells treated with FK228 or vehicle, where histones H2A and H3 served as corresponding controls. The data are expressed as mean±standard deviation (SD), and are representative of three separate experiments. ** *P*<0.01, * *P*<0.001.**

altered (Igarashi et al., 2014; Ise and Kurosaki, 2020). In our study, with the administration of FK228, the survival of secondary skin grafts was prolonged, and HE staining results showed that the number of infiltrated immunocytes was decreased. The levels of DSAs, including IgG and IgM, were significantly downregulated on Days 7 and 14. As TFH cells play an active role in promoting the differentiation of B cells to plasma cells via providing the co-stimulatory molecules CD40 ligand (CD40L) and inducible T cell co-stimulatory molecule (ICOS) (Dudreuilh et al., 2021), we analyzed the distribution of plasma cells and TFH cells in recipients' spleens using flow cytometry. The results revealed that the proportions of TFH and plasma cells in the FK228-treated group were markedly reduced.

To determine the underlying transcriptional mechanisms responsible for the decreased plasma cell and DSA levels in skin transplantation models, the impacts of FK228 treatment on the related gene transcription and the synthesis of DSA were determined. *Blimp-1*, *Irf4*, and *Xbp1* are the crucial transcription factors related to the differentiation of B cells in mice and humans (Kassambara et al., 2015; Nutt et al., 2015). Specifically, the expression of *Blimp-1* and *Irf4* acts on the initiation of B cells to plasma cells (Shapiro-Shelef et al., 2003; Klein et al., 2006; Sciammas et al., 2006), and *Xbp1* promotes the expression of genes involved in mitochondria and ER biosynthesis, such as the gene encoding choline cytidylyltransferase, which is a lipid component of the ER (Shaffer et al., 2004; Sriburi et al., 2004; Yang et al., 2021). Upon

XBP1 deletion in mouse models, the ER of plasma cells became swollen and the antibody secretion was inhibited (Reimold et al., 2001; McGehee et al., 2009). In our study, the mRNA levels of *Blimp-1* and *Xbp1* were significantly decreased compared with that of *Irf4* in FK228-treated recipients. This might be explained by the fact that *Blimp-1* and *Xbp1* are required for normal antibody secretion (Minnich et al., 2016; Tellier et al., 2016), and *Irf4* is mainly required for the upregulation of *Blimp-1* in the initial stage, whereas it is not essential for the whole sustained process (Trezise and Nutt, 2021). Thus, we speculated that FK228 might affect antibody secretion by inhibiting *Blimp-1* and *Xbp1* expression.

Once plasm cells are formed, their anabolism in the ER and Golgi complex is dramatically boosted to meet the requirement of mass antibody synthesis (Shi et al., 2015; Choi and Morel, 2020). This kind of hypermetabolism in plasma cells is primarily to redeploy three coordinated signaling branches of UPR, which could be triggered and initiated individually by the relevant stress sensor, namely, IRE1α, protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6), of which IRE1α is the most powerful and effective factor to produce antibodies (Yoshida et al., 2001; Ron and Walter, 2007; Aragon et al., 2012; Jurkin et al., 2014; Grootjans et al., 2016; Gong et al., 2017; Zhao et al., 2020). Our results showed that FK228 suppressed the ER UPR by decreasing the activation of IRE1α during antibody synthesis both in vitro and in vivo. As Blimp-1 is an upstream regulatory molecule of UPR and XBP1, we supposed that the downregulation of Blimp-1 by FK228 may subsequently enhance the inhibition of the IRE1α-XBP1 pathway.

Histone modification also contributes to the processes of antibody synthesis, including somatic hypermutation (SHM) and CSR (Crouch et al., 2007; Peng et al., 2011; Sheppard et al., 2018). AID, which is encoded by *Aicda* (Stavnezer et al., 2008), plays a crucial role in both CSR and SHM (Muramatsu et al., 2018). Activated AID converts cytosine to uracil, which initiates the transcription and subsequent expression of SHM- and CSR-relevant genes following the production of specific allogeneic antibodies (Hodgkin et al., 1996; Rush et al., 2005). We found that with the administration of FK228, the mRNA and protein levels of AID were downregulated consistently both in spleens and in vitro. Based on the above results, we speculated that FK228 could depress the differentiation of plasma cells via the reduction of AID expression along with the obstruction of CSR and SHM.

Except for malignancies, the modification of histone acetylation has also been recently reported to confer therapeutic effects in some diseases, specifically, improving pathological progression by reducing miR-149-5p expression in Alzheimer's disease (AD) (Chen et al., 2020), suppressing pathogenetic proliferation and extracellular matrix in Grave's disease (Ekronarongchai et al., 2021), expanding tolerogenic dendritic cells (DCs) and regulatory T cells (Tregs), and inhibiting autoimmune T cells and B cells in systemic lupus erythematosus (SLE) (da Costa et al., 2020; Zakzuk et al., 2020; Datta, 2021). Moreover, other kinds of HDACi, such as suberoylanilide hydroxamic acid and valproic acid, have been proved to possess the ability to inhibit B cell responses and attenuate AMR in transplant models (Ke et al., 2012; Zhang et al., 2013; Ye et al., 2016). To confirm the specific target of FK228 in this model, we examined the acetylation status in cultured B cells, and found that the expression of HDAC1 and HDAC2 was significantly decreased and that of acetylated H2A (Lys4 and Lys5) and H3 (Lys9, Lys27, and Lys56) was remarkedly increased in a concentration-dependent manner. Thus, we inferred that the decreased HDAC1 and HDAC2 and upregulated H2A/H3 acetylation might be the mechanism of FK228 leading to reduced DSA production (Cobaleda et al., 2007; Wu et al., 2019).

This study has some limitations. Firstly, we only used the morphological appearance and H&E staining to assess the histological characteristics of skin grafts. In future studies, we aim to perform immunohistochemical staining of specific markers to provide quantitative assessments of the extent of skin graft rejection and the specific cell types involved. In addition, more precise evaluations of plasma cells are required, as numerous subsets may be involved based upon characterizations using multiple markers. Although we established a model of AMR, this model did not eliminate the interference of T cell rejection. Moreover, it has not been distinctly revealed that how FK228 targets the regulation during the overall progress of antibody synthesis. These important issues need to be elucidated evidentially and comprehensively in the future investigations.

5 Conclusions

We showed that FK228 inhibited the production of antibodies in cultured B cells and DSA production in secondary skin transplantation mouse models via epigenetic regulation, followed by prolonged graft survival. These beneficial effects on transplant immunity may be attributed to the upregulation of H2A and H3 acetylation levels and the inhibition of Blimp-1-IRE1α-XBP1 signaling in the ER UPR. These findings provide a novel insight into the therapeutic potential of FK228 in ameliorating AMR and prolonging the long-term survival of transplanted organs in clinical therapy.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 81873511 and 81471587).

Author contributions

Yuliang GUO, Siyu SONG, Li TIAN, and Man ZHANG performed the experimental research and data analysis. Yuliang GUO, Xiaoxiao DU, and Sheng CHANG wrote and edited the manuscript. Yuliang GUO performed the establishment of animal models. Hongmin ZHOU, Zhonghua Klaus CHEN, and Sheng CHANG contributed to the study design, data analysis, writing and editing of the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Yuliang GUO, Siyu SONG, Xiaoxiao DU, Li TIAN, Man ZHANG, Hongmin ZHOU, Zhonghua Klaus CHEN, and Sheng CHANG declare that they have no conflict of interest.

All experiments were conducted according to the animal protection ethics and policies of Huazhong University of Science and Technology (Wuhan, China).

References

Aragon IV, Barrington RA, Jackowski S, et al., 2012. The specialized unfolded protein response of B lymphocytes: ATF6α-independent development of antibody-secreting B cells. *Mol Immunol*, 51(3-4):347-355. https://doi.org/10.1016/j.molimm.2012.04.001

Bezu L, Chuang AW, Liu P, et al., 2019. Immunological effects of epigenetic modifiers. *Cancers*, 11(12):1911. https://doi.org/10.3390/cancers11121911

Bondarev AD, Attwood MM, Jonsson J, et al., 2021. Recent developments of HDAC inhibitors: emerging indications and novel molecules. *Br J Clin Pharmacol*, 87(12):4577-4597. https://doi.org/10.1111/bcp.14889

Buelow R, Veyron P, Clayberger C, et al., 1995. Prolongation of skin allograft survival in mice following administration of ALLOTRAP. *Transplantation*, 59(4):455-460. https://doi.org/10.1097/00007890-199502270-00001

Chen FY, Chen HF, Jia YJ, et al., 2020. miR-149-5p inhibition reduces Alzheimer's disease β-amyloid generation in 293/APPsw cells by upregulating H4K16ac via KAt8. *Exp Ther Med*, 20(5):88. https://doi.org/10.3892/etm.2020.9216

Choi SC, Morel L, 2020. Immune metabolism regulation of the germinal center response. *Exp Mol Med*, 52(3):348-355. https://doi.org/10.1038/s12276-020-0392-2

Chong AS, 2019. New insights into the development of B cell responses: implications for solid organ transplantation. *Hum Immunol*, 80(6):378-384. https://doi.org/10.1016/j.humimm.2018.09.003

Clatworthy MR, Espeli M, Torpey N, et al., 2010. The generation and maintenance of serum alloantibody. *Curr Opin Immunol*, 22(5):669-681. https://doi.org/10.1016/j.coi.2010.08.018

Clotet-Freixas S, McEvoy CM, Batruch I, et al., 2020. Extracellular matrix injury of kidney allografts in antibody-mediated rejection: a proteomics study. *J Am Soc Nephrol*, 31(11):2705-2724. https://doi.org/10.1681/asn.2020030286

Cobaleda C, Schebesta A, Delogu A, et al., 2007. Pax5: the guardian of B cell identity and function. *Nat Immunol*, 8(5):463-470. https://doi.org/10.1038/ni1454

Crouch EE, Li ZY, Takizawa M, et al., 2007. Regulation of AID expression in the immune response. *J Exp Med*, 204(5):1145-1156. https://doi.org/10.1084/jem.20061952

da Costa TP, El-Cheikh MC, Carneiro K, 2020. Epigenetic therapy as a putative molecular target to modulate B cell biology and behavior in the context of immunological disorders. *J Immunol Res*, 2020:1589191. https://doi.org/10.1155/2020/1589191

Datta SK, 2021. Harnessing tolerogenic histone peptide epitopes from nucleosomes for selective down-regulation of pathogenic autoimmune response in lupus (past, present, and future). *Front Immunol*, 12:629807. https://doi.org/10.3389/fimmu.2021.629807

Dudreuilh C, Basu S, Scottà C, et al., 2021. Potential application of T-follicular regulatory cell therapy in transplantation. *Front Immunol*, 11:612848. https://doi.org/10.3389/fimmu.2020.612848

Ekronarongchai S, Palaga T, Saonanon P, et al., 2021. Histone deacetylase 4 controls extracellular matrix production in orbital fibroblasts from Graves' ophthalmopathy patients. *Thyroid*, 31(10):1566-1576. https://doi.org/10.1089/thy.2020.0948

Gong J, Wang XZ, Wang T, et al., 2017. Molecular signal networks and regulating mechanisms of the unfolded protein response. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 18(1):1-14. https://doi.org/10.1631/jzus.B1600043

Grootjans J, Kaser A, Kaufman RJ, et al., 2016. The unfolded protein response in immunity and inflammation. *Nat Rev Immunol*, 16(8):469-484. https://doi.org/10.1038/nri.2016.62

Gujral P, Mahajan V, Lissaman AC, et al., 2020. Histone acetylation and the role of histone deacetylases in normal cyclic endometrium. *Reprod Biol Endocrinol*, 18:84. https://doi.org/10.1186/s12958-020-00637-5

Hodgkin PD, Lee JH, Lyons AB, 1996. B cell differentiation and isotype switching is related to division cycle number. *J Exp Med*, 184(1):277-281. https://doi.org/10.1084/jem.184.1.277

Igarashi K, Ochiai K, Itoh-Nakadai A, et al., 2014. Orchestration of plasma cell differentiation by Bach2 and its gene regulatory network. *Immunol Rev*, 261(1):116-125. https://doi.org/10.1111/imr.12201

Ise W, Kurosaki T, 2020. Regulation of plasma cell differentiation. *In*: Wang JY (Ed.), B Cells in Immunity and Tolerance. Springer, Singapore, p.63-74. https://doi.org/10.1007/978-981-15-3532-1_6

Jurkin J, Henkel T, Nielsen AF, et al., 2014. The mammalian tRNA ligase complex mediates splicing of *XBP1* mRNA and controls antibody secretion in plasma cells. *EMBO J*, 33(24):2922-2936. https://doi.org/10.15252/embj.201490332

Kassambara A, Rème T, Jourdan M, et al., 2015. Genomicscape: an easy-to-use web tool for gene expression data analysis. Application to investigate the molecular events in the differentiation of B cells into plasma cells. *PLoS Comput Biol*, 11(1):e1004077. https://doi.org/10.1371/journal.pcbi.1004077

Ke Q, Yang RN, Ye F, et al., 2012. Impairment of liver regeneration by the histone deacetylase inhibitor valproic acid in mice. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 13(9):695-706. https://doi.org/10.1631/jzus.B1100362

Klein U, Casola S, Cattoretti G, et al., 2006. Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. *Nat Immunol*, 7(7):773-782. https://doi.org/10.1038/ni1357

Li J, Koerner J, Basler M, et al., 2019. Immunoproteasome inhibition induces plasma cell apoptosis and preserves kidney allografts by activating the unfolded protein response and suppressing plasma cell survival factors. *Kidney Int*, 95(3):611-623. https://doi.org/10.1016/j.kint.2018.10.022

Loupy A, Lefaucheur C, 2018. Antibody-mediated rejection of solid-organ allografts. *N Engl J Med*, 379(12):1150-1160. https://doi.org/10.1056/NEJMra1802677

Lyu X, Hu M, Peng JT, et al., 2019. HDAC inhibitors as antifibrotic drugs in cardiac and pulmonary fibrosis. *Ther Adv Chronic Dis*, 10:1-19. https://doi.org/10.1177/2040622319862697

McCaughan JA, Tinckam KJ, 2018. Donor specific HLA antibodies & allograft injury: mechanisms, methods of detection, manifestations and management. *Transpl Int*, 31(10):1059-1070. https://doi.org/10.1111/tri.13324

McGehee AM, Dougan SK, Klemm EJ, et al., 2009. XBP-1-deficient plasmablasts show normal protein folding but altered glycosylation and lipid synthesis. *J Immunol*, 183(6):3690-3699. https://doi.org/10.4049/jimmunol.0900953

Minnich M, Tagoh H, Bönelt P, et al., 2016. Multifunctional role of the transcription factor Blimp-1 in coordinating plasma cell differentiation. *Nat Immunol*, 17(3):331-343. https://doi.org/10.1038/ni.3349

Muramatsu M, Kinoshita K, Fagarasan S, et al., 2018. Pillars article: class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell*. 102:553-563. *J Immunol*, 201(9):2530-2540.

Nutt SL, Hodgkin PD, Tarlinton DM, et al., 2015. The generation of antibody-secreting plasma cells. *Nat Rev Immunol*, 15(3):160-171. https://doi.org/10.1038/nri3795

Peng LR, Yuan ZG, Ling HB, et al., 2011. SIRT1 deacetylates the DNA methyltransferase 1 (DNMT1) protein and alters its activities. *Mol Cell Biol*, 31(23):4720-4734. https://doi.org/10.1128/mcb.06147-11

Peng XP, Liao GC, Sun PH, et al., 2019. An overview of HDAC inhibitors and their synthetic routes. *Curr Top Med Chem*, 19(12):1005-1040. https://doi.org/10.2174/1568026619666190227221507

Pojani E, Barlocco D, 2021. Romidepsin (FK228), A histone deacetylase inhibitor and its analogues in cancer chemotherapy. *Curr Med Chem*, 28(7):1290-1303. https://doi.org/10.2174/0929867327666200203113926

Rafehi H, Karagiannis TC, El-Osta A, 2017. Pharmacological histone deacetylation distinguishes transcriptional regulators. *Curr Top Med Chem*, 17(14):1611-1622. https://doi.org/10.2174/1568026617666161104104341

Reimold AM, Iwakoshi NN, Manis J, et al., 2001. Plasma cell differentiation requires the transcription factor XBP-1. *Nature*, 412(6844):300-307. https://doi.org/10.1038/35085509

Ron D, Walter P, 2007. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol*, 8(7):519-529. https://doi.org/10.1038/nrm2199

Rush JS, Liu M, Odegard VH, et al., 2005. Expression of activation-induced cytidine deaminase is regulated by cell division, providing a mechanistic basis for division-linked class switch recombination. *Proc Natl Acad Sci USA*, 102(37):13242-13247. https://doi.org/10.1073/pnas.0502779102

Sciammas R, Shaffer AL, Schatz JH, et al., 2006. Graded expression of interferon regulatory factor-4 coordinates isotype switching with plasma cell differentiation. *Immunity*, 25(2):225-236. https://doi.org/10.1016/j.immuni.2006.07.009

Shaffer AL, Shapiro-Shelef M, Iwakoshi NN, et al., 2004. XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. *Immunity*, 21(1):81-93. https://doi.org/10.1016/j.immuni.2004.06.010

Shapiro-Shelef M, Lin KI, McHeyzer-Williams LJ, et al., 2003. Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells. *Immunity*, 19(4):607-620. https://doi.org/10.1016/s1074-7613(03)00267-x

Sheppard EC, Morrish RB, Dillon MJ, et al., 2018. Epigenomic modifications mediating antibody maturation. *Front Immunol*, 9:355. https://doi.org/10.3389/fimmu.2018.00355

Shi W, Liao Y, Willis SN, et al., 2015. Transcriptional profiling of mouse B cell terminal differentiation defines a signature for antibody-secreting plasma cells. *Nat Immunol*, 16(6):663-673. https://doi.org/10.1038/ni.3154

Sriburi R, Jackowski S, Mori K, et al., 2004. XBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum. *J Cell Biol*, 167(1):35-41. https://doi.org/10.1083/jcb.200406136

Stavnezer J, Guikema JEJ, Schrader CE, 2008. Mechanism and regulation of class switch recombination. *Annu Rev Immunol*, 26:261-292. https://doi.org/10.1146/annurev.immunol.26.021607.090248

Suliman BA, Xu DK, Williams BRG, 2012. HDACi: molecular mechanisms and therapeutic implications in the innate immune system. *Immunol Cell Biol*, 90(1):23-32. https://doi.org/10.1038/icb.2011.92

Tellier J, Shi W, Minnich M, et al., 2016. Blimp-1 controls plasma cell function through the regulation of immunoglobulin secretion and the unfolded protein response. *Nat Immunol*, 17(3):323-330. https://doi.org/10.1038/ni.3348

Tordera RM, Cortés-Erice M, 2021. Role of histone deacetylases in monocyte function in health and chronic inflammatory diseases. *In*: Pedersen SHF (Ed.), Reviews of Physiology, Biochemistry and Pharmacology. Springer, Cham, p.1-47. https://doi.org/10.1007/112_2021_59

Trezise S, Nutt SL, 2021. The gene regulatory network controlling plasma cell function. *Immunol Rev*, 303(1):23-34. https://doi.org/10.1111/imr.12988

Wu XM, Cao L, Nie P, et al., 2019. Histone H2A cooperates with RIP2 to induce the expression of antibacterial genes and MHC related genes. *Dev Comp Immunol*, 101:103455. https://doi.org/10.1016/j.dci.2019.103455

Yamaguchi T, Cubizolles F, Zhang Y, et al., 2010. Histone deacetylases 1 and 2 act in concert to promote the G1-to-S progression. *Genes Dev*, 24(5):455-469. https://doi.org/10.1101/gad.552310

Yang M, Chen G, Zhang X, et al., 2019. Inhibition of class I HDACs attenuates renal interstitial fibrosis in a murine model. *Pharmacol Res*, 142:192-204. https://doi.org/10.1016/j.phrs.2019.02.011

Yang X, Yu X, Zhao Z, et al., 2021. Endoplasmic reticulum stress is involved in retinal injury induced by repeated transient spikes of intraocular pressure. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 22(9):746-756. https://doi.org/10.1631/jzus.B2100053

Ye JS, Li J, Zhou MJ, et al., 2016. Modulation of donor-specific antibody production after organ transplantation by valproic acid: a histone deacetylase inhibitor. *Transplantation*, 100(11):2342-2351. https://doi.org/10.1097/tp.0000000000001197

Yoshida H, Matsui T, Yamamoto A, et al., 2001. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell*, 107(7):881-891. https://doi.org/10.1016/s0092-8674(01)00611-0

Zakzuk J, Acevedo N, Harb H, et al., 2020. IgE levels to *Ascaris* and house dust mite allergens are associated with increased histone acetylation at key type-2 immune genes. *Front Immunol*, 11:756. https://doi.org/10.3389/fimmu.2020.00756

Zhang X, Guo M, Kang YD, et al., 2013. SAHA, an HDAC inhibitor, attenuates antibody-mediated allograft rejection. *Transplantation*, 96(6):529-537. https://doi.org/10.1097/TP.0b013e31829b7bfc

Zhao D, Zou CX, Liu XM, et al., 2020. A UPR-induced soluble ER-phagy receptor acts with VAPs to confer ER stress resistance. *Mol Cell*, 79(6):963-977.e3. https://doi.org/10.1016/j.molcel.2020.07.019

Supplementary information

File S1; Table S1