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ORIGINAL ARTICLE

A De Novo Frameshift Mutation in *TNFAIP3* Impairs A20 Deubiquitination Function to Cause Neuropsychiatric Systemic Lupus Erythematosus

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Abstract

Purpose Genome-wide association study of systemic lupus erythematosus (SLE) revealed tumor necrosis factor alpha-induced protein 3 (*TNFAIP3*, A20) as a susceptibility gene. Here, we report a de novo mutation in *TNFAIP3* in a Chinese patient with neuropsychiatric SLE (NPSLE).

Methods Whole exome sequencing was performed for the patient and healthy members from the family. Suspected pathogenic variants were further analyzed and co-segregation was confirmed by Sanger sequencing. Real-time PCR and western blot were performed with peripheral blood mononuclear cells (PBMCs) and patient-derived T cells. Transfected HEK293T cells, human umbilical vein endothelial cells, normal human astrocytes, and microglia were used for in vitro studies.

Results A de novo frameshift mutation in *TNFAIP3* was found in the NPSLE patient. Western blot analysis showed activated NF-κB and mitogen-activated protein kinase pathways. Real-time PCR revealed elevated expression of pro-inflammatory cytokines. On immunoprecipitation assay, the mutant A20 altered the K63-linked ubiquitin level of TRAF6 via its ubiquitin-editing function.

Conclusions The mutant A20 may play a role in weakening the tight junction of the blood-brain barrier to cause neurologic symptoms. We report a rare variant of *TNFAIP3* in a patient with NPSLE and reveal its autoimmune disease–causing mechanism in both peripheral tissues and the central nervous system.

Keywords K63-linked ubiquitin · neuropsychiatric systemic lupus erythematosus · NF-κB · *TNFAIP3* · TRAF6 · blood-brain barrier

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune connective tissue disorder that mainly affects women at childbearing age [1]. The nomenclature neuropsychiatric SLE (NPSLE) refers to disorders in the central, peripheral, and autonomic nervous systems and syndromes in psychiatry [2]. Such symptoms include headache and mood disturbance in mild conditions as well as myelopathy, stroke, and acute confusion in severe conditions [3]. The prevalence of neuropsychiatric manifestations observed in SLE ranges from 12 to 95% because of various criteria used in diagnosis [4, 5]. Neuropsychiatric symptoms can occur along with the onset of SLE or be independent of serologic activity or other systemic symptoms [6].

SLE has been found strongly related to genetic components, and a large scale of genome-wide association studies have revealed many susceptibility alleles, but the etiologies of NPSLE are still undetermined. However, risk of developing neurologic disorders was found associated with an accumulation of susceptibility genes in SLE patients [7]. In addition, single nucleotide polymorphisms (SNPs) in TRPC6 [8], CD244 [9], ITAGM, FCγR IIIa, and FCγR IIIb [10] were found associated with NPSLE. These evidences strongly

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suggest that genetic factors play a significant role in the occurrence of NPSLE.

Genome-wide association study of SLE in Egyptian [11], Chinese Han [12, 13], Japanese [14], and Caucasian populations [15] revealed several SNPs of tumor necrosis factor alpha-induced protein 3 (*TNFAIP3*, A20) associated with disease onset [11, 12, 16]. A20 contains an ovarian tumor (OTU) deubiquitinating (DUB) domain in the N-terminus and seven carboxyterminal zinc finger (ZnF) domains thereafter. The OTU domain mediates NF-κB activation by hydrolyzing K63-linked ubiquitin (Ub) chains on RIP1, TRAF6, and IKKγ, while zinc finger domains are required for assembly of poly-Ub chains, and adding K48-linked Ub chains to substrates [17, 18]. Notably, ZnF4 selectively recognizes K63-linked Ub chains. Here, we report a Chinese NPSLE patient with a *TNFAIP3* mutation and investigated the underlying mechanism.

Methods

Subjects

The patient was evaluated in Qilu Hospital, Shandong University. All participated family members were enrolled after obtaining the approval of the ethics committee of the Qilu Hospital of Shandong University and the written consent of the family.

Genetic Analysis

DNA was extracted from peripheral blood samples collected from the proband and her parents. Whole exome sequencing was performed by BGI Shenzhen (Beijing Genome Institute, Shenzhen, China). The detailed procedure is described elsewhere. Briefly, exome capture involved use of the SureSelect Human All Exon Kit (Agilent, Santa Clara, CA, USA). Paired-end sequencing involved using the Hiseq2000 platform (Illumina, San Diego, CA, USA). Sequences of 10.5 Gb were generated, and at least 98.7% and 97.4% were obtained for × 4 and × 10 coverage for the examined sample. All variants were filtered by using dbSNP137, the 1000 Genomes Project, and HapMap8 databases. Compound heterozygote variants or a de novo variant was screened for Sanger sequencing.

Cell Cultures

Human embryonic kidney 293 cell line (HEK293T), normal human astrocytes (NHAs), human microglia (HMO6), and human umbilical vein endothelial cells (HUVECs) were cultured at 37 °C in DMEM (Life Technologies Laboratories, Grand Island, NY) complete media containing 10% fetal bovine serum (FBS). Peripheral blood mononuclear cells (PBMCs) from controls and patients were isolated using human lymphocyte separation medium (DAKEWE, Shenzhen, China) and cultured with RPMI 1640 (Hyclone, Logan, UT) containing 10% FBS for 1 day. Suspension cells were plated and stimulated with human anti-CD3 and anti-CD28 beads (Biolegend, San Diego, CA, USA) for 48 h in 6-well plate. Adherent cells were cultured in normal RPMI 1640. Cells were treated with TNF-α (50 ng/ml) for a time course.

RNA Extraction and Real-Time PCR

Total RNA was extracted by the Trizol method following the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). Reverse transcriptase (Thermo) was used to synthesize cDNA strands according to the manufacturer's protocol. Real-time PCR assays were performed as previously described [19]. The expression of U6 was an internal control; meanwhile, four duplicate wells were used for each subject. Primers were designed according to sequences on Ensembl GRCm38.p6. All primers are listed in Table 1.

Plasmid Construction and Cell Transfection

pCMV3-HA-*TNFAIP3* plasmid was purchased from Sino Biological Inc. (Beijing, HG12089-NY). Mutated *TNFAIP3* plasmid was constructed by PCR mutagenesis.

For immunoprecipitation assays, HEK293T cells were seeded 24 h before transfection. HA-K63-Ub and wild-type or mutant *TNFAIP3* plasmid were co-transfected by free medium with polyethylenimine, linear (Polysciences, Inc., Warrington, Philadelphia, USA), which was replaced by fresh medium after 6 h. HUVECs and NHAs were transfected by serum-free medium with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol and were replaced by fresh medium after 6 h.

Western Blot Analysis

Cells were lysed by radioimmunoprecipitation assay lysis buffer (Bioteke, Beijing, China) containing 1% protease inhibitor and 1% phosphatase inhibitors. Protein concentrations were determined by the bicinchoninic acid method (Thermo Scientific, Rockford, IL, USA). Details of immunoblotting assay were previously described [19]. Antibodies of TNFAIP3 N-terminus, Ub, and TRAF6 were from Santa Cruz. Phospho-IKKα/β, IKKα, IκBα, Phospho-IκBα, Phosopho-p65, p65, ZO-1, CDH1, Claudin-1, and VCAM-1 were from Cell Signaling Technology, Danvers, MA. MMP9, Phospho-P38, P38, phospho-JNK, and JNK1/2/3 were from Affinity Biosciences (Cincinnati, OH, USA). GAPDH and β-actin were from Abcam (Cambridge, UK). Enhanced chemiluminescence was used for immunodetection (Thermo Scientific, Rockford, IL, USA).

Table 1 Real-time quantitative RT-PCR primer sequences

	Forward	Reverse
IL1β	5′-ATGATGGCTTATTACAGTGGCAA-3′	5′-GTCGGAGATTCGTAGCTGGA-3′
IL2	5′-TCCTGTCTTGCATTGCACTAAG-3′	5′-CATCCTGGTGAGTTTGGGATTC-3′
IL6	5′-CCTGAACCTTCCAAAGATGGC-3′	5′-TTCACCAGGCAAGTCTCCTCA-3′
IL8	5′-ACTGAGAGTGATTGAGAGTGGAC-3′	5′-AACCCTCTGCACCCAGTTTTC-3′
IL12A	5′-CCTTGCACTTCTGAAGAGATTGA-3′	5′-ACAGGGCCATCATAAAAGAGGT-3′
IL17	5′-TCCCACGAAATCCAGGATGC-3′	5′-GGATGTTCAGGTTGACCATCAC-3′
IFN-γ	5′-TCGGTAACTGACTTGAATGTCCA-3′	5′-TCGCTTCCCTGTTTTAGCTGC-3′
Tbet	5′-GGTTGCGGAGACATGCTGA-3′	5′-GTAGGCGTAGGCTCCAAGG-3′
TNF	5′-CCTCTCTCTAATCAGCCCTCTG-3′	5′-GAGGACCTGGGAGTAGATGAG-3′
NOS2	5′-CCTTTGATGAGGGGACTGGG-3′	5′-CCGGGGTAAGGACAGTCAAA-3′

Immunoprecipitation Assays

HEK293T cells were used for immunoprecipitation assays. Cells were washed with cold PBS and lysed by using cold IP lysis buffer at 4 °C by ultrasonography. Cellular extracts were incubated with appropriate primary antibodies with Dynabeads™ Protein G (10004D Invitrogen) on a rotator at 4 °C for 3 h. Beads were then washed 3 times with IP lysis containing cocktail, and the immune complexes underwent SDS/PAGE and then were immunoblotted with secondary antibodies. Immunodetection was performed as previously described.

Statistical Analysis

Data analysis involved using GraphPad Prism (GraphPad Software, San Diego, CA). Quantitative data are expressed as mean ± SD. Differences between two groups were compared by Student's unpaired two-tailed *t* test. *P* value < 0.05 was considered statistically significant.

Results

Case Description

The proband (Fig. 1a) was a 36-year-old woman born in Shandong province. She was admitted to the hospital for fever, cough, headache, slow response, short-term memory loss, bilateral ptosis, and myalgia of limbs for 1 week, without any complain of rash or oral ulcer. The patient developed moderate polyarthritis at the age of 32 without treatment. At the age of 34, she was diagnosed with SLE on the basis of polyarthritis, pancytopenia, one episode of tonic-clonic epileptic seizure, and positive results of anti-nuclear and anti-Sm antibody testing. Steroid pulse therapy and prednisone were sequentially administrated after the seizure. On admission to the hospital, her vital signs and physical examination of skin, oral mucosa, joints, thorax, and abdomen were roughly normal. Neurological examination exhibited reduced facial expression, slow reaction, short-term memory impairment, and bradyphrasia. Cranial nerve examination revealed bilateral ptosis and difficulty looking upward. Neither tenderness nor weakness of the muscles was remarkable, except for symmetrically weakened deep tendon reflexes of bilateral extremities and positive bilateral Chaddock sign. Laboratory findings manifested leukopenia (2710 cells/μl; normal range 3500–9500 cells/μl), elevated erythrocyte sedimentation rate (26 mm/h; normal range 0–18 mm/h), and decreased C3 level (0.34 g/l; normal range 0.9–1.8 g/l) and C4 level (< 0.067 g/l; normal range 0.1–0.4 g/l). Anti-nuclear antibody (1:1280; normal range < 1:80), anti-double DNA antibody (1458.57 IU/ml; normal range < 100 IU/ml), and anti-Sm antibody were remarkably positive. Leucocyte count and protein level of cerebrospinal fluid examination were in normal range, while immunoglobulin G (71.4 mg/l; normally 0–34 mg/l) and immunoglobulin M (1.8 mg/l, normally 0–1.3 mg/l) were elevated. Oligoclonal bands of CSF and serum were negative. Chest CT revealed bilateral pneumonia and mild pericardial effusion. Brain MRI and susceptibility-weighted imaging were normal. Montreal Cognitive Assessment score was 20/30. Electroencephalography revealed generalized intermittent slow activity (mainly theta activity) and occasional sharp waves in the left centrotemporal region during a hyperventilation test. Electromyography showed myogenic damage and lack of H reflex on lower limbs. Thus, the diagnosis of NPSLE, simultaneously involving the central and peripheral nervous systems and muscles, was established. She was treated with systemic methylprednisolone 40 mg per day and hydroxychloroquine 200 mg twice a day. Antibiotics were administered to treat pneumonia. Two days later, her fever and cough disappeared. Ten days after these treatments, her headache and myalgia disappeared, while cognitive impairment and bilateral ptosis were improved. Montreal Cognitive Assessment (MOCA) score was improved to 23/30. Thus, oral steroid and antimalarials were continued and mycophenolate

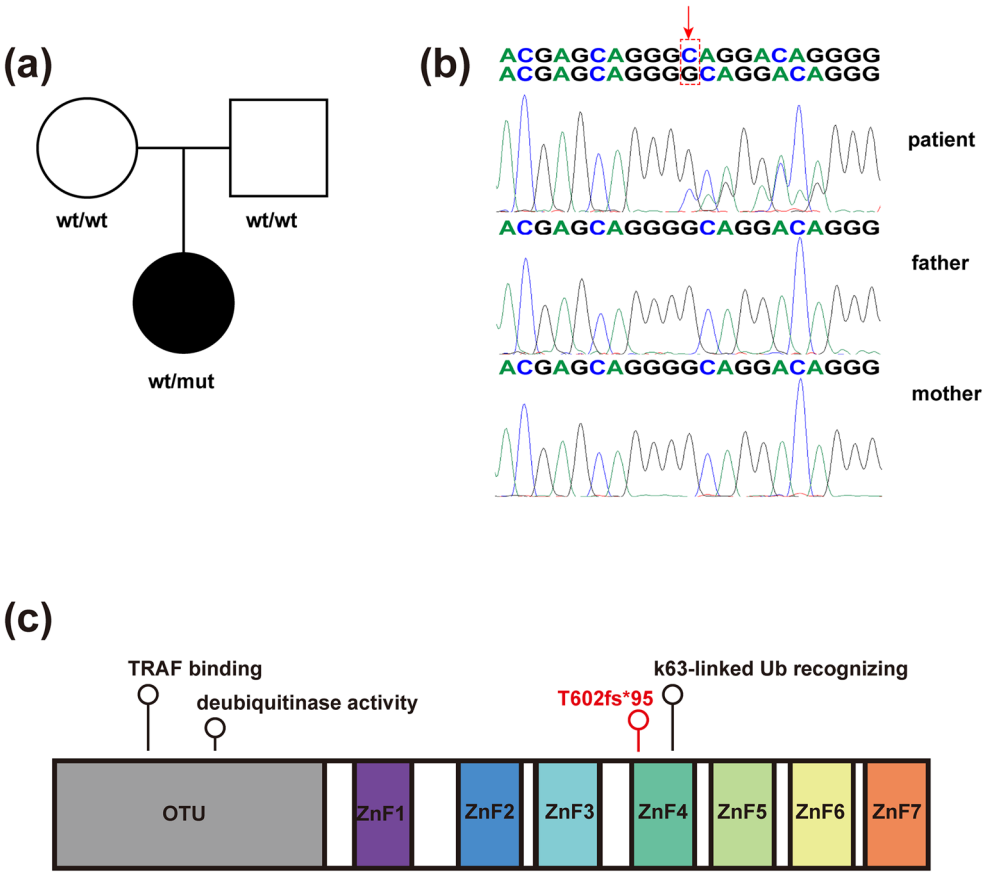

Fig. 1 Genetic analysis of the patient with neuropsychiatric systemic lupus erythematosus (NPSLE). **a** Tree of the NPSLE pedigree. **b** De novo heterozygous mutation in *TNFAIP3* confirmed by Sanger sequencing. **c** Protein structure and domain function of A20. The mutation and its location are marked in red. OTU, ovarian tumor domain; ZnF, zinc finger

mofetil 750 mg twice a day was administered. Nine months later, when tapering methylprednisolone to 8 mg per day, relapse of SLE occurred, manifesting as fever and one episode of grand mal seizure. After excluding reversible posterior leukoencephalopathy, infection, metabolic, and toxic disease, the diagnosis of NPSLE was established once again, and the treatment of intravenous methylprednisolone was raised to 80 mg per day, meanwhile replacing mycophenolate mofetil by cyclophosphamide 0.8 g once a month. Intrathecal injections of dexamethasone was given twice (10 mg per time). Her symptoms were alleviated gradually. The dosage of glucocorticoid was reduced gradually to prednisone 10 mg once a day, and immunosuppressant was switched back to mycophenolate mofetil 0.5 g twice a day after the cumulative dose of cyclophosphamide reached 4.8 g. She did not have another seizure after 15 months on follow-up. Blood and urine routine test, anti-dsDNA, and complement levels were almost normal with MOCA score remained at 23/30.

Genetic Analysis

Whole exome sequencing was performed for the patient and healthy family members (Fig. 1a). We identified a de novo mutation c.1806delG in *TNFAIP3* carried by the patient (Fig. 1b). The mutation located at the ZnF4 domain caused the early presence of a stop codon via a frameshift (p.T602fs*95), resulting in a truncated A20, 95 amino acids shorter than the wild type (Fig. 1c).

Impaired Cleavage of TRAF6 K63-Linked Ub Chain Is Caused by Interruption in TRAF6-A20 Interaction

A20 was previously found to interact with TRAF6 [20] and accordingly modifies TRAF6 K63-linked Ub moieties [18]. Therefore, we identified whether the binding and deubiquitinating functions were affected in cells with mutant A20. First, transfected HEK293T lysates were immunoprecipitated with A20 antibody and then blotted with TRAF6 antibodies. The result confirmed the wild-type and mutant A20 could bind to TRAF6 (Fig. 2a). Next, to determine A20 ubiquitination activity, HEK293T cells were co-transfected with K63-linked Ub and empty vector, wild-type, and mutant A20 constructs, respectively. The ubiquitin level with wild-type A20 transfection was considerably reduced, while mutant was similar to controls (Fig. 2b). Hence, although mutant A20 maintained its interaction with TRAF6, the K63-linked Ub chain hydrolyzing process was diminished because of an impaired recognition function in the mutated ZnF4 domain (Fig. 1c).

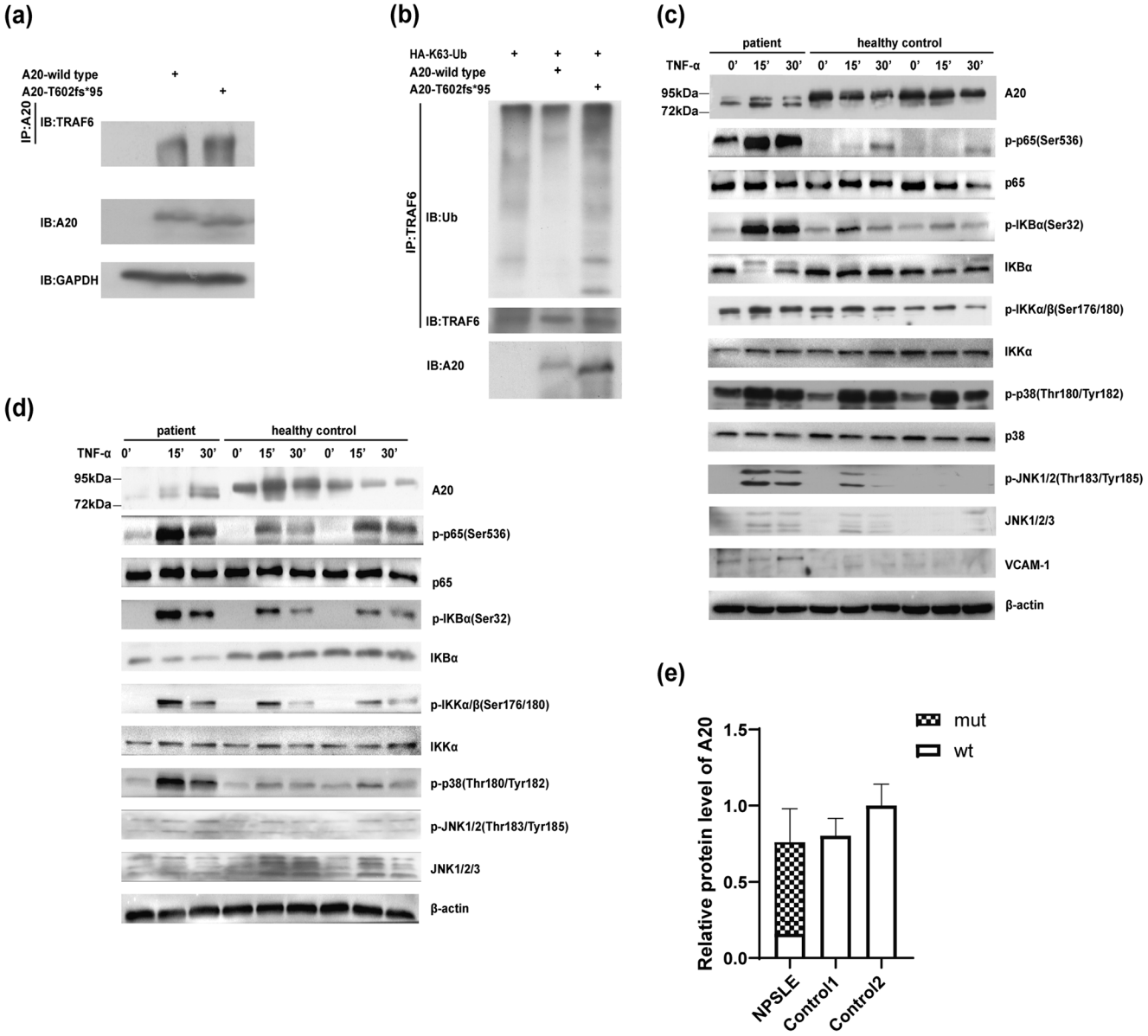

Fig. 2 Impaired ubiquitin editing function of mutant A20 activated NF-κB and MAPK pathway. **a** Both wild- and mutant-type A20 interact with TRAF6. HEK293T cells were transfected with wild- and mutant-type A20, respectively. Whole cell lysis was immunoprecipitated with A20 and detected with antibodies against TRAF6. Cell lysates were blotted with antibodies against A20 and GAPDH as internal controls. **b** Mutant A20 lost K63-linked Ub deubiquitination activity for TRAF6. HEK293T cells were co-transfected with HA-K63 Ub and wild- or mutant-type A20. Whole cell lysate was immunoprecipitated with TRAF6. High-molecular weight (MW) Ub aggregates (top panel) are indicated by immunoblotting with the antibody against Ub. **c** NF-κB and MAPK pathways were activated in stimulated patient-derived T cells. Patient-derived T cells were stimulated with TNF-α (50 ng/ml). Whole cell lysates were immunoblotted with respective target proteins. Healthy parents from the pedigree served as healthy controls

Activation of NF-κB Pathway in NPSLE Patient-Derived T Cells

We next examined whether the NF-κB pathway was activated in the NPSLE patient with mutated A20. Under normal conditions, the activity of NF-κB is inhibited by binding to IKK-mediated IKB. Phosphorylation of IKBα by activated IKK leads to the release of NF-κB and further phosphorylation as well as nuclear translocation. Patient-derived T cells were treated with TNF-α for 15 min and then 30 min separately to determine the induction of NF-κB in vivo. Upon TNF-α stimulation, the phosphorylation level of IKK and NF-κB was elevated, and IKB was downregulated in patient-derived T cells as compared with controls. VCAM-1, a downstream target of NF-κB, was also upregulated in the patient (Fig. 2c).

Activation Mitogen-Activated Protein Kinase Pathways (MAPKs) Pathway in NPSLE Patient-Derived T Cells

p38 and JNK, members of the MAPKs family, play important roles in mediating the expression of cytokines and regulating inflammation. Indeed, we found an increased phosphorylation state of p38 and JNK in patient-derived T cells after stimulation with TNF-α, thereby representing an activated state of MAPKs signaling pathway (Fig. 2c).

Elevated Cytokines Transcription in Patients with *TNFAIP3* Mutation

Because both NF-κB and MAPK activation could induce the transcription of several cytokines in vivo, we further monitored the changes in cytokine levels in the patient. As expected, the mRNA level of IFN-γ, TNF-α, T-bet, IL-1β, IL-2, IL-6, IL-8, and IL-17 was significantly increased in resting PBMCs from the NPSLE patient as compared with controls (Fig. 3a) and was higher in our NPSLE patient than other SLE patients. Therefore, the *TNFAIP3* mutation enhanced the over-reactive immune state as compared with other SLE patients.

To determine the ability of immune cells reacting to inflammation in vivo, we assessed the dynamic transcriptional changes of cytokines in the patient. By adding TNF-α to patient-derived T cells, the mRNA levels of cytokines showed a sudden increase, with a peak at 15 min (Fig. 3b). The changes in controls were stable or even more minimal, and the peak levels were lower. Therefore, the immune-related cytokines in the patient carrying the *TNFAIP3* mutation were transcriptionally activated and likely to be triggered by inflammatory circumstances.

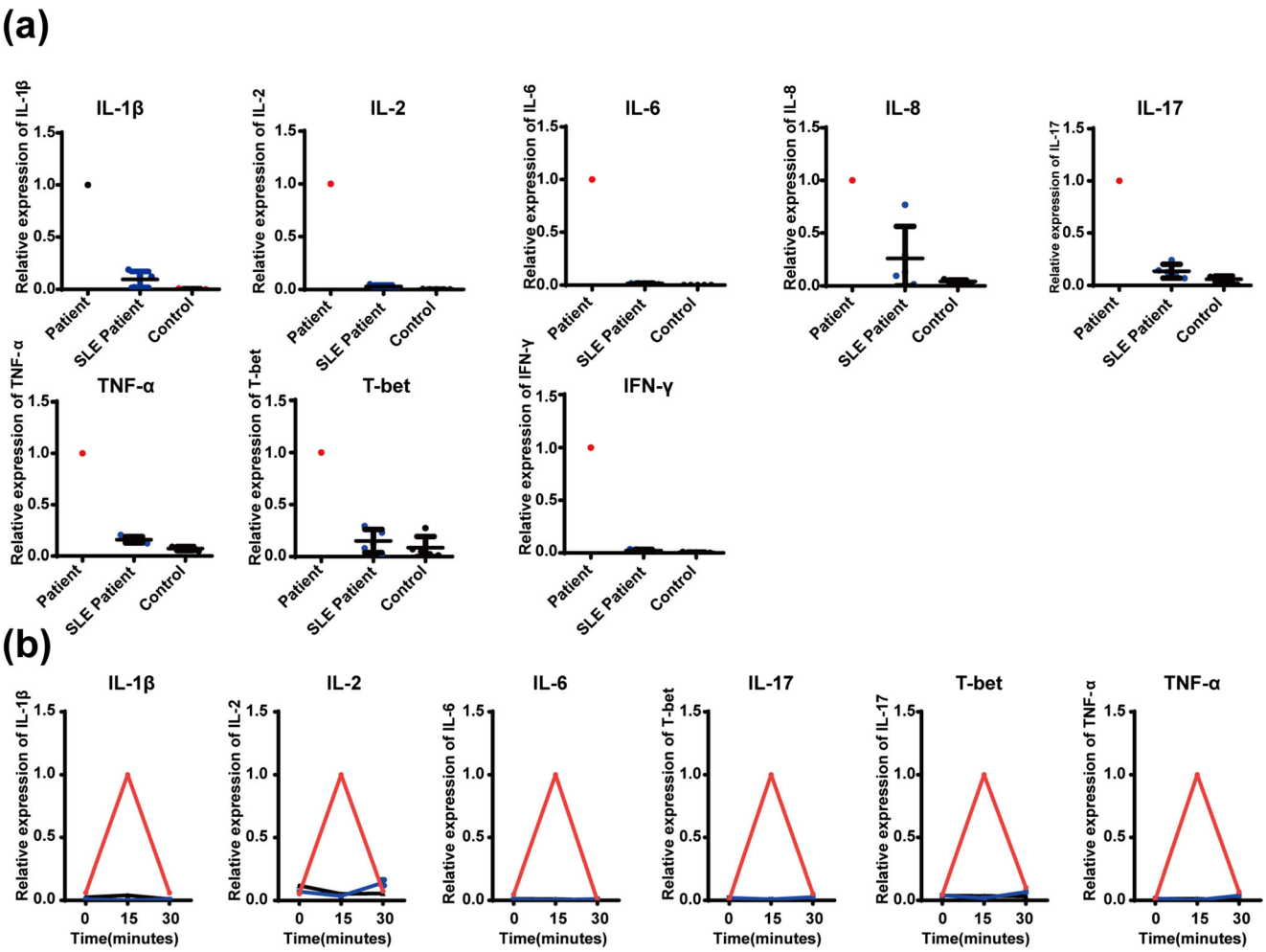

Fig. 3 Elevated levels of cytokines in the patient. **a** Real-time PCR analysis of mRNA levels of cytokines in PBMCs from A20-deficient patient and SLE patients ($n = 5$) and healthy controls ($n = 5$). Data are mean ± SD. $^*P < 0.05$. **b** Real-time PCR analysis of peak mRNA levels of cytokines in T cells from A20-deficient patient at 15 min after TNF-α (50 ng/ml) stimulation. Healthy parents from the pedigree served as controls. Data are mean ± SD

Mutant A20 Disrupts Blood-Brain Barrier and Activate Inflammation in Microglia

Besides the peripheral pro-inflammatory effect of the mutant A20 in SLE, we aimed to find a possible explanation for the central nervous system (CNS) susceptibility caused by mutant A20 in patients. NHAs and HUVECs were transfected with empty vector or wild-type or mutant A20. Protein markers of the tight junction were detected by western blot analysis. Both NHAs and HUVECs with mutant A20 transfection showed a pronounced decrease in levels of a series of tight junction–related proteins under TNF-α induction (Fig. 4a). These findings indicated a dysfunction of the mutant A20 in barrier maintenance, causing leakage of inflammatory cytokines into the CNS.

Microglia, served as tissue phagocytes in CNS, produce pro-inflammatory chemokines (IL-1, IL-6, IL-12A, IL-17, and TNF-α) and NO which contribute to local and widespread inflammation. Microglia transfected with mutant A20 exhibited high transcription level of several chemokines and iNOS (Fig. 4b). Based on these findings, we demonstrated that mutant A20 in patients could cause dysfunction of barrier maintenance which led to leakage of inflammatory cytokines from peripheral into CNS, while microglia exacerbated the neuroinflammation by secreting cytokines to further interfere CNS homeostasis.

Discussion

SLE is an autoimmune inflammatory disease that attacks multiple systems in the human body by excessive secretion of autoantibodies and cytokines [21]. As we previously mentioned, *TNFAIP3* is considered a susceptible gene for SLE; and recently, monogenic mutation in SLE patients has

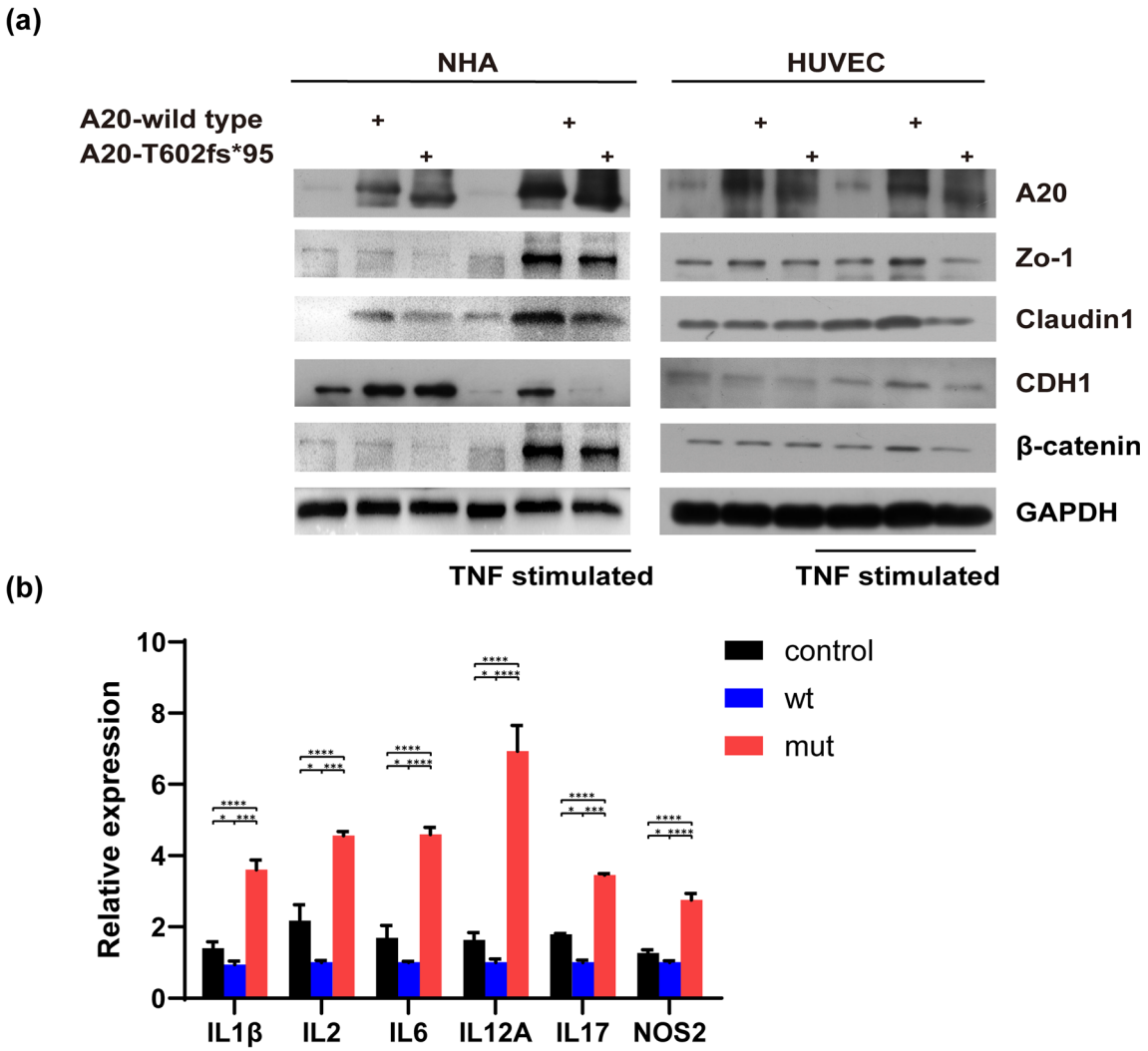

Fig. 4 Damaged blood-brain barrier and neuroinflammation caused by mutant A20. **a** Mutant A20 failed to regulate TNF-induced tight junction–related protein levels in NHA and HUVEC. Cells were transfected with wild- or mutant-type A20 and treated with TNF-α (50 ng/ml) for 2 h. Whole cell lysates were immunoblotted with target proteins. **b** Expression level of pro-inflammatory cytokines in microglia

been reported [22]. Zhou et al. reported several mutations in *TNFAIP3* that are haploinsufficient, causing an autoinflammatory syndrome resembling Behçet's disease [23]. Takagi et al. also reported a Japanese case with *TNFAIP3* mutation causing autoimmune lymphoproliferative syndrome [24]. Both studies demonstrated the activation of the NF-κB signaling pathway via the dual synergistic ubiquitin-related function of TNFAIP3 (A20), consequently altering immunologic characters and resulting in an inflammatory state in patients. Furthermore, several mutations in *TNFAIP3* have been reported pathogenic in juvenile onset of autoinflammatory disease, Behçet's disease, Still's disease, and Chronic Urticaria and Angioedema [25–27]. In this study, we identified a de novo frameshift mutation, c.1806delG (p.Thr.602fs*95), in *TNFAIP3* in a patient with NPSLE.

A20 consists of a DUB domain and seven ZnF domains; it is a bifunctional Ub-editing protein and therefore mediates the proteasome degradation of ubiquitinated or deubiquitinated substrates. ZnF4 selectively recognizes K63-linked Ub chains, and thus, mutations in ZnF4 binding sites could impair the A20 Ub-editing function [28]. TRAF6 functions as a signal transducer of TLR signaling to NF-κB via E3 ligase activity which is modified by A20 Ub-editing processes. Recruitment of TRAF6 to IL-1 receptor–associated kinase (IRAK) upon IL-1 stimulation led to autopolyubiquitination of TRAF6 and the formation of a critical complex that interacts with TGF-β-activated kinase (TAK) 1 and TAK1 binding protein (TAB)1/2, thus being intermediate between IL-1 receptor 1(IL-1R1) and NF-κB, JNK and p38 activation [29, 30]. The activation of the above molecules could trigger the transcription of cytokines while the patient with mutant A20 showing failure to attenuate the activated signaling pathways and thus leading to an overwhelming inflammatory status in vivo.

The symptoms of our patient during the disease onset and relapses all converged on CNS abnormalities. Whether the neuroinflammation pathogenesis is based on infiltrating cells from cerebral vessels, or originated from CNS, some evidence led us to consider the possibility of A20 disrupting the blood-brain barrier in the CNS. Previous studies have demonstrated the role of A20 in maintaining the intestinal epithelial barrier [31], and that TNFAIP3$^{-/-}$ mice have a greater intestinal permeability, A20 overexpression in intestinal epithelial cell protected tight junction (Tj) integrity [32]. In addition, this particular role of A20 is also associated with its deubiquitinating function; that is, it can interact with occludin and then deubiquitinate K63-linked Ub chains on occludin. In our study, NHAs and HUVECs were used to mimic the blood-brain barrier. TNF-α treatment notably enhanced the tight

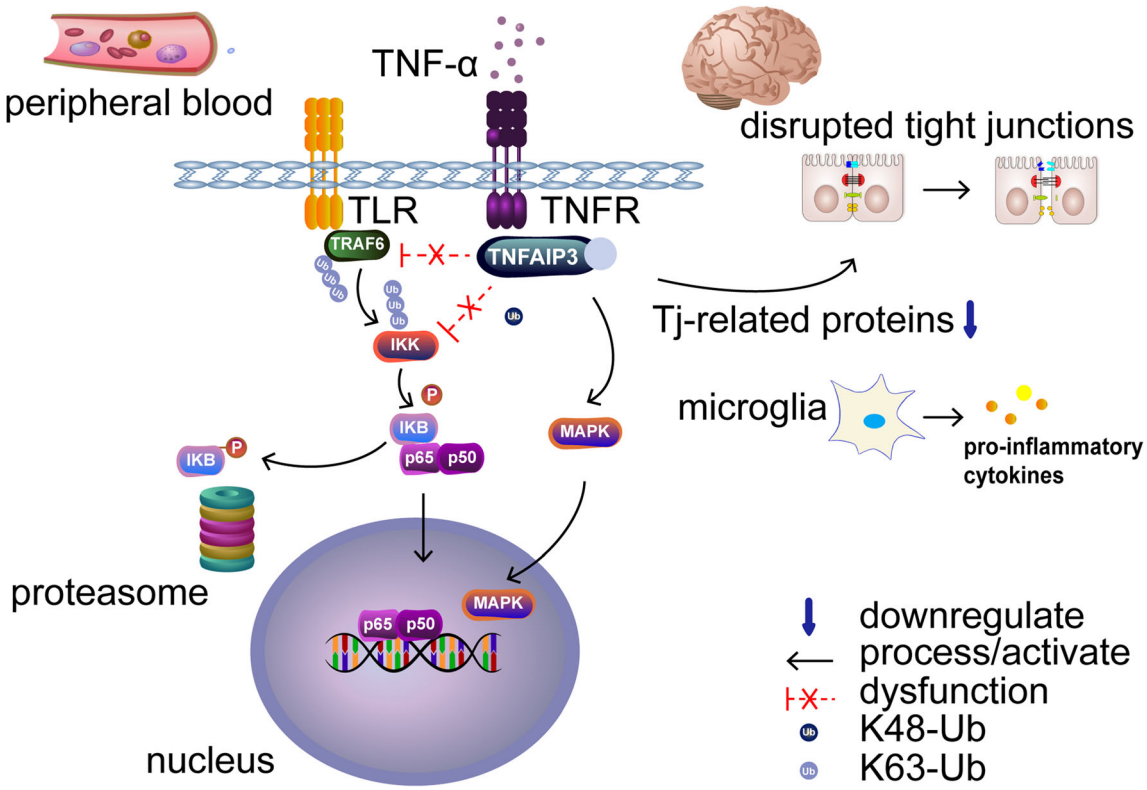

Fig. 5 Overview of impaired function of A20 mutants. Deubiquitinate dysfunction in patient-derived lymphocytes may activate NF-κB and MAPK pathways and subsequently regulate the transcription of varied cytokines. HUVEC and NHA cells transfected with mutant A20 displayed decreases in CNS barrier function. Mutant A20 triggered cytokine expression in HMO6 cells

junctions in cells with wild-type A20 but decreased levels of Tj-related molecules in cells expressing mutant A20. Therefore, A20 mutants failed to reverse the reduced barrier function triggered by TNF-α and could lead to inflammatory mediators disseminating from peripheral tissue to the CNS. However, whether the degradations of Tj-related molecules are caused by deubiquitinating impairment of A20 mutants still needs to be discovered. On the other hand, Sofie Voet et al. revealed that through affecting microglia, deficiency of A20 exacerbates neuroinflammation in EAE, a multiple sclerosis model in mice [33]. In our study, aside from peripheral immune cells, mutant A20 also induced expression of pro-inflammatory cytokines in microglia.

In conclusion, through its Ub-editing function, A20 mediates inflammatory responses in autoimmune diseases and possibly disrupts blood-brain barrier to cause neuropsychiatric symptoms (Fig. 5).

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Author Contribution RND and QL performed the main experiments, analyzed the data, and drafted the manuscript. QL collected clinical data from the patient. YL, SJW, SG, FL, PYL, and FG helped with experiments. XLB, XL, WJS, and QQY helped modifying experiments and approaches. JXL and QJL analyzed the data and reviewed the manuscript. All authors read and approved the final manuscript.

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Compliance with Ethical Standards All participated family members were enrolled after obtaining the approval of the ethics committee of the Qilu Hospital of Shandong University and the written consent of the family.

Conflict of Interest The authors declare that they have no conflict of interest.

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