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CASE REPORT

A novel compound heterozygous mutation of *MYSM1* gene in a patient with bone marrow failure syndrome 4

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Introduction

Bone marrow failure syndromes (BMFS) are a group of diseases in which the effective generation of mature red blood cells, granulocytes, and platelets in the bone marrow is disrupted [1,2]. Clinical BMFS can be divided into inherited (IBMFS) and acquired (ABMFS) types, the latter is the most prevalent and former is relatively rare. Bone marrow failure syndrome 4 (BMFS 4) is a rare type of IBMFS with incidence < 1/million. It is an autosomal recessive genetic disease characterized by early-onset anaemia, leucopenia, and B cell loss, and thrombocytopenia. Some patients may develop features such as facial deformities, bone abnormalities, and growth retardation [3]. In addition to routine clinical laboratory tests, the diagnosis of IBMFS includes chromosome fragility test, comet assay and the telomere related test [4].

BMFS failure (may involve all or a single lineage) may present at birth or at a variable time thereafter including in adulthood in some cases. They constitute an important group of disorders as recent advances in the genetics of some of these are beginning to unravel their pathophysiology.

MYSM1, located on chromosome 1, encodes a 828 amino acid deubiquitination enzyme composed of 3 domains. The N-terminal SANT domain binds to DNA, the middle domain binds to the histone tail, and the C-terminal JAMM domain has the activity of metalloprotease to hydrolyse ubiquitin [5–7]. In prostate cancer cells, MYSM1 forms a co-regulatory protein complex, the activity of deubiquitination enzyme is regulated by the acetylation state of histones to activate androgen receptor-dependent transcription [8]. Subsequent research on *MYSM1* has focused on its effects on the haematopoietic and immune systems. Mutations in *MYSM1* were recently linked to BMFS [9–11]. To date, only three pathogenic variants (p.Glu390*, p.Arg478*, and p.His656Arg) of *MYSM1* have been reported in nine patients, all of which are homozygous [3,12–16]. Recently, a study showed that a novel compound heterozygous variant in *MYSM1* (c.399 G > A, p.L133L, and c.1467 C > G, p.Y489*) was identified in a girl [17]. This study broadened our understanding of the role of the *MYSM1* gene in bone marrow failure and immune system genetics and disease progression. We present an additional case where abnormalities in *MYSM1* are linked to BMFS.

The patient

This study was approved by the Ethics Committee of Maternal and Child Hospital of Hubei province, Tongji Medical College Huazhong University of Science and Technology. We obtained written informed consent of the proband's parents. The term female was delivered by caesarean section due to endouterine asphyxia. The apgar score was unknown. The baby had slight yellow stains, no bleeding, no rash, irregular breathing, no dyspnoea, no abnormalities in heart and liver examination, and polydactylism with six fingers on the right hand. The baby's skin was pale after birth, and blood tests indicated that she was severely anaemic (Table 1). She received a 123 ml ABO/Rh compatible blood transfusion 4 hours after birth, and also included anti-infection and fluid rehydration treatment.

With little clinical or laboratory progress, on day 5 a more thorough investigation was undertaken (Table 1). These included liver and renal functions, haemolysis analysis, iron metabolism, virus detection (B19-DNA, EB-DNA and CMV-DNA), transfusion-associated contagion test, bone marrow cytology, echocardiography, abdominal ultrasonography, brain MRI, blood amino acids, urine organic acids, and hearing screening, etc. The results suggested mild jaundice, mild low magnesium, decreased unbound and total iron-binding capacity, atrial septal defect, mitral and tricuspid regurgitation, and slightly elevated pulmonary artery pressure. Bone marrow cytology revealed mild myelodysplasia with slight reductions in each cell linage, although the proportions were normal. The brain MRI

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Table 1. Laboratory data.

Items	Day 1	Day 5	Day 30	Day 60	Reference range
White blood cells ($\times10^9$/L)	1.5	2.2	2.0	2.0	3.5–9.5
Platelets ($\times10^9$/L)	184	70	223	291	100–300
Lymphocytes ($\times10^9$/L)	0.47	0.52	0.96	1.02	1.1–3.2
Monocytes ($\times10^9$/L)	0.02	0.31	0.26	0.05	0.1–0.6
Neutrophils ($\times10^9$/L)	0.99	0.52	0.84	0.8	1.8–6.3
Reticulocytes ($\times10^9$/L)	-	10.8	3.9	3.4	20–200
Reticulocytes (%)	-	0.26%	0.19%	0.17%	0.3–3.0
Haemoglobin (g/L)	52	132	65	72	115–150

revealed an increased T2 signal in bilateral parietal subcortical white matter, and a cyst (5 × 2 mm) in left lateral ventricle. The baby was diagnosed with leucopenia, thrombocytopenia, polydactylism, perinatal brain damage and a brain cyst. She was later discharged after treatment, including granulocyte colony-stimulating factor, phototherapy, etc.

Thirty days after birth, the baby was readmitted to our department with increasing pale skin. Weight had increased to 3500 g and height to 51.5 cm, both less than that expected from normal growth curves. Routine bloods reported leucopenia, erythropenia but normal platelets, a second bone marrow aspirate reported myelodysplasia with reduced number of nucleated cells and decreased numbers of megakaryocytes. As the infant has been hospitalized several times with transfusion-dependent anaemia, genetic studies were initiated.

By day 60, her weight (4250 g) and height (53 cm) were still less than expected and the routine bloods were unchanged. Immunological studies reported several abnormalities (some profound): IgA <0.07 g/L (reference range <0.34), IgG 3.6 g/L (2.0–6.9), IgM 0.04 g/L (0.06–0.66), complement C3 0.63 g/L (0.65–1.39), complement C4 0.16 g/L (0.16–0.38), NK cells 292 cells/μL (100–1400), CD3+ ve 647 cells/μL (1400–1800), CD4+ ve 301 cells/μL (900–5500), CD3+ ve/CD8+ ve 300 cells/μL (400–2300) and CD3-ve/CD19+ ve 32 cells/μL (600–3100).

Molecular genetics

The EDTA-treated peripheral blood was collected with informed consent of the patients. The Peripheral blood genomic DNA was extracted using the Blood Genome Column Medium Extraction Kit (Kangweishiji, China) according to the manufactural instructions. The extracted DNA samples were subjected to quality controlling using Qubit 2.0 fluorimeter and electrophoresis with 0.8% agarose gel for further protocol.

Protein-coding exome enrichment was performed using xGen Exome Research Panel v1.0 (IDT, Iowa, USA) that consists of 429,826 individually synthesized and quality-controlled probes, which targets 39 Mb protein-coding region (19,396 genes) of the human genome and covers 51 Mb of end-to-end tiled probe space. High-throughput sequencing was performed by Illumina NovaSeq 6000 series sequencer (PE150), and not less than 99% of target sequence was sequenced. The sequencing process was performed by Beijing Chigene Translational Medicine Research Center Co., Ltd, 100875, Beijing. Raw data were processed by fastp for adapters removing and low-quality reads filtering. The paired-end reads were performed using Burrows-Wheeler Aligner (BWA) to the Ensemble GRCh37/hg19 reference genome. Base quality score recalibration together with SNP and short indel calling was conducted using GATK. According to the sequence depth and variant quality, SNPs and Indels were screened, and high quality and reliable variants were obtained.

The online system independently developed by Chigene (www.chigene.org) was used to annotate database-based minor allele frequencies (MAFs), and ACMG practice guideline-based pathogenicity of the gene variant, and the system also provided serial software packages for conservative analysis and protein product structure prediction. The databases for MAFs annotation included 1,000 genomes. We used the OMIM, HGMD and ClinVar databases as references for annotations on the pathogenicity of the ACMG guidelines. To predict functional change of variants on the splicing sites, MaxEntScan, dbscSNV and GTAG software packages were used. Mutation Taster predicted the harmfulness of mutation sites.

Results

We identified a compound heterozygous variation in the proband, including a frameshift mutation (c.1607_1611delAAGAG, p.Glu536Glyfs*7) and a nonsense mutation (c.1432 C > T, p.Arg478*, rs748065332). Sanger sequencing showed that the deletion mutation in the proband was inherited from her mother, who was in a heterozygous state with c.1607_1611delAAGAG (p.Glu536Glyfs*7) in exon 12. The SNP in the patient was inherited from her father, who was in a heterozygous state carrying c.1432 C > T (p.Arg478*) in exon 10 (Figure 1(a)). According to American College of Medical Genetics ratings, both mutations were pathogenic. The mother of the proband was wild-type at c.1432, while the father was wild-type at c.1607_1611. The parents of the proband were normal and had no clinical problems. The mutation of the proband was compound heterozygous, which was consistent with the pathogenesis of autosomal recessive compound heterozygous genetic disease. The phenotype and genotype of the proband and her family members conformed to the genetic law of segregation. There was no family history of BMFS4 and related syndromes. Her parents are nonconsanguineous. The variant for c.1607_1611delAAGAG (p.Glu536Glyfs*7) was reported in patients for the first time (Figure 1(b)). Through public population databases

research, the variant c.1432 C > T (p.Arg478*) was registered with a very low allele frequency in the Genome Aggregation Database (gnomAD) database (1/248644, GnomAD_exome) and the Exome Aggregation Consortium (ExAC) database (1/107514, ExAC). Predicted by Mutation Taster, the results of two mutation sites in our article were disease causing. *MYSM1* contains 20 exons, encoding an 828 amino acid protein, which has three main domains, such as SANT, SWIRM and JAB. Both c.1607_1611delAAGAG and c.1432 C > T variants truncated MYSM1, resulting in the loss of JAB domain (Figure 2). Table 2 summarizes reports of *MYSM1* mutations.

Discussion

BMFS 4 is an extremely rare condition (ORPHA: 508542, https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng= en&Expert = 904) We described a patient with recurrent severe anaemia, leucopenia, neutropenia, intermittent thrombocytopenia, polydactyly, physical development retardation, B-lymphocyte immune deficiency, and so suspected BMFS type 4. Surprisingly, a novel compound heterozygous mutation of *MYSM1*, one from each parent, was identified.

MYSM1 includes 3 domains: SANT, SWIRM and JAB. The SANT domain is present in nuclear receptor co-repressors and in the subunits of many chromatin-remodelling complexes [18]. It has a strong structural similarity to the DNA-binding domain of Myb-related proteins [19]. Despite the overall similarity there are differences that indicate that the SANT domain is functionally divergent from the canonical Myb DNA-binding domain [20]. SWIRM domain is a small alpha-helical domain of about 85 amino acid residues containing a helix-turn helix motif and binds to DNA [21]. Members of JAB family are found in proteasome regulatory subunits, eukaryotic initiation factor 3 (eIF3) subunits and regulators of transcription factors. This family is also known as the MPN domain [22] and PAD-1-like domain [23] JABP1 domain [24] or JAMM domain [25]. These are metalloenzymes that function as the ubiquitin isopeptidase/deubiquitinase in the ubiquitin-based signalling and protein turnover pathways in eukaryotes [25].

In recent years, the reports of *MYSM1* mainly focused on immune related aspects. Jiang et al. first found that *MYSM1* deficiency leads to the inhibition of early B cell differentiation and the deficiency of EBF1 and other B lymphoid genes expression in progenitor B cells, which affects the maturation of B cells [26]. Subsequently, Nijnik and others revealed the key role of *MYSM1* in haematopoiesis and lymphocyte differentiation [27]. Firstly, compared with normal mice, *MYSM1* deficient mice showed smaller body size and lighter weight, abnormal deformity of hind limbs and tail, lymphopenia, anaemia, and thrombocytosis. These symptoms are (mostly) consistent with those of our proband [5]. B cells decreased in the pre-pro-b-cell stage, and the development of T cells was seriously defective, that is, the decrease of thymocytes, $CD4^+$ and $CD8^+$ single positive, double positive and double negative cells. In line with the findings, the proband in this study also showed a decrease in the number of $CD4^+$ T cells [28]. *MYSM1* also has an effect on T cells after they leave the thymus. In addition, *MYSM1* deficient lymphocytes

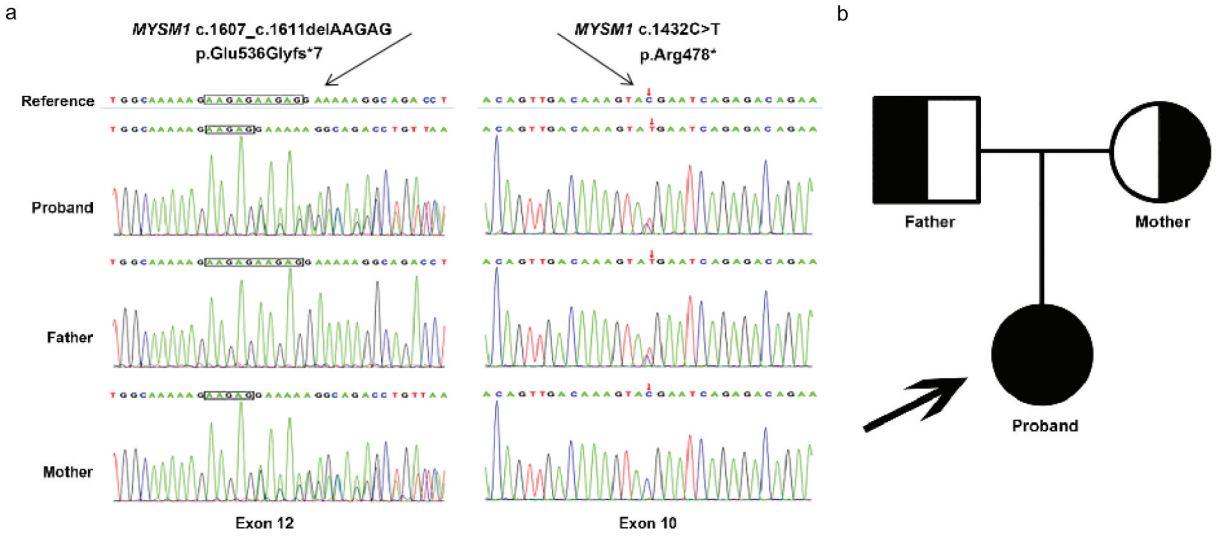

Figure 1. Compound heterozygous variants in *MYSM1* with BMFS4. (a) The c.1607_1611delAAGAG deletion in exon 12 and c.1432 C > T substitution in exon 10 were confirmed in family members by Sanger sequencing. (b) Familial pedigree of BMFS4. ◧ represents the normal phenotype male with heterozygous mutation. ◑ represents the normal phenotype female with heterozygous mutation. ● represents the female patients with compound heterozygous mutations. The arrow indicates the proband.

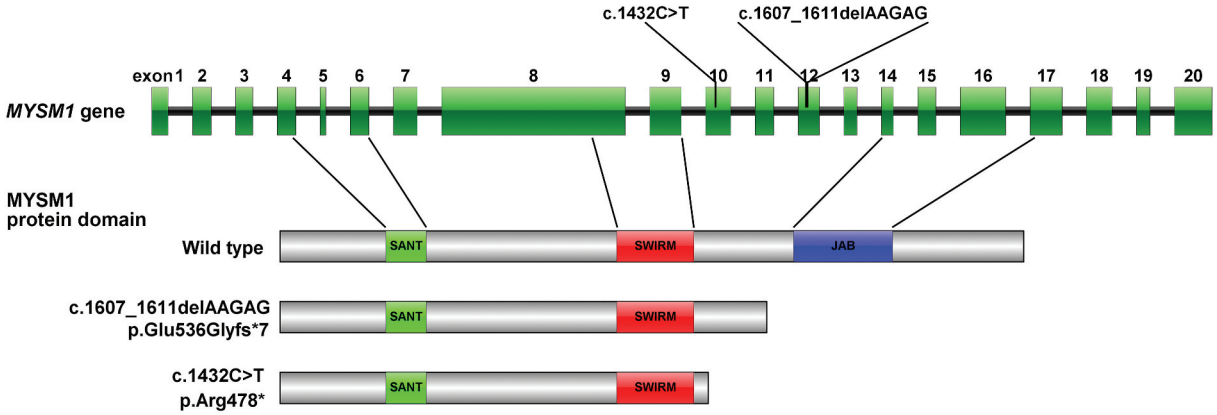

Figure 2. The schematic diagram of the gene structure and protein domain of *MYSM1*. *MYSM1* gene consists of 20 exons. MYSM1 protein mainly includes SANT, SWIRM and JAB domains.

Table 2. *MYSM1* gene variations linked to BMFS4 reported in the literatures.

Homozygous/ Heterozygous	Nucleotide change	Protein change	Exon (s)	Reference
Homozygous	c.1168 G > T	p.Glu390*	9	[3,13]
Homozygous	c.1967A>G	p.His656Arg	16	[12,14,16]
Homozygous	c.1432 C > T	p.Arg478*	10	[15]
Compound heterozygous	Paternal: c.399 G > A, Maternal: c.1467 C > G	p.Leu133Leu, p.Tyr489*	6,10	[17]
Compound heterozygous	Paternal: c.1432 C > T, Maternal: c.1607_1611 delAAGAG	p.Arg478*, p.Glu536Glyfs*7	10, 12	This study

also have defects in response to stimulation. The function of haematopoietic progenitor cells (HSC) in *MYSM1* deficient mice is impaired [29].

The mutation of *MYSM1* found in patients with hereditary BMFS causes anaemia, thrombocytopenia, NK, and B cell reduction. The results of CNVseq and whole exome sequencing showed that these haematopoietic changes were indeed related to the defects of *MYSM1*. Li et al. described a female with bone marrow failure, whose peripheral blood mainly showed leukocytopenia and granulocytopenia. Whole exome sequencing revealed a novel complex heterozygous mutation in *MYSM1*. One was a novel nonsense variant (c.1467 C > G, p.Y489*) in exon 10, which triggered nonsense-mediated mRNA degradation. The other was a novel *MYSM1* mRNA transcript (missing exons 5 and 6) in human blood cells, which was the first report of a synonymous splicing mutation that led to post-transcriptional jumping of exons 6 leading to a bone marrow failure syndrome phenotype [17].

Based on the above studies, previous reports on patients with *MYSM1* deficiency suggested its importance in haematopoiesis and B-cell development. We extended these observations and suggest that *MYSM1* deficiency is associated with bone marrow abnormalities and developmental abnormalities. *MYSM1* deficiency drives haemopoietic stem cells to enter the fast cell cycle from a resting state, increases the proportion of apoptosis, and leads to the exhaustion of stem cell pool, the ability of HSC self-renewal and pedigree reconstruction is impaired [30]. We also speculated and suggested that haematopoietic defects in patients with *MYSM1* deficiency could be cured by allogeneic HSCT. The role of *MYSM1* in maintaining the function and homoeostasis of stem cells has been confirmed, but its mechanism is still unclear. In the present study, the mutation c.1432 C > T (p.Arg478*) in exon10 was identified, which has been reported [15]. A pathogenic truncated mutation c.1607_1611delAAGAG (p.Glu536Glyfs*7) was found, which was reported in patients for the first time. Polydactyly, white matter change and ventricular cyst may reflect the characteristic phenotype of the disease in addition to pancytopenia. This will contribute to the differential diagnosis of similar diseases in the future, but its specific mechanism needs to be further explored.

Taken together, we have identified a novel compound heterozygous mutation of *MYSM1*, which enriched the mutation database and further enhanced our understanding of the BMFS 4 disease caused by *MYSM1* mutation.

Disclosure statement

The authors declare that they have no competing interests.

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References

[1] Biswajit H, Pratim PP, Kumar ST, et al. Aplastic anemia: a common hematological abnormality among peripheral pancytopenia. N Am J Med Sci. 2012;4:384–388.

[2] Young NS. Current concepts in the pathophysiology and treatment of aplastic anemia. Hematology Am Soc Hematol Educ Program. 2013;2013:76–81.

[3] Bahrami E, Witzel M, Racek T, et al. Myb-like, SWIRM, and MPN domains 1 (MYSM1) deficiency: genotoxic stress-associated bone marrow failure and developmental aberrations. J Allergy Clin Immunol. 2017;140:1112–1119.

[4] Dietz AC, Savage SA, Vlachos A, et al. Late effects screening guidelines after hematopoietic cell transplantation for inherited bone marrow failure syndromes: consensus statement from the second pediatric blood and marrow transplant consortium international conference on late effects after pediatric HCT. Biol Blood Marrow Transplant. 2017;23:1422–1428.

[5] Nijnik A, Clare S, Hale C, et al. The critical role of histone H2A-deubiquitinase Mysm1 in hematopoiesis and lymphocyte differentiation. Blood. 2012;119:1370–1379.

[6] Belle JI, Langlais D, Petrov JC, et al. p53 mediates loss of hematopoietic stem cell function and lymphopenia in Mysm1 deficiency. Blood. 2015;125:2344–2348.

[7] Zhu P, Zhou W, Wang J, et al. A histone H2A deubiquitinase complex coordinating histone acetylation and H1 dissociation in transcriptional regulation. Mol Cell. 2007;27:609–621.

[8] Sun J, Hu X, Gao Y, et al. MYSM1-AR complex-mediated repression of Akt/c-Raf/GSK-3β signaling impedes castration-resistant prostate cancer growth. Aging. 2019;11:10644–10663.

[9] Belle JI, Petrov JC, Langlais D, et al. Repression of p53-target gene Bbc3/PUMA by MYSM1 is essential for the survival of hematopoietic multipotent progenitors and contributes to stem cell maintenance. Cell Death Differ. 2016;23:759–775.

[10] Petrov JC, Nijnik A. Mysm1 expression in the bone marrow niche is not essential for hematopoietic maintenance. Exp Hematol. 2017;47:76–82.

[11] Förster M, Belle JI, Petrov JC, et al. Deubiquitinase MYSM1 is essential for normal fetal liver hematopoiesis and for the maintenance of hematopoietic stem cells in adult bone marrow. Stem Cells. 2015;24:1865–1877.

[12] Le Guen T, Touzot F, André-Schmutz I, et al. An in vivo genetic reversion highlights the crucial role of Myb-Like, SWIRM, and MPN domains 1 (MYSM1) in human hematopoiesis and lymphocyte differentiation. J Allergy Clin Immunol. 2015;136:1619–1626.

[13] Alsultan A, Shamseldin HE, Osman ME, et al. MYSM1 is mutated in a family with transient transfusion-dependent anemia, mild thrombocytopenia, and low NK- and B-cell counts. Blood. 2013;122:3844–3845.

[14] Bluteau O, Sebert M, Leblanc T, et al. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. Blood. 2018;131:717–732.

[15] Ulirsch JC, Verboon JM, Kazerounian S, et al. The genetic landscape of diamond-blackfan anemia. Am J Hum Genet. 2019;104:356.

[16] Al-Herz W, Chou J, Delmonte OM, et al. Comprehensive genetic results for primary immunodeficiency disorders in a highly consanguineous population. Front Immunol. 2018;9:3146.

[17] Li N, Xu Y, Yu T, et al. Further delineation of bone marrow failure syndrome caused by novel compound heterozygous variants of MYSM1. Gene. 2020;757:144938.

[18] Aasland R, Stewart AF, Gibson T. The SANT domain: a putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIIB. Trends Biochem Sci. 1996;21:87–88.

[19] Grüne T, Brzeski J, Eberharter A, et al. Crystal structure and functional analysis of a nucleosome recognition module of the remodeling factor ISWI. Mol Cell. 2003;12:449–460.

[20] Boyer LA, Latek RR, Peterson CL. The SANT domain: a unique histone-tail-binding module? Nat Rev Mol Cell Biol. 2004;5:158–163.

[21] Da G, Lenkart J, Zhao K, et al. Marmorstein R: structure and function of the SWIRM domain, a conserved protein module found in chromatin regulatory complexes. Proc Natl Acad Sci U S A. 2006;103:2057–2062.

[22] Hofmann K, Bucher P. The PCI domain: a common theme in three multiprotein complexes. Trends Biochem Sci. 1998;23:204–205.

[23] Ponting CP, Aravind L, Schultz J, et al. Eukaryotic signalling domain homologues in archaea and bacteria. Ancient ancestry and horizontal gene transfer. J Mol Biol. 1999;289:729–745.

[24] Kouranti I, McLean JR, Feoktistova A, et al. A global census of fission yeast deubiquitinating enzyme localization and interaction networks reveals distinct compartmentalization profiles and overlapping functions in endocytosis and polarity. PLoS Biol. 2010;8:e1000471.

[25] Verma R, Aravind L, Oania R, et al. Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome. Science. 2002;298:611–615.

[26] Jiang XX, Nguyen Q, Chou Y, et al. Control of B cell development by the histone H2A deubiquitinase MYSM1. Immunity. 2011;35:883–896.

[27] Förster M, Farrington K, Petrov JC, et al. MYSM1-dependent checkpoints in B cell lineage differentiation and B cell-mediated immune response. J Leukoc Biol. 2017;101:643–654.

[28] Förster M, Boora RK, Petrov JC, et al. A role for the histone H2A deubiquitinase MYSM1 in maintenance of CD8(+) T cells. Immunology. 2017;151:110–121.

[29] Wang T, Nandakumar V, Jiang XX, et al. The control of hematopoietic stem cell maintenance, self-renewal, and differentiation by Mysm1-mediated epigenetic regulation. Blood. 2013;122:2812–2822.

[30] Huo Y, Li BY, Lin ZF, et al. MYSM1 is essential for maintaining hematopoietic stem cell (HSC) quiescence and survival. Med Sci Monit. 2018;24:2541–2549.