The expression characteristics of cytochrome C oxidase subunit I in mitochondrial of MRL/lpr lupus mice

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Abstract

Objective

We sought to analyse the expression characteristics of cytochrome C oxidase subunit I in mitochondrial of MRL/lpr lupus mice.

Methods

The whole blood of MRL/lpr lupus mice was detected for whole mitochondrial genome sequencing performed by Illumina HiSeq PE150 instrument, compared with house mouse (NC_005089.1) and screened for the maximum difference gene, MT-CO1. Quantitative real-time polymerase chain reaction (qRT-PCR) and western blot were used to detect the mRNA and protein expression of MT-CO1 in lupus mice and control mice. The total antioxidant capacities of lupus mice and control mice were measured using the rapid 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) method.

Results

The mitochondrial genome sequencing showed that five mitochondrial genes had base differences and MT-CO1 was the maximum difference gene, 31 in total. Among the 31 base difference sites, 2 were missense mutations and 29 were synonymous_variant. qRT-PCR test results showed that the MT-CO1 expression in lupus mouse blood was statistically lower than that in control mice blood ($t=4.333$; $p=0.0003$). Western blot test results revealed that the expression of MT-CO1 was lower in the lupus mice compared with the control mice at the protein level. Serum total antioxidant capacity testing showed that: the serum total antioxidant capacity of lupus mice was statistically lower than that of the control mice ($t=9.957$; $p<0.0001$).

Conclusion

High mutation rate and decreased expression of MT-CO1 in MRL/lpr lupus mice accompanied the decrease of antioxidant capacity, which indicated that abnormal MT-CO1 might be involved in the pathogenesis of SLE and the production of anti–dsDNA antibodies.

Key words

mitochondrial DNA, systemic lupus erythematosus, mitochondrial cytochrome C oxidase subunit 1

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Introduction

Systemic lupus erythematosus (SLE) is a common autoimmune disease and anti-double-stranded DNA (dsDNA) antibody is recognised as the most significant SLE-specific landmark antibody (1). Although some new biomarkers of SLE have been published in the past year, such as ollagen triple helix repeat containing-1 (CTHRC1) and aminoacyl-tRNA synthetase-interacting multifunctional protein-1 (AIMP1), which were correlated with systemic disease activity, anti-dsDNA antibody is always the most specific (2). Mitochondria, transformed from bacteria billions of years ago with heterogeneous bacterial dsDNA, are organelles that generate energy in cells and are the only organelles with genetic material besides the nucleus (3). During the energy production process, mitochondrial DNA (mtDNA), lacking histone protection, is prone to mutation under reactive oxygen species (ROS) conditions (4), which most possibly involves conversion to a potential antigen for anti-dsDNA antibodies. In this study, we screened mutation gene by sequencing the whole genome of mtDNA of MRL/lpr lupus mice, studied the expression of mitochondrial cytochrome C oxidase 1 (MT-CO1) with the high-rate mutant, and analysed the correlation to the total antioxidant capacity of serum in lupus mice.

Materials and methods

Animals

Female MRL/lpr mice (n=16, 12-weeks old) as experimental mice and female Balb/c mice (n=12, 12-weeks old) as control mice were used in this study. The MRL/lpr mice were obtained from the Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China), and the Balb/c mice were obtained from the Guangdong Experimental Animal Center (Guangdong, China). All of the mice were raised in the Guangdong Experimental Animal Center. After one week of adaptation, the MRL/lpr mice and the control mice were anesthetised by intraperitoneal injection of 10% chloralhydrate 1 mL/kg and the right eyeball from each was removed for blood collection. The collection of whole blood from all of the mice was performed using an ethylenediaminetetraacetic acid anticoagulant tube. The whole blood of four MRL/lpr lupus mice was used for mtDNA sequencing. The remaining whole blood samples of 12 MRL/lpr lupus mice and 12 control mice were placed at 37°C for 30 minutes and centrifuged for 15 minutes at 1,000 g/min, and then the serum was separated and collected before being preserved at -80°C. The remaining blood cells were extracted the total RNA and protein used to perform MT-CO1 messenger RNA (mRNA) and protein expression detection. The kidney tissues of the mice were also harvested and fixed in formaldehyde. All of the animal experiments were approved by the Institutional Animal Care and Use Committee at the Shanghai Laboratory Animal Center (certification no. SCXK2017-0005).

Laboratory reagents

Total antioxidant capacity assay kit with ABTS method (S0119; Beyotime, China), Trizol (Ambion, Austin, TX, USA), reverse transcriptase enzyme (Takara Bio Inc., Kusatsu, Japan), KAPA polymerase chain reaction (PCR) Master Mix kit (Kapa Biosystems, Wilmington, MA, USA), radioimmunoprecipitation assay (RIPA) buffer (ab156034: Abcam, Cambridge, UK), enhanced chemiluminescence western blot detection kit (WBKLS0100; Millipore, Burlington, MA, USA); MT-CO1 antibody (ab45918; Abcam, Cambridge, UK), β-actin Rabbit pAb (30102ES40; Yeasen, China), peroxidase-conjugated goat anti-rabbit immunoglobulin G (H+L) (33101ES6; Yeasen, Shanghai, China), and a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA) were used in this study. Mouse anti-double stranded DNA antibody(IgM)ELISA Kit (Cusabio Biotech Co., Ltd.). Primers were obtained from (Sangon Biotech, Shanghai, China) and are MT-CO1 primer sequence: forward 5'-TATGTTCTATCAATGGGAGC-3', reverse: 5'-GTAGTCTGAGTAGCGTCGTG-3' and housekeeping gene b-actin: forward: 5'-TCGCTGCGCTGGTCGTC-3', reverse: 5'-GGCCTCGTCACCCACATAGGA-3'.

The whole mitochondrial genome sequencing

The qubit™ dsDNA HS assay kit was used to accurately quantify the genomic concentration to determine the total amount of DNA added to the library construction. DNA samples were randomly broken into 500 bp insert length to construct library following the protocol of NEB Next® Ultra™ DNA Library Prep Kit for Illumina®. Samples were sequenced by Illumina HiSeq PE150 instrument. Raw FASTQ files were processed and filtered to acquire clean reads for downstream analysis. Filtered clean reads were aligned to the reference house mouse (NC_005089.1) using BBTools software (v. 37.48). For these samples, single-nucleotide polymorphism (SNP) and insertion and deletion (INDEL) were analysed and annotated by GATK (v. 3.8) and SnpEff software (v. 4.3s).

Periodic Acid-Schiff (PAS) staining of the kidney in MRL/lpr lupus mice

The kidney tissues of MRL/lpr lupus mice were embedded in paraffin, sectioned, dewaxed, and hydrated, and stained with PAS. Semiquantitative grading of the renal pathology of lupus was then performed as follows: grade 0, normal glomerulus without interstitial infiltration; grade 1, mild renal interstitial infiltration; grade 2, moderate renal interstitial infiltration, a small amount of tubular type, mild to moderate glomerular hyperplasia; and grade 3, severe renal interstitial infiltration, massive tubular type, severe glomerular hyperplasia with segmental or glomerular sclerosis, or crescent formation.

RNA extraction and real-time PCR analysis

Total RNA from the whole blood cell of lupus mice and control mice was extracted using Trizol. RNA was transcribed into single-stranded DNA using the RT-PCR reagent kit and complementary DNA (cDNA) as a template. PCR for MT-CO1 was performed using the KAPA PCR Master Mix kit. mRNA expression was determined by quantitative real-time PCR (qRT-PCR). mRNA expression levels of the endogenous housekeeping gene β-actin were used to normalise MT-CO1 expression levels. The relative gene abundance was calculated using the $2^{-\Delta\Delta CT}$ formula. qRT-PCR conditions were as follows: 65°C for 3 minutes, then 95°C for 3 seconds, and 60°C for 30 seconds for 40 cycles.

Table I. MT-CO1 mutations in MRL/lpr lupus mice.

Gene	Type	Postion	Effect	Change of amino acid
COX1	snp	NC_005089.1:5433	synonymous	c.106T>C,p.Leu36Leu
COX1	snp	NC_005089.1:5462	synonymous	c.135T>C,p.Gly45Gly
COX1	snp	NC_005089.1:5561	synonymous	c.234T>C,p.Phe78Phe
COX1	snp	NC_005089.1:5576	synonymous	c.249C>A,p.Val83Val
COX1	snp	NC_005089.1:5588	synonymous	c.261C>T,p.Ile87Ile
COX1	snp	NC_005089.1:5630	synonymous	c.303T>C,p.Ser101Ser
COX1	snp	NC_005089.1:5732	synonymous	c.405T>C,p.Asn135Asn
COX1	snp	NC_005089.1:5753	synonymous	c.426A>C,p.Ser142Ser
COX1	snp	NC_005089.1:6128	synonymous	c.801T>C,p.Pro267Pro
COX1	snp	NC_005089.1:6134	synonymous	c.807C>A,p.Gly269Gly
COX1	snp	NC_005089.1:6155	synonymous	c.828A>C,p.Ala276Ala
COX1	snp	NC_005089.1:6161	synonymous	c.834G>A,p.Met278Met
COX1	snp	NC_005089.1:6173	synonymous	c.846T>C,p.Phe282Phe
COX1	snp	NC_005089.1:6248	synonymous	c.921A>T,p.Ser307Ser
COX1	snp	NC_005089.1:6290	synonymous	c.963T>C,p.Phe321Phe
COX1	snp	NC_005089.1:6311	synonymous	c.984C>T,p.His328His
COX1	snp	NC_005089.1:6317	synonymous	c.990T>A,p.Gly330Gly
COX1	snp	NC_005089.1:6332	synonymous	c.1005T>C,p.Ser335Ser
COX1	snp	NC_005089.1:6362	synonymous	c.1035T>C,p.Ile345Ile
COX1	snp	NC_005089.1:6404	synonymous	c.1077C>T,p.Ser359Ser
COX1	snp	NC_005089.1:6443	synonymous	c.1116T>C,p.Tyr372Tyr
COX1	snp	NC_005089.1:6522	synonymous	c.1195T>C,p.Leu399Leu
COX1	snp	NC_005089.1:6527	synonymous	c.1200T>C,p.Phe400Phe
COX1	snp	NC_005089.1:6543	missense	c.1216G>A,p.Asp406Asn
COX1	snp	NC_005089.1:6581	synonymous	c.1254C>T,p.Phe418Phe
COX1	snp	NC_005089.1:6812	synonymous	c.1485T>G,p.Leu495Leu
COX1	snp	NC_005089.1:6818	synonymous	c.1491C>A,p.Gly497Gly
COX1	snp	NC_005089.1:6845	synonymous	c.1518G>A,p.Glu506Glu
COX1	snp	NC_005089.1:6851	synonymous	c.1524A>T,p.Pro508Pro
COX1	snp	NC_005089.1:6852	missense	c.1525A>T,p.Thr509Ser
COX1	snp	NC_005089.1:6866	synonymous	c.1539A>T,p.Val513Val

Western blot

The whole blood cell of lupus and control mice were extracted by RIPA and the total protein concentrations were quantitatively detected with a BCA kit. Equal amounts of protein were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by transference to polyvinylidene fluoride membrane. The membrane was blocked with 3% bovine serum albumin and probed with primary antibody overnight at 4°C, then reacted with 1:10,000 diluted second antibody at room temperature for 2 hours. The electrochemical luminescence (ECL) method and ImageJ software (National Institutes of Health, Bethesda, MD, USA) were used to read the grey values of each protein band and analyse the MT-CO1 protein expression.

Detection of total antioxidant capacity

The total antioxidant capacity of the samples was detected using the rapid ABTS kit and operated according to the instructions. The OD value of the 734-nm wavelength was read on the spectrophotometer.

Detection of serum serum anti-dsDNA antibody

The concentrations of anti-dsDNA antibody in the serum of mice were determined by mouse anti-dsDNA antibody ELISA kit, and the related operations were carried out according to the instructions of the kit. The OD value of the 450-nm wavelength was read on the spectrophotometer.

Statistical analysis

The data are presented as means and standard deviation. Statistical analyses between groups were performed using two independent-samples *t*-tests after performing the data normality test. Results were considered statistically significant if *p*-values were less than 0.05.

Results

Results of mtDNA sequencing in MRL/lpr lupus mice

Mutations were detected in five mitochondrial genes which included MT-CO1, MT-CO2, MT-CO3, MT-ATP6 and MT-ND3. Among them, MT-CO1 has the most mutation sites, 31 in total. Among the 31 base mutation sites, 2 were missense mutations and 29 were synonymous_variant (Table I).

Results of PAS staining in kidney tissue of MRL/lpr lupus mice

The kidney tissue of MRL/lpr lupus mice showed grade 3 pathological changes with mesangial cell and matrix hyperplasia and the formation of small crescents in the glomerulus (Fig. 1).

Expression results of MT-CO1 mRNA in the two mouse groups

The expression of MT-CO1 (t=4.333; p=0.0003) was significantly lower in the lupus model group than in the control group (Fig. 2).

Expression results of MT-CO1 protein in the two mouse groups

The expression of MT-CO1 was lower in the MRL/lpr lupus mice as compared with in the control mice at the protein level through western blot (Fig. 3).

Total antioxidant capacity in the serum of lupus and control mice

The total antioxidant capacity (t=9.957; p<0.0001) was lower in the MRL/lpr lupus mice as compared with in the control mice (Fig. 4).

The results of serum anti-dsDNA antibody level detection

The serum anti-dsDNA antibody level (t=6.383; p<0.0001) was significantly higher in the lupus model group as compared with in the control group (Fig. 5).

Correlation analysis of total antioxidant capacity and MT-CO1 mRNA expression level in MRL/lpr lupus mice

The results of correlation analysis showed that there was a positive correlation between total antioxidant capacity and MT-CO1 expression in the serum of MRL/lpr lupus mice (r=0.8170; p=0.0001) (Fig. 6).

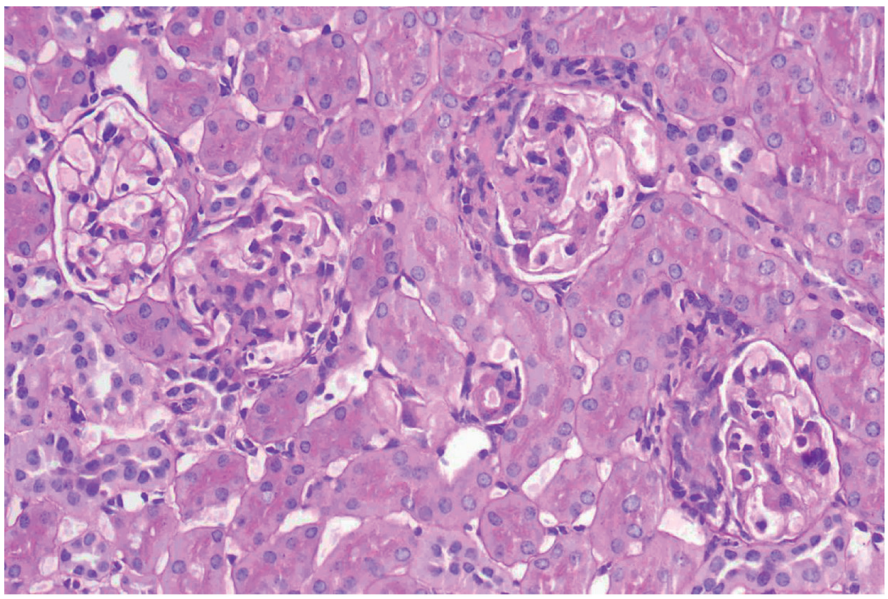

Fig. 1. PAS staining of kidney tissue in lupus mice (400×).

Discussion

In mammalian cells, only the nucleus and mitochondria are the organelles with genetic material that can be copied, transcribed, and translated. mtDNA of mice, about 16.6 kb in size, is a closed-ring, dsDNA that consists of a gene-coding region and noncoding D-ring region. There are 37 coding genes: 2 rRNA genes, 22 tRNA genes, and 13 polypeptide chain genes (5). Mitochondrial functional proteins are mostly transcribed and translated by nuclear genes, while a small part is transcribed and translated by the mitochondrial genes themselves. The genetic material DNA of the nucleus and mitochondria is dsDNA, but the nucleus DNA appears as a linear double-helix, while the mtDNA is circular. Anti-dsDNA antibodies can bind with dsDNA to form immune complexes, deposit in tissues and organs, and trigger an immune response, causing tissue and organ damage. Theoretically, the anti-dsDNA antibody is induced by immunogenic dsDNA, which may include nucleus DNA and mtDNA. However, the antigenicity of mammalian nuclear dsDNA is weak and the induction of anti-dsDNA antibodies has been largely unsuccessful (6-7). mtDNA is a source of bacteria with heterogeneity and can easily evolve to become an autoantigen due to the natural bacteria dominant sequence and structure (3). Lupus mice constitute a good model mouse for the study of SLE with spontaneous onset, which can gradually develop anti-dsDNA antibodies and renal damage.

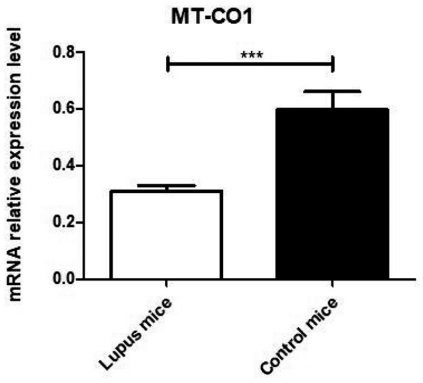

Fig. 2. The expression of MT-CO1 mRNA in lupus mice and control mice by qRT-PCR analysis of MT-CO1 mRNA expression. *Represents a statistically significant difference between the two groups of mice, p<0.05.

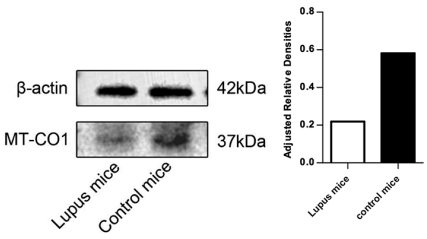

Fig. 3. Detection of MT-CO1 protein expression in lupus and control mice by western blot.

Unlike nuclear DNA, mtDNA lacks histone and double-helix structures, such as introns, to protect mtDNA from damage. Therefore, in the oxidative

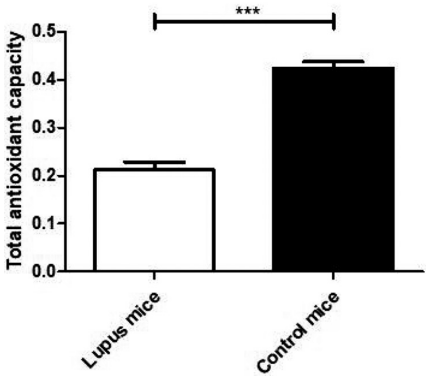

Fig. 4. Test results of total antioxidant capacity in the serum of lupus and control mice.*Represents a statistically significant difference between the two groups of mice, $p<0.05$.

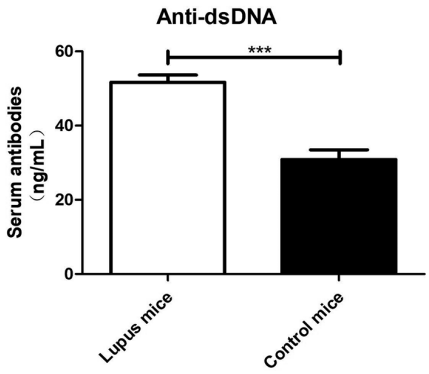

Fig. 5. Test results of serum anti-dsDNA antibody level of lupus and control mice.*Represents a statistically significant difference between the two groups of mice, $p<0.05$.

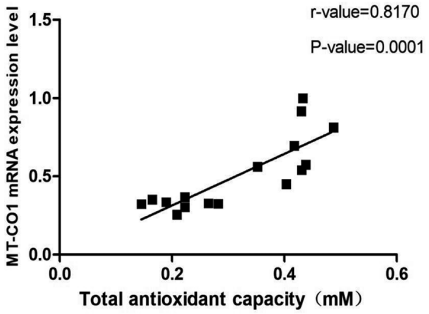

Fig. 6. Correlation analysis of serum total antioxidant capacity and MT-CO1 mRNA expression in lupus mice.

stress environment, mtDNA continues to replicate asymmetrically and efficiently, making it vulnerable to ROS damage caused by the electron transport chain (4).

In mtDNA base differences of lupus mice, our results showed that there were five gene as follows MT-CO1, MT-CO2, MT-CO3, MT-ATP6, and MT-ND3 genes.The maximum difference gene was the MT-CO1 gene, including 31 base differences sites, especially the 2 missense base differences were all located at MT-CO1 region which might lead peptide chain and function of MT-CO1 change. Previous studies have shown, the expression profiles of adult C57BL/6 mouse heart obtained by serial analysis of gene expression and cDNA arrays, numerous quantitative discrepancies of base mutation could be observed for, arrays, MT-CO1 > MT-CO2 > MT-CO3; SAGE, MT-CO1 >> MT-CO3 > or = MT-CO2 (8), which is consistent with our present study that most of the base differences were located in MT-CO1. Meanwhile post research showed base differences of MT-CO1 was related to some diseases, T6459C in the MT-CO1 gene is associated with sepsis susceptibility in the Chinese Han population (9-11), in the MT-CO1, the mutation of G6150A is an important cause of primary open-angle glaucoma (10-11). In addition, in tumour studies, C7028T in the MT-CO1 gene had an ascending frequency from borderline (8%) to stages III/IV (75%) among the three ovarian cancer subtypes and stages (12). Three point mutations (A6052G, A9545G, and G9575A) of MT-CO1 are risk factors for endometrial cancer (13).

Our mtDNA sequencing results suggested, when lupus mice compared to house mouse (NC_005089.1), there were many base differences in mtDNA, our results also showed that the serum total antioxidant capacity of lupus mice was decreased, we supposed the aetiology of base differences was ROS. Actually, the post studies reported that ROS was increased in SLE patients (14-15). ROS are important active molecules in organisms which usually caused by a lower antioxidant capacity. They readily caused oxygen toxicity to biological macromolecules, especially a high mutation rate of mtDNA (16). However, in rodents, because the base sequence of MT-CO1 is highly conservative, researchers had tried to establish the MT-CO1 DNA barcode as a method of species identification in complex populations (17). Thus we considered that all of sequencing results might be mainly related to the pathological condition such as ROS, meanwhile we could not eliminate the cause of sequencing results related to differences between different species of mice. In addition, the MRL/lpr mice used in this study, as the classical animal model of SLE, had some differences between themselves and SLE patients. The pathogenesis of MRL / lpr mice is mainly due to apoptosis related mutations of Fas gene, which leads to apoptosis defects of T cells, B cells and macrophages, and then produces autoimmune disease symptoms (18), which is very similar to human SLE symptoms. It is characterised by the production of a large number of ANA, anti dsDNA, anti ssDNA and other autoantibodies, and the occurrence of glomerulonephritis and other symptoms. However, MRL / lpr mice could not represent SLE caused by genetic factors, estrogen and environmental factors. Therefore, the mutation of lupus mouse gene detected in the study does not fully represent the situation of SLE patients. We believe that the mutation rate of mitochondrial genes, including MT-CO1, in SLE patients will be higher and more complex than that in lupus mice. Therefore, we should take the research results of mtDNA of lupus mice as the research basis and guidance, and make a more specific and targeted in-depth study of mtDNA of SLE patients.

Especially, in a biological sense, missense mutations could change the amino acid type and sequence, which could change the MT-CO1-encoded peptide chains and push the encoded protein to lose its original function. Considering the oxidative respiratory chain of the mitochondria, the glutamate residue (E242) in the center of MT-CO1 can import and export protons directionally, which eventually forms an electron gradient in the respiratory chain and enables oxidizing oxygen molecules to water molecules on cytochrome C oxidase 1. Moreover, at MT-CO1, the energy generated by the electron gradient to form ATP, the body's most important energy substance, so the abnormal expression and dysfunction of MT-CO1 not only leads to the abnormal generation of ATP but also increases the generation potential of ROS. Because ATP

and ROS all are so important bioactive molecule and could be affect by the MT-CO in organism, that it was necessary and reasonably to focus and deeply study MT-CO1.

Our qPCR results showed that MT-CO1 expression in lupus mice decreases. Our results indicated, it's no coincidence that the base differences sites of the mitochondrial gene in lupus mice were mainly concentrated in MT-CO1. Obviously, this decrease of MT-CO1 expression in lupus mice was benefit for supporting that the base differences of MT-CO1 could mainly relate to the base mutation, especially missense differences. So, as expected, we utilised the whole mitochondrial genome sequencing and screened successfully MT-CO1 gene as a maximum base difference gene in mtDNA, and finally confirmed the abnormal expression of MT-CO1 gene, it was clear that our experimental results was reasonable and mutual corroborated.

In fact, MT-CO1 is the core group in the whole process of electron transport of the oxidative respiratory chain of cells and plays a most important role. Previous studies have shown that MT-CO1 could react very sensitively and rapidly to various physiological conditions. When myocardial cells were heated, the expression of MT-CO1 reached its peak in 60 minutes and, when they recovered, the upregulated MT-CO1 decreased to a physiological state after 60 minutes, Vogt, S speculated the ATP might play a decisive negative feedback role to the respiratory chain during the myocardial cells sensitive reaction (19), another research had revealed the influence of COX deficiency on mtDNA biosynthesis was a consequence of insufficient ATP synthesis by analyzing the cytochrome C oxidase (COX)-positive and COX-negative areas in 19 parathyroid mtDNA (20). In precancerous lesion, the abnormal expression of MT-CO1 leads to the abnormal generation of ATP and increases the generation potential of ROS (21). However, there are few studies on the relationship between mitochondrial gene abnormality and SLE, especially the abnormal mechanism of MT-CO1 in SLE. We supposed that the abnormality of MT-CO involves the formation of ATP and ROS, the important active substances for many biological functions in organism, abnormal MT-CO levels in lupus mice will inevitably lead to the abnormal production of ATP and ROS, the abnormal ATP and ROS would have unknown feedback and impact on MT-CO1, finally lead abnormal MT-CO1 expression. It was clear that further study for mechanism of MT-CO1 abnormality would be beneficial to the prevention and treatment of SLE.

We also studied the expression of MT-CO1 in lupus mice at the translation level and found MT-CO1 in lupus mice was decreased. This result had showed the MT-CO1 was decreased in both gene expression and protein level, which would induce declining function of MT-CO1. The literature shows that the expression of mitochondrial genes is regulated not only by the mitochondrial genes themselves but also by nuclear genes, and there are more than 1,000 proteins in mitochondria, only 13 proteins are encoded by mitochondrial genes, while the MT-CO1 transcription and translation could be accomplished independently in mitochondria (22). It was clear that the decrease of MT-CO1 expression in lupus mice would inevitably have an important impact on the mitochondrial respiratory chain, which could induce series pathological reactions of the body and might trigger and aggravate SLE. In a double-blind controlled study, MT-COI was abnormally expressed in patients with hearing impairment, diabetes mellitus, and congenital visual loss (23), a study about sporadic frontotemporal lobar degeneration with TDP-43 pathology disclosed the downregulation of MT-CO1, which provides robust information about the downregulation of genes involved in vital biochemical pathways and in synaptic neurotransmissions (24). The regulation of MT-CO1 by P2 peptide is essential for efficient reverse transcription in the early stage of HIV-1 infection (25), the abnormality of MT-CO1 involves a variety of diseases. However, up to now, there has been little research on MT-CO1 in autoimmune diseases. Ishikawa reported the expressions of MT-CO1 and MT-CO2 were decreased in systemic juvenile idiopathic arthritis patients (26). In this study, the base differences rate of MT-CO1 in lupus mice increased but its gene expression and protein level was decreased. Theoretically, the gene mutation could induce lower expression of gene. So it was reasonable that the decreased expression of MT-CO1 went hand in hand with the base differences of MT-CO1. We speculated that the abnormal expression of MT-CO1 would inevitably have a crucial impact on the pathogenesis of SLE, although current studies have not demonstrated the exact relationship between abnormal MT-CO1 expression and SLE, which might be the reason for why the pathogenesis of SLE is unknown until now. A thorough study on the relationship between MT-CO1 and SLE might be of great value to the core pathogenesis of SLE.

Meanwhile, up to now, there has not been any direct evidence to demonstrate that mtDNA was the antigen of anti-dsDNA antibody, but many researches have revealed that antimitochondrial antibodies (AMAs) has close relationship with SLE (27). AMAs is a group autoantibodies to protein, RNA and ds-DNA of mitochondrial (28). We were willing to believe that the base mutation of mtDNA in lupus mice could be caused by lupus damage especially ROS. The antigenicity might increase as mitochondrial base mutations caused by ROS, which could induce anti-dsDNA antibodies.

Essentially, the antimitochondrial antibodies have been confirmed both in lupus patients and lupus model mice (28-29). The research made by Becker *et al*. suggested that different mitochondrial components are immunogenic in SLE and supported the view that extracellular mitochondria may be an important source of circulating autoantigens in SLE (27). The latest literature also revealed that mtDNA is an important component of neutrophil extracellular traps (NETs), which is a new form of programmed cell death (30). In theory, mtDNA, as an important component of NETs, is likely to become a potential antigen of anti-dsDNA antibody, even though there is no direct and clarify in the relationship between antimitochon-

drial and anti-dsDNA antibodies at this time. Anti-dsDNA antibody is recognized as a marker of SLE. Currently, the Systemic Lupus International Collaborating Clinics (SLICC) group suggested that patients with lupus nephritis could be diagnosed as SLE if anti-dsDNA antibody was found in kidney biopsy (31). Therefore, it was important to clarify the formation mechanism of anti-dsDNA antibody for SLE research. We supposed that MT-CO1 had an important biological function and participates in the formation of ROS and ATP, could induce the change of mtDNA, finally might be closely related to the formation of anti-dsDNA antibodies and core mechanism of SLE.

Because MT-CO1 was the core group of mitochondrial respiratory chain in mitochondrion where is mainly region for ROS production. In order to clarify the relationship between abnormal MT-CO1 and oxidative stress, we used a rapid ABTS method to detect the total antioxidant capacity in the serum of lupus mice and analyse the relationship between the MT-CO1 base differences and its abnormal mRNA expression.

Our results showed that the serum total antioxidant capacity of lupus mice was decreased. Post Studies suggested that ROS increase and antioxidant capacity decrease in SLE (32), which is a pathological mechanism that could induce a series of inflammatory reactions, lead to lupus activity and organ damage. The ABTS method is a common method for antioxidant detection. The antioxidant scavenging capacity is determined by the ratio of ABT absorbance to standard antioxidants. The higher the ratio, the stronger the antioxidant capacity (33). Our results confirm the reported increase in ROS and decreased antioxidant capacity could be coexistence in SLE.

We analysed the correlation between the total antioxidant capacity and the mRNA expression of MT-CO1, and the results showed that the total antioxidant capacity was positively correlated with the mRNA expression of MT-CO1, while the more strong antioxidant capacity the lower the oxidative stress, our result indicated that the abnormal expression of MT-CO1 might be closely related to the oxidative stress caused by ROS in mitochondria. The abnormality of MT-CO1 leads to the ROS in mitochondria being unable to utilise the electronic potential difference of the respiratory chain to form ATP and water molecules, thus the ROS increased in the body. ROS are extremely active biomolecules. A large number of studies have shown that ROS could affect the replication, transcription, and modification of mitochondrial gene (16, 34), which could lead to multiple types of mutations in mitochondrial genes, sequence changes, and functional abnormalities of peptide chains. A large number of reports revealed that the effect of ROS on mitochondrial genes may lead to an abnormality of MT-CO1. We speculated that MT-CO1 and ROS might influence each other and form a vicious circle, this change trend might coincide with the development of SLE and help to elucidate SLE pathogenesis, search for new therapeutic targets and strategies of SLE. Although we used animal models to study the variation and expression of MT-CO1, the MRL/lpr mice we used are classic SLE mice models, which are very similar to SLE patients in pathogenesis. Therefore, we believe that the results of this study are not only of significance to the pathogenesis of lupus mouse model, but also of reference and guidance to the pathogenesis of human SLE. Based on this, the research on human SLE mtDNA should be more comprehensive and in-depth.

Conclusion

In conclusion, in lupus mice, MT-CO1 encodes the core terminal enzymes of the electron transport chain in mitochondrial oxidative respiration. The abnormal expression of MT-CO1 could cause abnormal production of ROS and ATP as well as numerous biological effects and complex immune abnormalities. The abnormal expression of MT-CO1 is closely related with lupus. We believe that the abnormal expression of MT-CO1 in lupus mice is of great significance in the pathogenesis of SLE. Meanwhile, mtDNA, including MT-CO1, are dsDNA whose structure and function are easy to change under ROS which boasts stronger antigenicity of mtDNA. We speculated that MT-CO1 might play an important role in the pathogenesis of SLE and the production of anti-dsDNA antibodies, and could be an important inducer of anti-dsDNA antibody.

Key messages

In this report, by using the MRL/lpr mice model, we found a few points to prove that MT-CO1 is involved in the pathogenesis of SLE.

- The antioxidative ability of lupus mice was decreased.
- The expression of MT-CO1 was decreased in lupus mice.
- We speculated that the decrease of MT-CO1 expression was related to the decrease of antioxidant capacity in lupus mice.
- We could not confirm that the characteristics of antioxidative ability and MT-CO1 expression in lupus mice were similar in SLE patients.

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