Effectiveness of sirolimus in a patient with a novel heterozygous FAS mutation leading to severe autoimmune lymphoproliferative syndrome

Hao Gu
Beijing Children's Hospital https://orcid.org/0000-0002-4278-8559
Zhenping Chen
Beijing Children's Hospital
Jie Ma
Beijing Children's Hospital
Jingyao Ma
Beijing Children's Hospital
Lingling Fu
Beijing Children's Hospital
Rui Zhang
Beijing Children's Hospital
Tianyou Wang (wangtianyou@bch.com.cn)
Beijing Children's Hospital
Runhui Wu (runhuiwu@hotmail.com)
Beijing Children's Hospital, Capital Medical University https://orcid.org/0000-0003-4030-209X

Research

Keywords: FAS、ALPS、DNT、Treg、Sirolimus

Posted Date: February 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-173979/v1

Abstract

Here we report a patient with a novel mutation in the FAS gene associated with a severe phenotype of the autoimmune lymphoproliferative syndrome. FAS gene identified as a novel spontaneous somatic heterozygous missense mutation (c.857G > A, p.G286E) in exon 9, causing an amino acid exchange was identified using sequencing. Consequently, the treatment with sirolimus (1mg/d, blood concentration 5-15ng/L) was initiated. Subsequently, the patient's platelet count and clinical condition improved rapidly. Moreover, our *in vitro* data showed that G286E could inhibit cell proliferation and induce apoptosis compared with the wild type. To sum up, these data indicated that defective apoptosis might contribute to the clinical phenotype of lymphoproliferation in FAS deficient patients. The polarization of DNT/Treg axis may be an operative target of the sirolimus application.

1. Introduction

Apoptosis induced by the Fas-Fas ligand (FasL) pathway is vital in the regulation of the immune system. The interaction of Fas and FasL (homotrimeric transmembrane counter-receptors) results in the association of the adaptor protein FADD (Fas-associated death domain) and the recruitment of procaspases 8 and 10, which leads to the formation of a multimolecular signaling complex called DISC (death-inducing signaling complex) (Fiona L Scott et al.,2009). Extracellular-region FAS mutations can induce low FAS expression due to nonsense-mediated RNA decay or protein instability, resulting in defective death-inducing signaling complex formation and impaired apoptosis (Hye Sun Kuehn et al.,2011).

The autoimmune lymphoproliferative syndrome (ALPS) is an inherited disorder characterized by defective function of the Fas death receptor, which results in chronic, nonmalignant lymphoproliferation and autoimmunity accompanied by elevated numbers of double-negative (DN) T cells (T-cell receptor α/β + CD4 − CD8−) and an increased risk of developing malignancies later in life(Joao B Oliveira et al.,2010). ALPS-FAS is most frequently caused by heterozygous somatic or germline mutations that generate mutant FAS proteins, often with defective death domains. Herein, we reported data from an ALPS patient with a novo FAS-exon 9 mutation, his parents, and healthy donors, including their clinical and laboratory findings.

2. Material And Methods

2.1 Patients

This study enrolled the patient, his parents and healthy donors. After obtaining written informed consent, all experiments were performed and were approved by the local Ethics committee of Beijing Children's Hospital, Capital Medical University.

2.2 DNA isolation and Sequencing

Genomic DNA was isolated using the DNA Blood kit (Qiagen, Shanghai, China). Eight hundred sixteen genes associated with blood disease were selected by a gene capture strategy, using the GenCap custom enrichment kit. After sequencing the target area, the readout was filtered and processed to remove the low-quality reads (<80bp) from the sequencing data. The data obtained were analyzed by standard process information, and the results of single nucleotide polymorphisms (SNPs) and insertion-deletion mutation (InDels) were obtained. The goal was to identify rare (MAF $\leq$0.05, based on 1000 genome, ESP6500, and ExAc database) and damaging variants predicted algorithms (SIFT, PolyPhen-2, MutationTaster, GERP++). Parental testing was performed through targeted analysis by Sanger sequencing.

2.3 Immunophenotyping

Immunophenotyping was carried out by employing the following antibodies: (PerCP)-labelled antiCD3 (clone SK7, BD Pharmingen, San Jose, CA, USA), fluorescein isothiocyanate (FITC)-labelled anti-CD4 (BD Pharmingen, San Jose, CA, USA), allophycocyanine (APC)-labelled antiCD8 (clone SK1, BD Pharmingen, San Jose, CA, USA), APC-labelled anti-CD25 (clone 2A3, BD Pharmingen, San Jose, CA, USA), and PE-labelled anti-Foxp3 (clone:PCH101, eBioscience, San Diego, CA, USA). Using the FACSDiva software, all samples were analyzed in FACS Canto II flow cytometer (BD Biosciences).

2.4 Design, Construction, and Packaging of Mutant and Wild-type FAS Gene Overexpression Lentiviral Vectors

The National Center of Biotechnology Information (NCBI) website database was used to query and obtain the sequence encoding the gene of interest. The gene was then cloned into a lentivirus expression backbone plasmid vector to complete the construction of the lentivirus overexpression plasmid. Lentivirus packaging was performed using constructed gene overexpression lentiviral plasmid, HEK 293FT cells, and lentivirus packaging kit.

2.5 Measurement of apoptosis

PI/annexin V-FITC apoptosis detection kit was used for cell apoptosis detection (Ebioscience, Thermo Fisher). 72 hours after seeding, cells were resuspended at a density of 1×10^6 cells/ml. Then, 2ul PI and 5μl annexin V-APC was added into 100 μl of the cell suspension. The mixture was incubated for 15 min at room temperatureand then were analyzed in FACS Canto II flow cytometer (BD Biosciences)

2.6 Measurement of Cell viability and cell cycle distribution

The CCK kit was used for cell viability analysis. In brief, 2000 HCC cells were seeded in 100 ul DMEM medium into the 96-well plates. After 1, 2, 3, and 4 days, 10 ul CCK solution was added into the well and incubated for 3 hours. Forty-eight hours later, the nuclei of HCC cells were stained with PI.

3. Results

3.1 The patients presented with a severe phenotype of ALPS

Herein, we present a case of patient with clinical and genetic characteristics of ALPS and TCRαβ + CD4/CD8 double-negative T cells (DNT) elevation. From Sep, 2016, he showed unexplained large-scale splenomegaly and lymphadenopathy, along with severe thrombocytopenia. The patient was unresponsive to steroids, immunoglobulins, platelet transfusions, and recombinant human platelet growth factors. The elevated proportion of DNTs/CD3+ (5.8%) made us consider the dignosis of ALPS. however, he was positive for anti-platelet antibodies, anti-Epstein-Barr viruses, rubella viruses, cytomegalovirus, herpes simplex virus immunoglobulin G, and antinuclear antibodies. In addition, the analysis of markers for measles virus, parvovirus B19, schistosomiasis, and Leishmania donovani infection was negative. T lymphocytes (CD3+CD19-) and T helper cells (CD3 + CD4 +) in peripheral blood samples were slightly increased. However, Treg/CD4+ ratio was significantly decreased (2.5%). Moreover, the patient displayed amplified proportions of cytokine IFN-γ, IL-10, and IL-6.

Histological analysis of the bone marrow and spleen did not show signs of hemophagocytosis with obviously splenomegaly. Because of the severe clinical manifestation, we tried rapamycin mTOR signal pathway inhibitor before the NGS genetic results, after which platelet count was restored, and spleen retracted quickly.

3.2 Immunophenotyping after sirolimus

Routine blood results indicated thrombocytopenia (0-5×10^9/L). Four days after the application of rapamycin, the platelet count returned to 150×10^9/L. The patient displayed persisting T-cell lymphocytosis with an increased proportion of DNT, while NK and Treg cells (CD3+CD4+CD25+Foxp3+) were reduced. After 4 weeks rapamycin treatment, DNTs/CD3+ (3.5%) was decreased; however, it was still not within normal levels, unlike Treg and the serum levels of TNF, IL-6, and IL-10, which returned to a normal level.

3.3 ALPS was associated with a novel type of FAS mutation

After receiving informed consent from the parents, we sequenced the ALPS-related genes (FAS, FASLG, CASP8, CASP10) and genes known to cause common variable immunodeficiency. We identified novel spontaneous somatic heterozygous missense mutations on the FAS gene (c.857G>A, p.G286E) in exon 9, causing an amino acid exchange. Sanger sequencing verification of peripheral blood and nail confirmed the somatic mutation. The identified variation, which has been previously observed, is the first disease-causing mutation detected in the extracellular domain of the Fas (neither in the 1000 Genomes Project, the HapMap Project, Exome Variant Server data sets nor in dbSNP database or ExAC-Asian database). No mutations were detected by Sanger sequencing of the FASLG、CASP8, CASP10 gene.

3.4 The abnormal ability of the mutant to inhibit cell proliferation

According to the results of CCK8, after 96 h, higher inhibition of Jurkat cell proliferation was observed in the overexpressing wild-type FAS (LW1866) and FAS-G286E (LW1579) groups compared with the NC group. However, the G286E had less ability to inhibit compared to wild-type.

3.5. Cell cycle results: mutant vs. wild-type

According to the flow cytometry results, compared with the NC group, the proportion of overexpressed wild-type FAS (LW1866) and FAS-G286E (LW1579) G2 cells increased, indicating that overexpressed wild-type FAS (LW1866) and FAS-G286E (LW1579) could block Jurkat cells in G2 phase. Although the trend of the G286E was more obvious, there was no significant difference between them.

3.6. Mutant and wild-type apoptosis results

Compared with the NC group, the over-expressing wild-type FAS (LW1866) and FAS-G286E (LW1579) groups could increase Jurkat cells' early number. The proportion of apoptotic cells in the G286E was lower than that in the wild type. Compared to the overall cells, the degree of speculation of these three groups was not obvious.

3.7.Clinical course

Medical history, clinical presentation, and laboratory tests results led to the diagnosis of the autoimmune lymphoproliferative syndrome (ALPS). Consequently, the treatment with sirolimus (1mg/d, blood concentration 5-15ng/L) was initiated. Subsequently, the patient’s platelet count and clinical condition improved rapidly.

4. Discussion

FAS mutations can appear in a heterozygous and homozygous state. The first one has differencing clinical penetrance, while homozygous mutations have been associated with early-onset and severe clinical manifestations (Bleesing JJH.,2006). In this study, we reported a single case of a patient with typical ALPS caused by a heterozygous mutation in the FAS gene's intracellular domain, a member of the TNF/TNFR superfamily that contains nine exons spanning 26kb on chromosome10q24.1 (Locksley et al., 2001). The first 5 exons encode the extracellular portion containing 3 cysteine-rich domains that control receptor trimerization and FASL binding, which could induce proliferation of murine CD4 + T cells. The frequency of missense mutations in exon 9 is rare (Roy U., 2016). To determine whether the mutations would impair protein function, we predicted the score of PolyPhen2 and SIFT (0.822 and 0.016, respectively). The frequency of EXAC-Asian (c.857G > A, p.G286E) was less than 0.00001.

Both G286E and wild-types inhibited T cell line proliferation and induced apoptosis. Still, the effect of G286E was lower than the wild-types. Moreover, more cells of the G286E and the wild type stayed in the G2 phase compared with the normal control cells. Although the trend of the G286E was more obvious, there was no statistical difference between the G286E and wild-types. The above shows that the G286E

has a certain degree of defects in inhibiting cell proliferation and inducing apoptosis compared with the wild type.

The ALPS suspicion was facilitated by the significant polarization of the DNT/Treg axis in this patient. It has been reported that the acquisition of a somatic FAS mutation, which is typically enriched in DNT cells, can precipitate the development of full-blown ALPS (Völkl S et al., 2001). The inhibition of Treg may be due to the upregulation of IL-10 caused by DNT accumulation Zhang (Zhang ZX et al., 2011).

In this case, we treated with sirolimus. This case highlights the importance of studying ALPS-FAS within the context of interconnected immune dysregulation disorders that have been previously described to share overlapping clinical and histopathological features, including Castleman disease, Rosai-Dorfman disease, X-linked lymphoproliferative disease, Dianzani autoimmune lymphoproliferative disease, Kikuchi-Fujimoto disease, caspase 8 deficiency syndrome, CTLA-4 haploinsufficiency with autoimmune infiltration and lipopolysaccharide-responsive vesicle trafficking, beach- and anchor- containing.

The molecular mechanism and physiological purpose of interaction between FAS mutation and sirolimus application remain to be determined in future analyses. It is possible that this pathway serves to shut down immune reactions to avoid excessive responses and/or limit autoimmune reactions, especially in ALPS.

5. Conclusions

We described a novel somatic FAS mutation associated with a severe clinical phenotype of ALPS. The mutation causes effects lymphocytes apoptosis and proliferation. Our study indicates that defective apoptosis may contribute to the clinical phenotype of lymphoproliferation in Fas deficient patients. The polarization of DNT/Treg axis may be a target of sirolimus application.

Abbreviations

ALPS: Autoimmune lymphoproliferative syndrome

FasL: Fas ligand

FADD: Fas-associated death domain

DISC: death-inducing signaling complex

mTOR: mammalian target of rapamycin

DNT：TCRαβ + CD4 / CD8 double-negative T cells

IFN：interferon

IL：interleukin

Treg：regulatory T cells

Th：T helper cell

Foxp3+：frokhead-box P3+

CVID: Common Variable Immune Deficient

IVIG：intravenous immunoglobulin

DALD：Dianzani Autoimmune/lymphoproliferative Disease

Declarations

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81970111), Beijing Natural Science Foundation of China (No. 7192064), the Pediatric Medical Coordinated Development Center of Beijing Municipal Administration of Hospitals (No.XTZD20180205), and the National Science and Technology Key Projects (No. 2017ZX09304029001).

Declaration of Interest Statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company.

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Figures

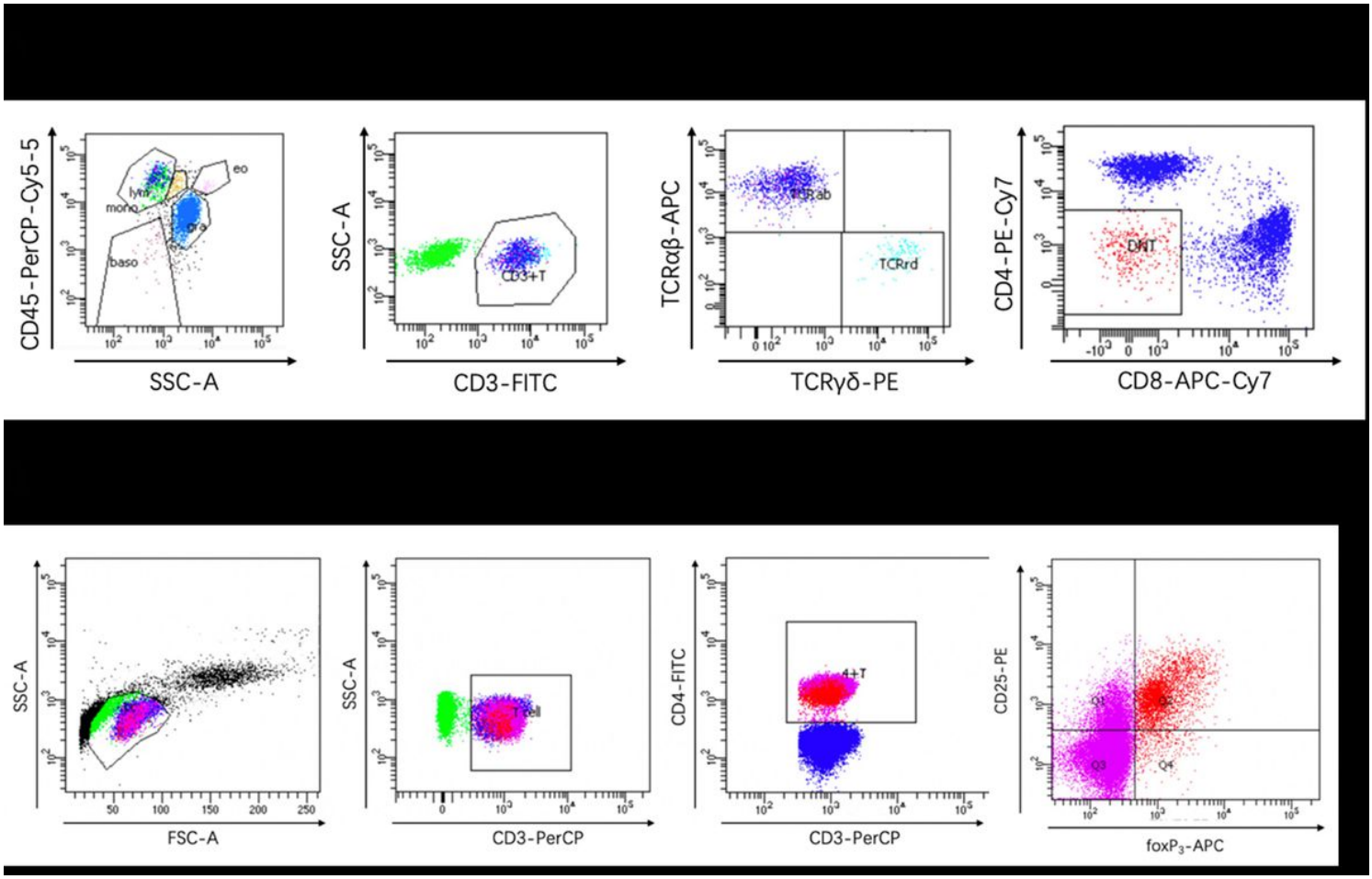

Figure 1

Ratio of DNT and Treg cells. A. A representative dot plot analysis of CD4-CD8-TCRαβ+ cells gated in the CD3+ cell fraction. B. A representative dot plot analysis of CD25+foxP3+ cells gated in the CD4+ cell fraction. Q2 section (upper-right quadrant) represents Treg cells.

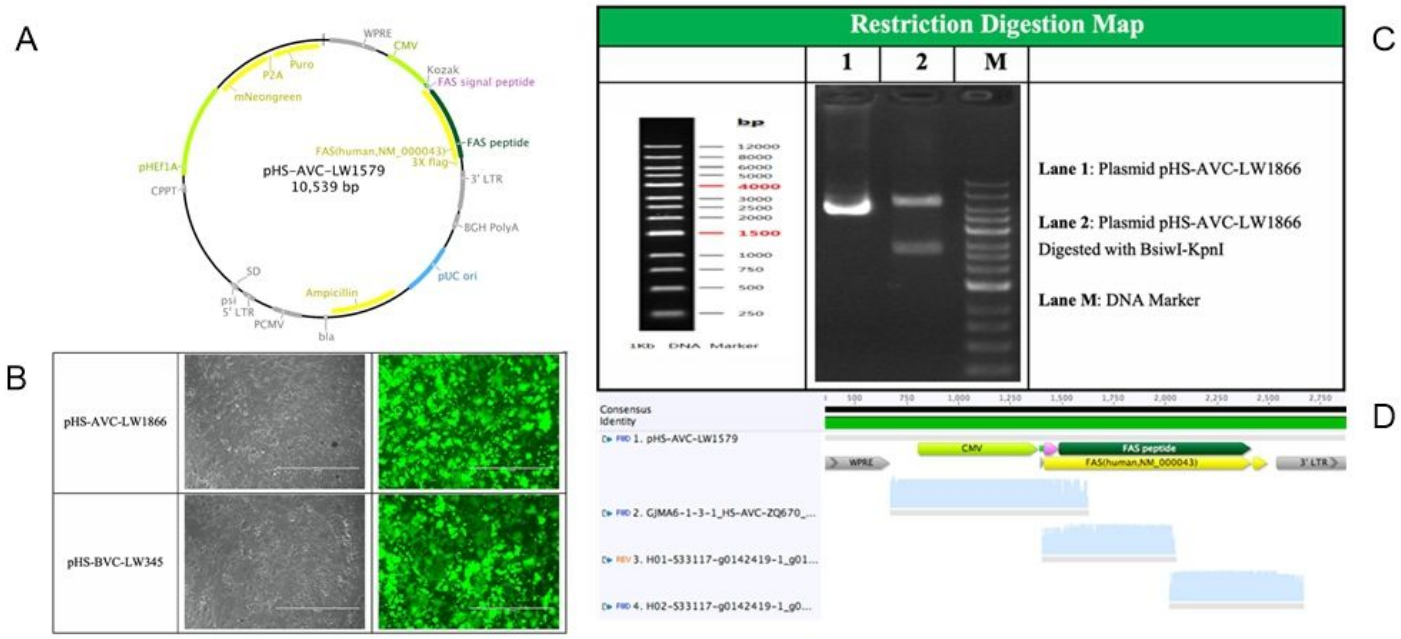

Figure 2

Mutant and Wild-type FAS Gene Overexpression Lentiviral Vectors. A.Overexpression lentiviral plasmid schematic diagram. B. Lentivirus titer: LW1866 lentivirus titer=2.17×108TU/mL；LW345 lentivirus titer=2.14×108TU/mL C. Plasmid digestion. D. Gene sequencing comparison.

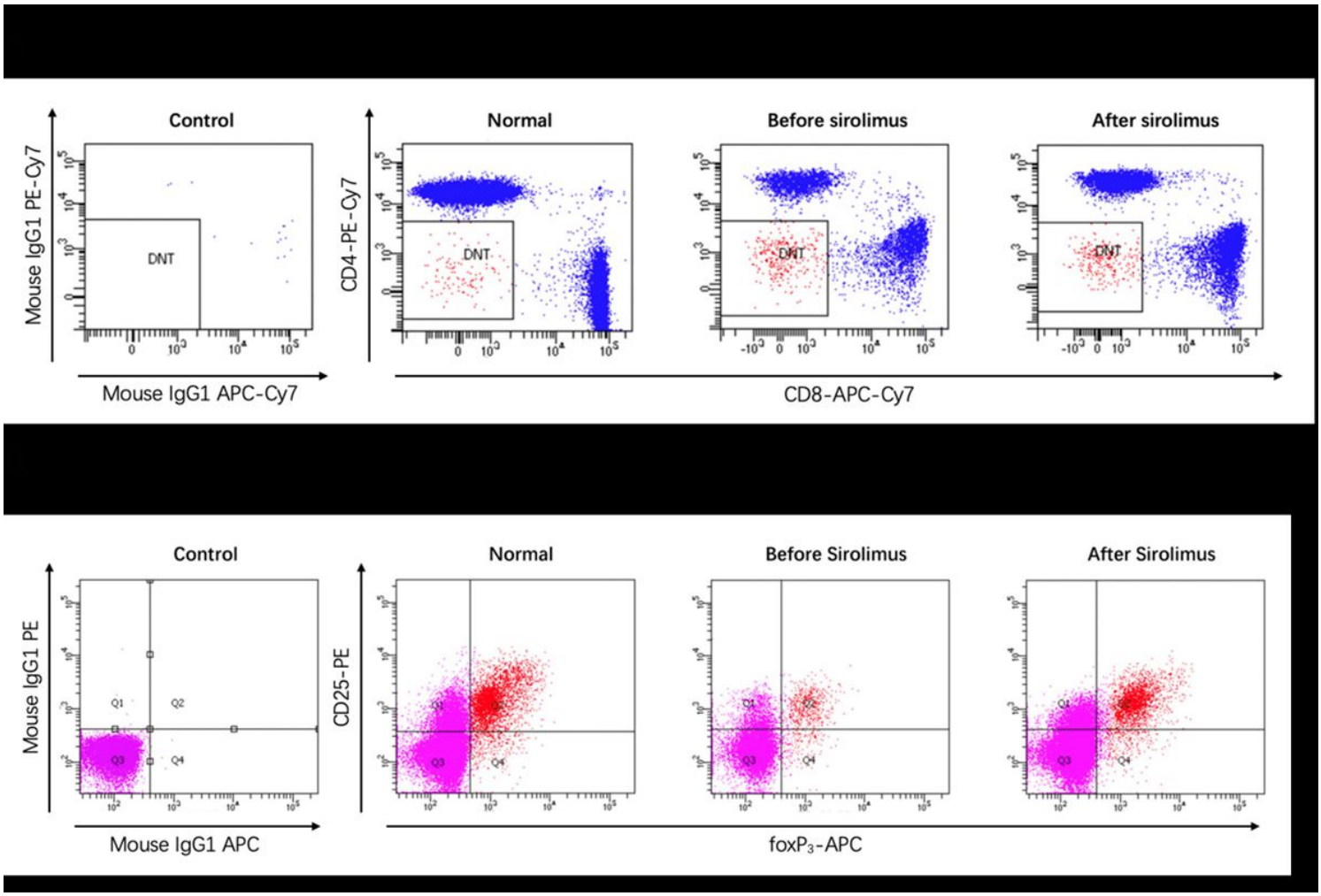

Figure 3

The ratio of DNT and Treg after sirolimus. A. DNT/CD3+ cell proportion of tested patients before and after 6 months of treatment. B. Treg/CD4+ T cell proportion of tested patients before and after 6 months of treatment.

A

Genetic	cDNA mutation	Protein level mutation	Zygosity	Protein function	Method	OMIM no.	PolyPhen2	SIFT	EXAC-EAST Asian
FAS	c.857G>A	p.G286E	Heterozygous	Likely LOF	TNGS	601859	0.822	0.016	-

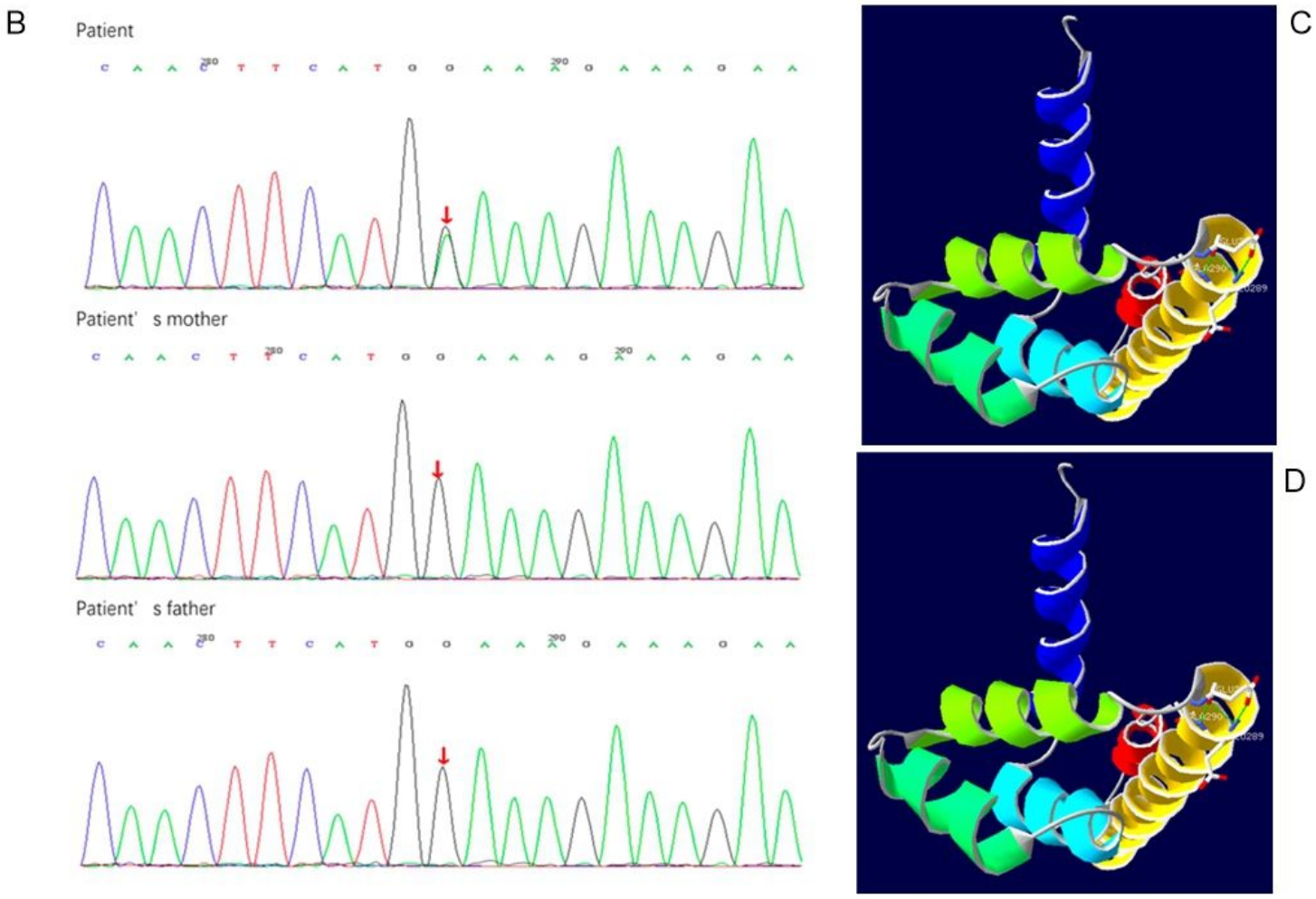

Figure 4

A.FAS gene mutation. B. Sanger sequencing of cDNA. C. A hydrogen bond is formed between amino acids 286G and 289E; A hydrogen bond is formed between amino acid 286G and 290A; D. Two hydrogen bonds are formed between amino acids 286E and 289E; A hydrogen bond is formed between amino acid 286E and 290A

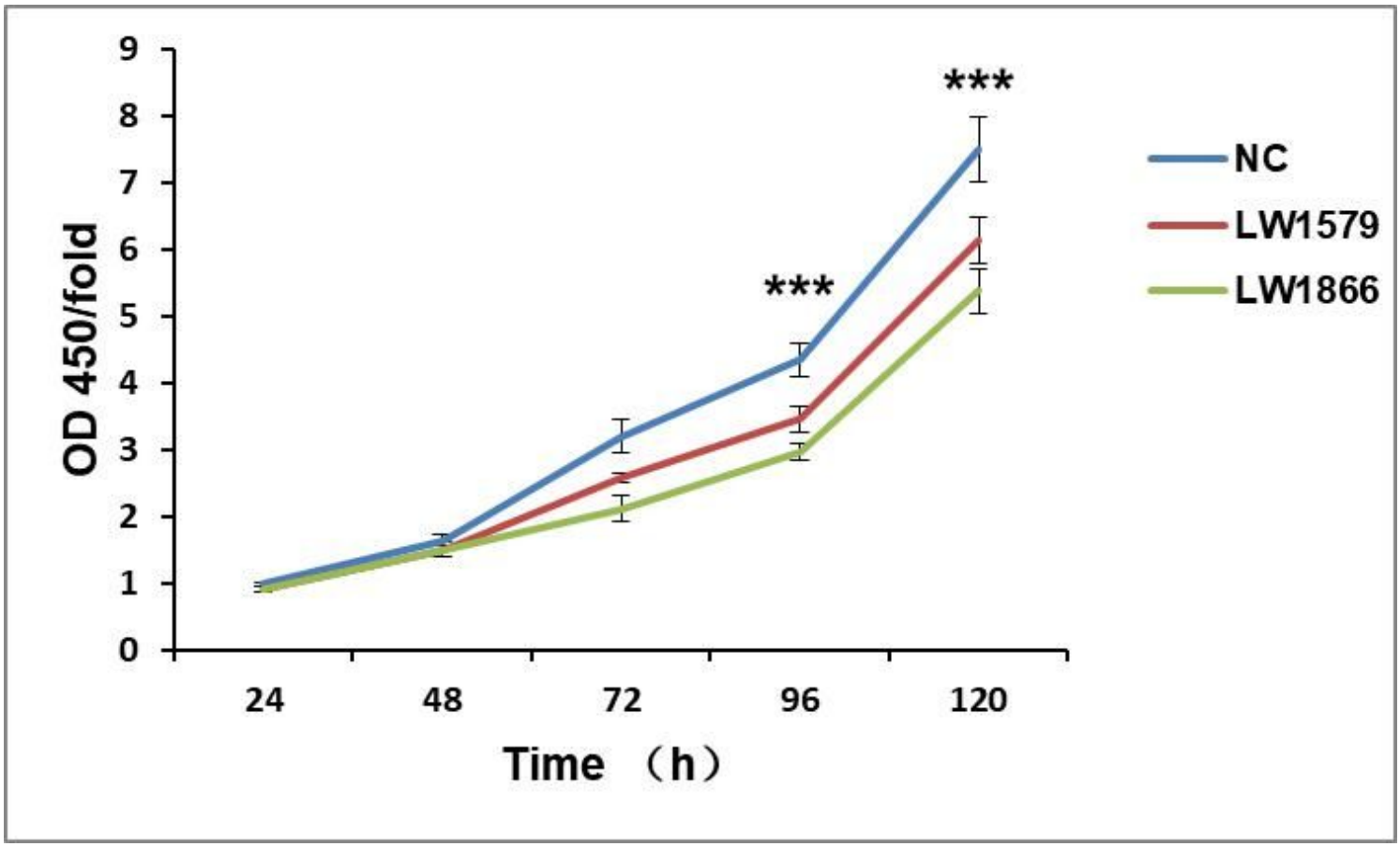

Figure 5

The results of cell proliferation. Higher inhibition of Jurkat cell proliferation was observed in the overexpressing wild-type FAS (LW1866) and FAS-G286E (LW1579) groups compared with the NC group

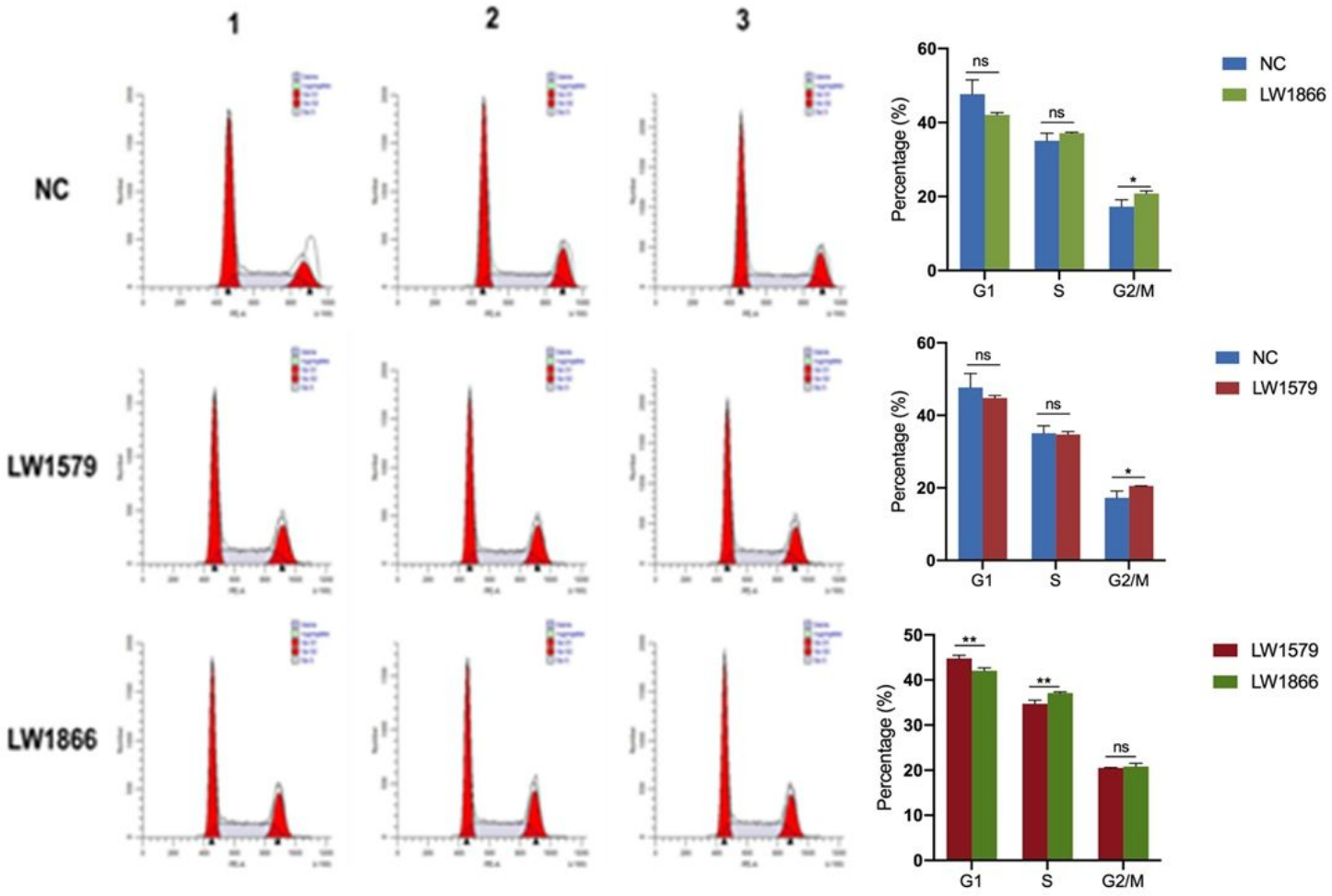

Figure 6

cell cycle results:compared with the NC group, the proportion of overexpressed wild-type FAS (LW1866) and FAS-G286E (LW1579) G2 cells increased

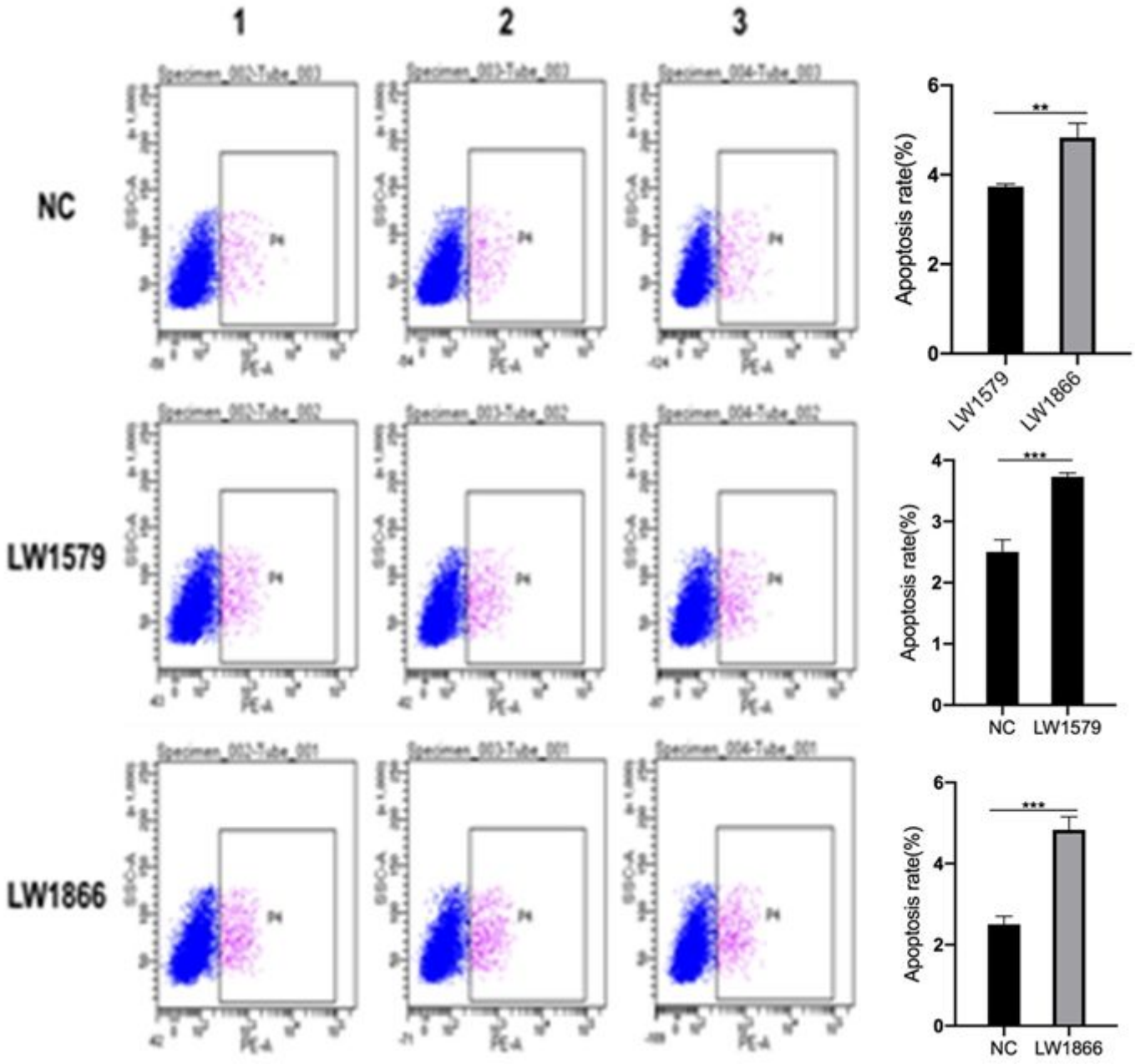

Figure 7

apoptosis results:The proportion of apoptotic cells in the G286E was lower than that in the wild type.