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Original Article

Deubiquitinase USP29 correlates RORγt expression and its association with thymoma myasthenia gravis

Deyu Xia[1*], Liyan Pan[1*], Xiaochen Fu[2*], Yiran Meng[1], Min Li[1], Wei Wang[1], Zhongkui Wang[1]

Departments of [1]Neurology, [2]Intervention Therapy, Hebei Yanda Hospital, Langfang 065201, Hebei, P. R. China. [*]Equal contributors.

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Abstract: Objective: The objective of this study was to examine the expression of deubiquitylases USP29 in thymomas with myasthenia gravis (MG) and research associated immunological processes. Methods: 69 MG patients with thymomas, 21 thymoma patients without MG, and 31 healthy controls were classified into three groups (categories): group with MG-associated thymoma (MG-T), group with non-MG-associated thymoma (NMG-T), and group with healthy controls (HC). In thymomas, the mRNA and protein levels of RORγt and USP29 were examined by real-time reverse transcription polymerase chain reaction (real-time RT-PCR) and western blotting. Th17 cell counts in MG patients with thymomas were investigated by flow cytometry. Results: In MG-related thymomas, the mRNA and protein levels of deubiquitylases USP29 were substantially elevated. USP29 post-transcriptionally regulated RORγt. In MG patients with thymomas, the expression of USP29 was positively linked to the RORγt expression and Th17 cell frequency. Conclusion: This work exhibited that the elevated USP29 enhanced RORγt expression, which in turn affected the Th17 cell growth in thymomatous MG. Our data suggest that USP29 might take part in the regulation of RORγt expression and Th17 cell generation and constitute an innovative regulatory function for USP29 in autoimmune disease.

Keywords: Deubiquitinase, USP29, myasthenia gravis, thymoma, Th17 cells, RORγt

Introduction

The presence of antibodies against the acetylcholine receptor (AchR) or additional components of the neuromuscular junction's postsynaptic muscle endplate results in myasthenia gravis (MG) - which is an autoimmune illness that is T cell-reliant and B cell-mediated [1]. MG is an autoimmune disease that also affects organs. Pathogenesis of MG is substantially influenced by thymus abnormalities, which can manifest as thymoma or hyperplasia [2].

Thymomas (TM), which are often in the form of type B1 or B2 thymic epithelial tumors, are detected in nearly 10-15% of patients with generalized AChR-MG [3]. Autoimmune processes and the onset and advancement of thymomas are strongly correlated. In the pathogenesis of MG, several immunoregulatory cell populations, including $CD4^+FoxP3^+$ regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and $CD4^+$ T helper 17 cells (Th17s), are involved [4]. In MG patients with thymomas who had peripheral blood mononuclear cells (PBMCs), Th17 cells and their related cytokines are enhanced, according to our earlier findings [5]. The severity of the disease may be characterized by the rise in Th17 cells. However, the mechanisms responsible for Th17s proliferation in MG patients with thymomas are still unclear.

The orphan nuclear receptor RORγt is a crucial transcription element that controls the advancement and function of inflammatory Th17s [6]. Therefore, it is important to thoroughly understand the upstream regulators of RORγt. Evidence has also shown that several DUBs including Ubiquitin-specific protease 4 (USP4), tumor necrosis factor receptor-associated factor 5 (TRAF5), and USP17 have a clear effect on Th17 differentiation and function by stabilizing RORγt [7-9].

Ubiquitination is a crucial posttranslational alteration that modulates the activity or cellular

localization of proteins or can target them for degradation [10]. Deubiquitylases (DUBs) encompass a family of about 90 cysteine proteases and metalloproteases [11]. Previous studies have confirmed that DUBs regulate the pathogenesis of a variety of tumors [12-15]. Nevertheless, investigations focusing on the relationship between expression levels of DUBs and the pathogenesis of thymoma or MG are few.

By comparing genes dysregulated in autoimmune diseases from two GSE profiles (GSE81066 and GSE79702) [16, 17], we found only USP29 was enriched in both sequencing data. USP29 was reported to regulate the checkpoint adaptor claspin in tumor [18]. It was further found to regulate the stability of HIF1alpha and related with tumor progression and sorafinib resistance [19, 20]. USP29 can also regulate anti-viral immune responses by deubiquitination of cGAS [21]. However, its role in MG has not been demonstrated till now.

Here we report that USP29 expression in MG correlates with the RORγt and Th17 cell expression levels, suggesting the roles of USP29 in Th17-mediated autoimmunity.

Materials and methods

Patients and control samples

Employing the clinical criteria mentioned below, MG patients were recruited into this research [22]: (1) fatigable and fluctuating weakness of axial, limb, bulbar, and/or extraocular muscles; (2) positive neostigmine test results; (3) a gradual decline in the magnitude of compound muscle action potentials induced by 3-Hz repetitive nerve stimulations of a peripheral nerve; and/or (4) positive AChR-binding antibodies or muscle-specific kinase (MUSK) antibodies. Patients with a history of cancer, additional autoimmune diseases, or other CNS diseases, were excepted. The patients were not treated with any immunomodulatory therapy at the time of the blood draw. The research group (MG-associated thymoma group, MG-T) comprised 69 MG patients with thymomas (30 males, 39 females; age 45±22 years) and 31 healthy controls (13 males, 18 females; age 49±17 years) who were registered from tissue specimens of thymoma and serum samples that were acquired from MG patients admitted to the Hebei Yanda Hospital and the 309 Hospital of Chinese People's Liberation Army. In addition, matching thymoma tissue samples were randomly resected from 21 MG-negative patients (10 males, 11 females; age 43±14 years) who had undergone surgery in the cardiothoracic surgery, comprising the group with the positive control (non-MG-associated thymoma group, NMG-T). Age, gender, and ethnicity did not substantially differ amongst the group with MG-T, the group with NMG-T, and the healthy control group. Hebei Yanda Hospital's Institutional Ethics Board granted its approval for the research.

Extraction of RNA and quantitative real-time PCR (qRT-PCR)

In liquid nitrogen, primary thymic tumor pieces were immediately snap-frozen following the resection and kept at -80°C. Subsequently, 8 μm slides were cut from a frozen tumor specimen that had been embedded in an optimal cutting temperature compound (OCT). The manufacturer's instructions were followed for the extraction of total RNA utilizing a standard Trizol (Invitrogen, Carlsbad, CA, USA). The GAPDH, housekeeping gene, served as an internal control. The manufacturer's instructions were followed for performing a reverse transcription reaction on 2 μg of total RNAs from individual samples utilizing a reverse transcription system (Invitrogen) with a reaction volume of 20 μl. The sequences of the gene-specific primers, which were produced in accordance with the sequences available in Gene Bank, are presented below: GAPDH (forward: 5'-GTG AAG GTC GGA GTC AAC G-3'; reverse: 5'-TGA GGT CAA TGA AGG GGT C-3'). Rorc (forward: 5'-GTGGGGACAAGTCGTCTGG-3'; reverse: 5'-AGTGCTGGCATCGGTTTCG-3'). USP29 (forward: 5'-GAAACTCGGGCCTTCATTCAA-3'; reverse: 5'-CTGTGCTCTGGTTCCAATGG-3'). In addition, we diluted 20 μl of cDNA synthesis reaction to 100 μl, and 5 μl was utilized in each PCR reaction after diluting 20 μl of cDNA synthesis reaction to 100 μl. In an individual sample, the levels of GAPDH were utilized to normalize RORγt and USP29 levels, which were then, analyzed employing the 2-standard curve approach.

Western blotting analysis

By means of western blot analysis, RORγt and USP29 expression levels in thymomas were evaluated. Harvested tissues were extracted

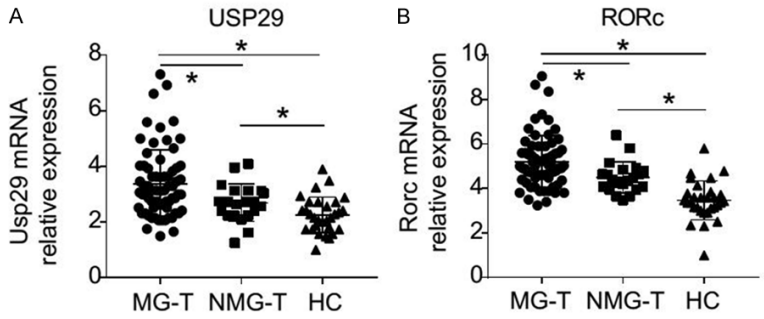

Figure 1. The mRNA level of USP29 and Rorc in patients with MG. The expression of USP29 (A) and Rorc (B) were examined by qRT-PCR. The expression levels were normalized by Gapdh. The sample with the lowest gene expression was set as a reference. Gene's relative expression was computed by comparing it with the reference sample. *P<0.05. MG: Myasthenia Gravis.

utilizing a RIPA buffer (pH 7.5, 150 mM NaCl, 0.5% sodium deoxycholate, 25 mM Tris-HCl, 0.1% SDS, 1% Nonidet P-40). SDS-PAGE was employed to separate the samples, and then the membranes made of polyvinylidene difluoride (Bio-Rad) were utilized to transfer proteins. Membranes were blocked in TBS comprising 0.1% Tween 20 for 1 h, followed by overnight incubation with appropriate primary AB at 4°C and rinsing with TBS comprising 0.1% Tween 20. The following antibodies and their dilutions were used in the study: anti-USP29 (Invitrogen, PA5-104441, 1:500), anti-RORγt antibody (Invitrogen, 14-6988-82, 1:500).

Flow cytometry

With the aid of the Ficoll-Paque PLUS (GE Healthcare) gradient centrifugation, the isolation of Peripheral blood mononuclear cells (PBMC) was performed from the study subjects' peripheral blood. Cells were stimulated for the cytokine staining procedure with 50 ng/ml PMA, 2 μM monensin (Enzo, Plymouth, PA, USA), and 1 μg/ml ionomycin (Sigma, StLouis, MO, USA) at 37°C for 4 h. Cells were harvested, and surface-stained first for 15 min with fluorescein isothiocyanate-conjugated antihuman CD4 antibodies, followed by fixing and permeabilization using Perm/Fix solution and final intracellular staining utilizing phycoerythrin (PE)-conjugated antihuman IL-17A antibodies. From eBioscience (San Diego), all antibodies were procured. Employing CELLQUEST software and FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA), stained samples were analyzed.

ELISA

A human AChRab ELISA kit (R&D, USA) was employed for measuring the levels of anti-AchR antibodies in the sera of the MG patients following the manufacturer's instructions. The assay's concentration ranged from 20 pmol/L to 500 pmol/L.

Statistical analysis

All of the data are shown as the means ± SD. For statistical analysis, Windows software of SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) was employed. For comparisons across groups, the nonparametric Mann-Whitney U test was utilized. A Bonferroni/Dunn multiple-comparison test was employed to conduct post-hoc analyses. *P*-values <0.05 indicated a significant level.

Results

USP29 and RORγt mRNAs were upregulated in thymoma tissues from patients with thymoma-associated myasthenia gravis

Employing quantitative real-time PCR, USP29 mRNA and Rorc mRNA (which encodes RORγt) human thymoma expression was tested both in MG-positive and -negative thymoma patients as well as in normal thymus. In terms of the housekeeping gene (GAPDH), the values were determined as copy numbers of the interesting gene. **Figure 1A** depicts the relative quantification values (RQ values) of USP29 mRNA. USP29 mRNA-related RQ values in the group with MG-T (3.37±1.23, P<0.05) and in the group with NMG-T (2.69±0.67, P<0.05) were substantially higher in comparison with those in the normal thymus (2.27±0.62). Furthermore, USP29 mRNA expression levels in the group with MG-T were increased substantially compared to the group with NMG-T (P<0.05) values. Meanwhile, the Rorc mRNA-related RQ values in the group with MG-T (5.20±1.18, P<0.05) and in the group with NMG-T (4.49±0.70, P<0.05) were elevated substantially than those in the normal thymus (3.52±0.77) (**Figure 1B**).

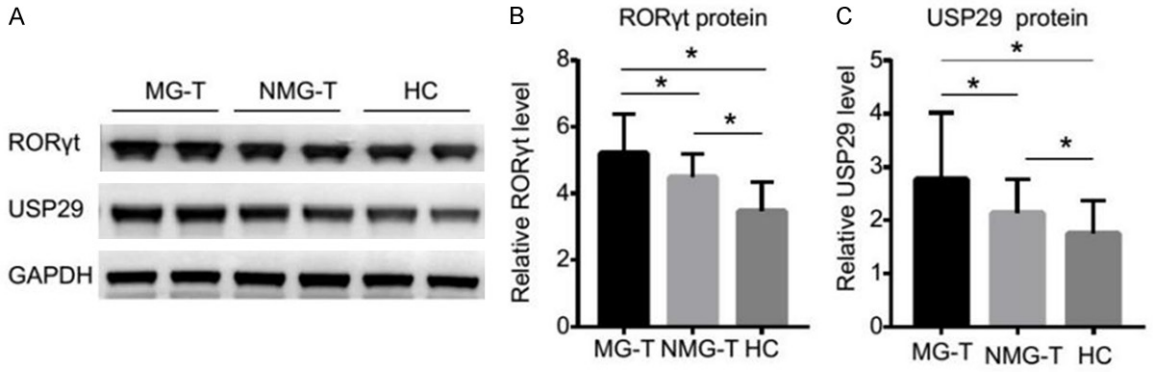

Figure 2. USP29 and RORγt protein level in patients with MG. A. The protein level of USP29 and RORγt were determined by SDS-PAGE and immunoblot assay. The expression of USP29 and RORγt were normalized by GADPH. B, C. Statistic analyses of RORγt and USP29 protein expression. *P<0.05. MG: Myasthenia Gravis.

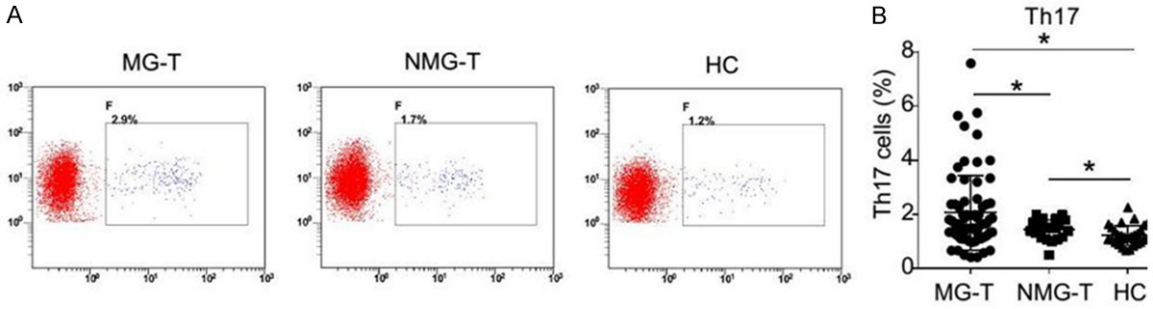

Figure 3. Th17 cells in the PBMC of patients with MG. (A) PBMC from patients were stimulated in vitro with PMA plus ionomycin for 4 h, followed by staining with anti-CD4 and anti-IL-17A as stated in materials and methods. Representative flow cytometry plots were shown; (B) Statistical analysis of Th17 cell percentages in (A). *P<0.05. MG: Myasthenia Gravis; PBMC: Peripheral Blood Mononuclear Cell.

Increased USP29 and RORγt proteins in thymoma tissues from thymoma-associated MG patients

In thymoma tissues, in order to investigate whether USP29 was linked to the RORγt protein level, we next evaluated the levels of USP29 and RORγt proteins in the MG patients with thymoma. Human thymoma expression of USP29 and RORγt proteins was determined (normalized to GAPDH) by using western blot analysis. USP29 production in the group with MG-T (2.77±1.25, P<0.01) and in the group with NMG-T (2.14±0.63, P<0.05) were increased substantially compared with the normal thymus (1.75±0.62). USP29 expression levels in the group with MG-T were increased substantially versus those in the group with NMG-T (P<0.05). Our findings, however, revealed a remarkable upregulation of RORγt in the group with MG-T (5.21±1.18, P<0.01) and in the group with NMG-T (4.49±0.70, P<0.05) than healthy controls (3.52±0.77). The expression levels of RORγt in the group with MG-T were substantially higher compared to those in the group with NMG-T (P<0.05) (**Figure 2**).

Elevated frequency of Th17 cells in thymoma-associated MG patients

In order to assess the distribution profiles of Th17 cell subsets in PBMCs of MG patients with thymoma, a standard flow cytometry assay was developed to test on PBMCs from MG patients with thymoma, thymoma without MG and healthy controls (**Figure 3A**). As depicted in **Figure 3B**, TH17 cells were found at a greater percentage in the group with MG-T (2.06± 1.38, P<0.05) and in the group with NMG-T (1.44±0.37, P<0.05), than healthy controls (1.22±0.36). The Th17 cell proportions in the MG-T group were substantially higher compared to those in the group with NMG-T (P<0.05).

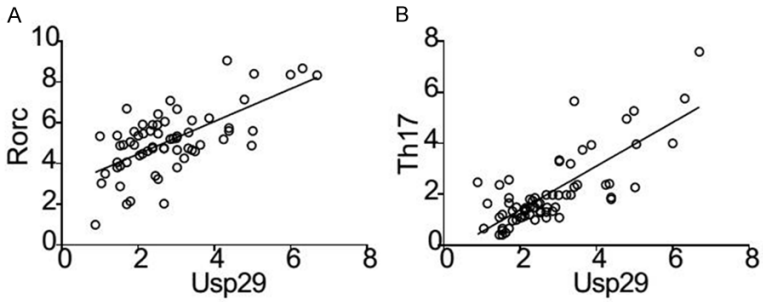

Figure 4. USP29 expression correlated with Rorc and Th17 cells in patients with MG. MG: Myasthenia Gravis.

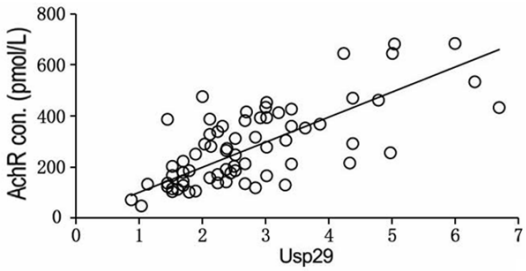

Figure 5. USP29 expression correlated with levels of anti-AChR Ab in patients with MG. MG: Myasthenia Gravis.

USP29 expression levels correlate with the RORγt expression levels and the frequency of Th17 cells in thymoma-associated MG patients

In total, 68 tumoral tissues and PBMCs from MG patients with thymoma were examined for a better understanding of the link between expression levels of USP29 proteins and RORγt and the frequency of Th17 cells in vivo. We discovered a substantial correlation between the USP29 protein expression levels and the RORγt protein expression levels (R^2=0.45, P<0.05), as shown in **Figure 4A**. Meanwhile, a correlation was also observed between the USP29 protein expression levels and the frequency of Th17 cells in thymoma-associated MG patients (R^2=0.6, P<0.05), as shown in **Figure 4B** (P<0.0001). These findings suggest that the USP29 proteins may partially characterize the RORγt protein expression levels and affect the differentiation and growth of Th17 cells in patients with thymoma-associated MG.

The relationship between USP29 expression and levels of anti-AChR Ab in MG patients

Among the healthy controls and MG patients, 67/69 MG sera were positive in the ELISA, whilst 0/31 control serum samples were positive. Additionally, we discovered a substantial positive correlation between USP29 protein expression levels and AChR antibody concentrations in MG patients (R=0.69, P<0.05) (**Figure 5**). According to the findings, the AChR-Ab concentration is related to the USP29 protein expression levels in MG patients.

Discussion

Myasthenia gravis is one of the most understood autoimmune disorders that affect neuromuscular transmission. Autoantibodies against AChR, MuSK, Titin, and LRP4 alter neuromuscular transmission in MG differentially. Previous studies have demonstrated that dysregulation of Th17 cells, conventional T cells, T regulatory cells, and other immune cells that facilitate the production of autoantibodies also participate in the pathogenesis of MG [5, 23, 24]. Nevertheless, the inducing processes by which the immune response is modulated in MG are largely unknown. According to recent research, deubiquitinase may be crucial for the post-translational regulation of T-helper cell differentiation and growth [25].

Ubiquitination has emerged as a critical mechanism that regulates the growth, activation, and differentiation of T cells. According to recent research, several DUBs stimulate cancer cell proliferation and tumor growth [26, 27]. The relationship between DUB and thymoma, however, remained poorly understood. In the current research, we analyzed the USP29 expression levels in MG-T, NMG-T, and healthy controls based on 121 subjects. According to the findings of our research, the USP29 mRNA and protein expression levels were upregulated in MG-positive and -negative thymoma tissues in comparison with the normal thymus. These findings suggested that USP29 may play post-translational regulatory roles in thymoma pathogenesis. The amount of USP29 protein in the thymoma tissue correlated largely with USP29 mRNA abundance, suggesting that elevated USP29 in thymoma is due to transcriptional upregulation. Moreover, we also confirmed that the USP29 mRNA and protein

expression levels in MG-T were elevated substantially in comparison with those in NMG-T. These findings indicate that USP29 may be used as a biological indicator for judging thymoma with MG.

RORγt has been proven to be a crucial factor for the differentiation of Th17 cells [28, 29]. Here, we demonstrated that the RORγt mRNA and protein expression levels were substantially elevated in MG-positive and -negative thymoma. Concurrently, the RORγt protein expression levels were positively correlated with USP29 protein expression. Therefore, the results indicated a novel potential role for USP29 in regulating the expression of RORγt proteins.

Previous studies have demonstrated that Th17 cells influence the immunopathogenesis of MG. In MG patients, Yang et al [30] discovered higher frequencies of cTfh-Th17 cells and cTfh cell counterparts. Anti-AChR Ab concentrations were positively linked to cTfh-Th17 cell frequencies in MG patients. In line with the earlier investigation, we discovered a greater percentage of Th17 cells expressing IL-17A in thymoma with and without MG. Furthermore, in MG patients with thymoma, we discovered that the ratio of TH17 cells and USP29 were correlated positively with each other. Collectively, these findings imply that USP29 can regulate RORγt expression, which in turn influences the growth of Th17 cells and the expression of Th17-associated cytokines, including IL-17.

In summary, our research showed that USP29 is crucial in the tumorigenesis of thymoma by promoting the RORγt expression protein and in the pathogenesis of MG. As far as we are aware, this is the first research on the post-transcriptional regulation mechanism of DUBs in MG. The levels of USP29 proteins and the serum AChR-Ab concentration were also shown to be positively correlated with each other. We, therefore, propose that USP 29 may enhance Th17 cells' autoreactivity, which would then affect the production of self-reactive antibodies and worsen the disease. There is one limitation in this study. Thymic epithelial cells surrounded by T-cells that develop in response to stimulation of epithelial cells make up the mediastinal tumors known as thymomas. Because the thymoma cell line is not a single tumor cell, the radioimmunoassay test of USP29 and RORγt in the thymoma cell line was not performed. Therefore, the regulation mechanism of USP29 on RORγt expression will be included in our future studies.

Acknowledgements

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhongkui Wang, Department of Neurology, Hebei Yanda Hospital, No. 6 Sipulan Road, Langfang 065201, Hebei, P. R. China. Tel: +86-10-03163306640; Fax: +86-10-03163306640; E-mail: ctzlwzk@163.com

References

[1] Ciafaloni E. Myasthenia gravis and congenital myasthenic syndromes. Continuum (Minneap Minn) 2019; 25: 1767-1784.

[2] Meriggioli MN and Sanders DB. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. Lancet Neurol 2009; 8: 475-490.

[3] Evoli A, Minisci C, Di Schino C, Marsili F, Punzi C, Batocchi AP, Tonali PA, Doglietto GB, Granone P, Trodella L, Cassano A and Lauriola L. Thymoma in patients with MG: characteristics and long-term outcome. Neurology 2002; 59: 1844-1850.

[4] Wu Y, Luo J and Garden OA. Immunoregulatory cells in myasthenia gravis. Front Neurol 2020; 11: 593431.

[5] Wang Z, Wang W, Chen Y and Wei D. T helper type 17 cells expand in patients with myasthenia-associated thymoma. Scand J Immunol 2012; 76: 54-61.

[6] Chen Z, Lin F, Gao Y, Li Z, Zhang J, Xing Y, Deng Z, Yao Z, Tsun A and Li B. FOXP3 and RORγt: transcriptional regulation of Treg and Th17. Int Immunopharmacol 2011; 11: 536-542.

[7] Yang J, Xu P, Han L, Guo Z, Wang X, Chen Z, Nie J, Yin S, Piccioni M, Tsun A, Lv L, Ge S and Li B. Cutting edge: ubiquitin-specific protease 4 promotes Th17 cell function under inflammation by deubiquitinating and stabilizing RORγt. J Immunol 2015; 194: 4094-4097.

[8] Wang X, Yang J, Han L, Zhao K, Wu Q, Bao L, Li Z, Lv L and Li B. TRAF5-mediated Lys-63-linked

polyubiquitination plays an essential role in positive regulation of RORγt in promoting IL-17A expression. J Biol Chem 2015; 290: 29086-29094.
[9] Han L, Yang J, Wang X, Wu Q, Yin S, Li Z, Zhang J, Xing Y, Chen Z, Tsun A, Li D, Piccioni M, Zhang Y, Guo Q, Jiang L, Bao L, Lv L and Li B. The E3 deubiquitinase USP17 is a positive regulator of retinoic acid-related orphan nuclear receptor γt (RORγt) in Th17 cells. J Biol Chem 2014; 289: 25546-25555.
[10] Grabbe C, Husnjak K and Dikic I. The spatial and temporal organization of ubiquitin networks. Nat Rev Mol Cell Biol 2011; 12: 295-307.
[11] Clague MJ, Urbé S and Komander D. Breaking the chains: deubiquitylating enzyme specificity begets function. Nat Rev Mol Cell Biol 2019; 20: 338-352.
[12] Li J, Yuan S, Norgard RJ, Yan F, Yamazoe T, Blanco A and Stanger BZ. Tumor cell-intrinsic USP22 suppresses antitumor immunity in pancreatic cancer. Cancer Immunol Res 2020; 8: 282-291.
[13] Du T, Li H, Fan Y, Yuan L, Guo X, Zhu Q, Yao Y, Li X, Liu C, Yu X, Liu Z, Cui CP, Han C and Zhang L. The deubiquitylase OTUD3 stabilizes GRP78 and promotes lung tumorigenesis. Nat Commun 2019; 10: 2914.
[14] Prieto-Garcia C, Hartmann O, Reissland M, Braun F, Fischer T, Walz S, Schülein-Völk C, Eilers U, Ade CP, Calzado MA, Orian A, Maric HM, Münch C, Rosenfeldt M, Eilers M and Diefenbacher ME. Maintaining protein stability of ΔNp63 via USP28 is required by squamous cancer cells. EMBO Mol Med 2020; 12: e11101.
[15] Wu Y, Zhang Y, Wang D, Zhang Y, Zhang J, Zhang Y, Xu L, Wang T, Wang S, Zhang Q, Liu F, Zaky MY, Li Q, Sun Q, Guo D, Liu S, Zou L, Yang Q and Liu H. USP29 enhances chemotherapy-induced stemness in non-small cell lung cancer via stabilizing Snail1 in response to oxidative stress. Cell Death Dis 2020; 11: 796.
[16] Torre S, Polyak MJ, Langlais D, Fodil N, Kennedy JM, Radovanovic I, Berghout J, Leiva-Torres GA, Krawczyk CM, Ilangumaran S, Mossman K, Liang C, Knobeloch KP, Healy LM, Antel J, Arbour N, Prat A, Majewski J, Lathrop M, Vidal SM and Gros P. USP15 regulates type I interferon response and is required for pathogenesis of neuroinflammation. Nat Immunol 2017; 18: 54-63.
[17] Kathania M, Khare P, Zeng M, Cantarel B, Zhang H, Ueno H and Venuprasad K. Itch inhibits IL-17-mediated colon inflammation and tumorigenesis by ROR-γt ubiquitination. Nat Immunol 2016; 17: 997-1004.
[18] Martín Y, Cabrera E, Amoedo H, Hernández-Pérez S, Domínguez-Kelly R and Freire R. USP29 controls the stability of checkpoint adaptor Claspin by deubiquitination. Oncogene 2015; 34: 1058-1063.
[19] Gao R, Buechel D, Kalathur RKR, Morini MF, Coto-Llerena M, Ercan C, Piscuoglio S, Chen Q, Blumer T, Wang X, Dazert E, Heim MH, Hall MN, Tang F and Christofori G. USP29-mediated HIF1α stabilization is associated with Sorafenib resistance of hepatocellular carcinoma cells by upregulating glycolysis. Oncogenesis 2021; 10: 52.
[20] Tu R, Kang W, Yang M, Wang L, Bao Q, Chen Z, Dong Y, Wang J, Jiang J, Liu H and Qing G. USP29 coordinates MYC and HIF1α stabilization to promote tumor metabolism and progression. Oncogene 2021; 40: 6417-6429.
[21] Zhang Q, Tang Z, An R, Ye L and Zhong B. USP29 maintains the stability of cGAS and promotes cellular antiviral responses and autoimmunity. Cell Res 2020; 30: 914-927.
[22] Hehir MK and Silvestri NJ. Generalized myasthenia gravis: classification, clinical presentation, natural history, and epidemiology. Neurol Clin 2018; 36: 253-260.
[23] Balandina A, Lécart S, Dartevelle P, Saoudi A and Berrih-Aknin S. Functional defect of regulatory CD4(+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. Blood 2005; 105: 735-741.
[24] Gradolatto A, Nazzal D, Truffault F, Bismuth J, Fadel E, Foti M and Berrih-Aknin S. Both Treg cells and Tconv cells are defective in the Myasthenia gravis thymus: roles of IL-17 and TNF-α. J Autoimmun 2014; 52: 53-63.
[25] Rutz S, Kayagaki N, Phung QT, Eidenschenk C, Noubade R, Wang X, Lesch J, Lu R, Newton K, Huang OW, Cochran AG, Vasser M, Fauber BP, DeVoss J, Webster J, Diehl L, Modrusan Z, Kirkpatrick DS, Lill JR, Ouyang W and Dixit VM. Deubiquitinase DUBA is a post-translational brake on interleukin-17 production in T cells. Nature 2015; 518: 417-421.
[26] Li XY, Wu HY, Mao XF, Jiang LX and Wang YX. USP5 promotes tumorigenesis and progression of pancreatic cancer by stabilizing FoxM1 protein. Biochem Biophys Res Commun 2017; 492: 48-54.
[27] Zhang X, Meng T, Cui S, Feng L, Liu D, Pang Q and Wang P. Ubiquitination of nonhistone proteins in cancer development and treatment. Front Oncol 2020; 10: 621294.
[28] Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ and Littman DR. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 2006; 126: 1121-1133.

[29] Chang H, Zhao F, Xie X, Liao Y, Song Y, Liu C, Wu Y, Wang Y, Liu D, Wang Y, Zou J and Qi Z. PPARα suppresses Th17 cell differentiation through IL-6/STAT3/RORγt pathway in experimental autoimmune myocarditis. Exp Cell Res 2019; 375: 22-30.

[30] Yang Y, Zhang M, Ye Y, Ma S, Fan L and Li Z. High frequencies of circulating Tfh-Th17 cells in myasthenia gravis patients. Neurol Sci 2017; 38: 1599-1608.