Proteome analysis of lysine 2-hydroxyisobutyrylation in the peripheral blood of systemic lupus erythematosus patients

Xianqing Zhou
   Nephrology department of Guilin No.924 Hospital,Guangxi key laboratory of metabolic diseases research,Guilin key laboratory of kidney diseases research

Hua Lin
   Nephrology department of Guilin No.924 hospital,Guangxi key laboratory of metabolic diseases research,Guilin key laboratory of kidney diseases research

Ruohan Zhang
   Nephrology department of Guilin No.924 hospital,Guangxi key laboratory of metabolic diseases research,Guilin key laboratory of kidney distases research

Weiguo Sui
   Nephrology department of Guilin No.924 hospital,Guangxi key laboratory of metabolic diseases research,Guilin key laboratory of kidney diseases research

Yue Zhang
   Nephrology department of Guilin No.924 hospital,Guangxi key laboratory of metabolic diseases research,Guilin key laboratory of kidney diseases research

Jiejing Chen
   Nephrology department of Guilin No.924 hospital,Guilin key laboratory of metabolic diseases research,Guilin key laboratory of kidney diseases research

Wen Xue
   Nephrology department of Guilin No.924 hospital,Guangxi key laboratory of metabolic diseases research,Guilin key laboratory of kidney diseases research,Guilin key laboratory of kidney diseases research

Qiang Yan
   Nephrology department of Guilin No.924 hospitoal,Guangxi key laboratory of metabolic diseases,Guilin key laboratory of kidney diseases research

Chunhong Li
   Nephrology department of Guilin No.924 hospital,Guangxi key laboratory of metabolic diseases research,Guilin key laboratory of kidney diseases research

Huixuan Xu
   Clinical medical research,The second clinicla medical college of Jinan Univerisity(Shenzhen People's hospital)

Shaoying Huang
   Clinical medical research center,The second clinical medical college of Jinan University(Shenzhen People's hospital)

Werer Dai
   Clinical medical research center,The second clinical medical college of Jinan University(Shenzhen People's hospital).College of Natural Sciences,The University of Texas at Austin

Donge Tang
   Clinical medical research center,The second clinical medical college of Jinan Univerisity(Shenzhen People's hospital)

Yong Dai

Research

Keywords: SLE, 2-hydroxyisobutyrylation, Mass spectrometry, Bioinformatics

Abstract

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that affects multiple organs, the pathogenic mechanism is related to many factors, but the specific pathogenic mechanism has not been clarified yet. Protein lysine modifications play important roles in gene regulation, transcription, metabolism and other biological processes. The lysine 2-hydroxyisobutyrylation(K hib) histone mark has recently been discovered as a novel protein modification. In this study, patients in the active SLE group were examined (N=8), while the control group was healthy (N=20).Utilizing antibody-based affinity enrichment and nano-HPLC/MS/MS analyses of K hib peptides, we identified 156 upregulated proteins(fold change>1.5),124 downregulated proteins(fold change<1/1.5), including 220 K hib sites that were upregulated and 187 K hib sites that were downregulated. Our data demonstrate that proteins with K hib sites were localized in the cytoplasm. Functional enrichment analysis revealed that proteins with K hib sites are broadly involved in a wide range of biological processes, cellular components and molecular functions. The 03010 Ribosome pathways may exert important influence on the SLE pathogenic mechanism, according to a KEGG analysis. The functional analysis of K hib is of value for important future investigations of SLE pathogenesis.

Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease produced by many factors, such as heredity, medications, and the environment, and clinical manifestations of the disease involve many organs and the adaptive immune system(Regunathan-Shenk and Radhakrishnan 2018). Posttranslational modifications (PTMs) promote the formation of covalent bonds between chemical groups/peptides and target proteins and constitute a mechanism that ultimately results in changes in protein structure and function (Marcelli et al. 2018). Lysine 2-hydroxyisobutyrylation (K_{hib}) is a newly identified PTM found in animal and yeast cells. Previous research suggested that histone K_{hib} is involved in male cell differentiation and plays a critical role in the regulation of chromatin functions in animals(Yu et al. 2017). The removal of the K_{hib} group from H4K8 is mediated by the histone lysine deacetylases Rpd3p and Hos3p in vivo. In addition, eliminating modifications at this site by alanine substitution alters transcription in carbon transport/metabolism genes and results in a reduced chronological life span (CLS). Furthermore, consistent with the glucose-responsive H4K8hib regulation, proteomic analysis of K_{hib} revealed a large set of proteins involved in glycolysis/gluconeogenesis(Huang et al. 2017). Dong reported comprehensive identification of K_{hib} in Proteus mirabilis, with K_{hib} sites involved in metabolic pathways, such as the pentose phosphate pathway, and in purine metabolism and glycolysis/gluconeogenesis. The modification of carbon sources can affect the occurrence of K_{hib}. Furthermore, they observed that K_{hib} on K343 had a negative regulatory effect on enolase (ENO) activity, and K_{hib} may change the binding formation of ENO and its substrate, 2-phospho-D-glycerate (2PG), to prevent the substrate from coming close to the active sites of the enzyme(Dong et al. 2018). He's study revealed that EP300-catalyzed K_{hib} and further indicated that EP300 has the intrinsic ability to select short-chain acyl-CoA-dependent protein substrates by regulating cellular glucose metabolism(He et al.). Meng and coworkers provided the first systematic analysis of K_{hib} modifications in developing rice (Oryza sativa) seeds, and functional annotation analyses indicated that proteins modified by K_{hib} are preferentially targeted in glycolysis/gluconeogenesis, TCA cycle metabolism, starch biosynthesis, lipid metabolism, protein biosynthesis and other biological processes. Research has revealed that K_{hib} sites are conserved in the histone proteins H3 and H4 in humans, mice and Physcomitrella patens and are novel sites in the histone proteins H1, H2A and H2B in Physcomitrella patens.

Here, we identified that 156 upregulated proteins(fold change > 1.5),124 downregulated proteins(fold change < 1/1.5), including 220 Khib sites that were upregulated and 187 Khib sites that were downregulated. In addition, functional enrichment analysis revealed that proteins modified by K_{hib} were broadly involved in a wide range of biological processes, cellular components and molecular functions. Our research on the K_{hib} modifications of the histones in SLE will facilitate the understanding of the pathogenic mechanism of SLE.

Materials And Methods

With informed consent and under the direction of a protocol approved by the Guangxi Key Laboratory of Metabolic Diseases Research Ethics Committee, we collected 3.5 ml peripheral blood (PBMC) samples from the normal control group, which consisted of 20 healthy people and 8 SLE patients; the information regarding these patients is displayed in Table 1. PBMCs were isolated by density gradient centrifugation using Ficoll-Hypaque and sonicated three times on ice using a high-intensity ultrasonic processor (Scientz) in lysis buffer (8 M urea and 1% protease inhibitor cocktail). (Note: For PTM experiments, the inhibitors were also added to the lysis buffer, e.g., 50 mM NAM and 3 μM TSA for acetylation). The remaining debris was removed by centrifugation at 12,000 × g and 4 °C for 10 min. Finally, the supernatant was collected after centrifugation and the protein concentration was determined by a BCA kit according to the manufacturer's instructions.

NO	Gender	Age	WBC	RBC	HGB	PLT	MPV	Urea	Cr	UA	IgG	IgA	IgM	C3	C4	CRP	ANA
P1	man	48	5.82	4.55	124	136	11.2	6.2	73	228	16.92	4.30	1.28	0.26	0.01	<5.0	1:10000 +
P2	women	55	11.2	3.42	108	166	8.6	9.9	139	217	5.48	1.52	0.17	1.08	0.24	8.77	1:3200 +
P3	women	17	7.64	4.92	135	371	11.0	2.5	60	336	11.38	1.51	0.89	1.18	0.12	42.34	1:3200 +
P4	man	80	6.83	2.44	79	269	7.1	22.3	729	216	19.1	0.83	0.40	0.90	0.13	<5.0	1:1000 +
P5	women	60	6.37	3.05	88	124	11.0	10.2	63	231	11.11	1.90	0.75	0.75	0.01	-	-
P6	women	57	8.92	3.52	102	301	-	-	-	-	17.64	3.86	0.86	0.59	0.09	13.38	1:10000 +
P7	women	28	9.14	3.58	109	117	8.4	11.9	146	494	-	-	-	-	-	<5.0	1:100 +
P8	women	25	6.49	2.13	61	115	7.7	24.8	436	257	-	-	-	-	-	<5.0	1:1000 +

Table 1. Detailed information for all identified K_{hib} of SLE patients.

Trypsin Digestion

The protein solution was reduced with 5 mM dithiothreitol (56 °C for 30 min) and alkylated with 11 mM iodoacetamide (room temperature in darkness, 15 min). The protein sample was then diluted by adding 100 mM TEAB to a urea concentration that was less than 2 M. Finally, trypsin was added at a 1:50 trypsin-to-protein mass ratio for the first digestion, which was completed overnight, and a 1:100 trypsin-to-protein mass ratio for a second digestion, which lasted 4 h.

Antibody-based Ptm Enrichment:

To enrich the K_{hib}-modified peptides, tryptic peptides dissolved in NETN buffer (100 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, and 0.5% NP-40 at pH 8.0) were incubated with prewashed antibody beads at 4 °C overnight with gentle shaking. Then, the beads were washed four times with NETN buffer and twice with H_2O. The bound peptides were eluted from the beads with 0.1% trifluoroacetic acid. Finally, the eluted fractions were combined and vacuum dried. For the LC-MS/MS analysis, the resulting peptides were desalted with C18 Zip Tips (Millipore) according to the manufacturer's instructions.

Lc-ms/ms Analysis

Trypsin hydropeptides were dissolved in 0.1% formic acid and analyzed in a reversed-phase column. They were run through an Easy-NLC 1000 system with a constant flow rate set at 400 nL/min. The mobile phase A aqueous solution was prepared with 0.1% formic acid and 2% acetonitrile. The mobile phase B aqueous solution was prepared with 0.1% formic acid and 90% acetonitrile aqueous solution. The following liquid gradient setting was used: 8%-22% B phase, 0 ~ 38 min; 322%-35% B phase, 8 ~ 52 min; 35%-80% B phase, 52–56 min; 80% B phase, 56 ~ 60 min. The flow rate was maintained at 800 nL/min.

The peptides were isolated by an ultra-high-performance liquid phase system and subsequently ionized in an NSI source at an ion source voltage of 2.0 kV. The ionized peptides were analyzed by Q Exactive TM Plus mass spectrometry (MS/MS). The high-resolution Orbitrap was used to detect and analyze the peptide parent ions and their secondary ion fragments. The peptide ions were first scanned by first-order mass spectrometry at a scanning range of 350–1800 m/z and a resolution of 70,000. The secondary mass spectrometry scanning was performed at a scanning range of 100 m/z and a resolution of 17,500. Data were collected using a data-dependent scan (DDA) program. The mass spectrometer had a set automatic gain control (AGC) parameter of 5e4 with a signal threshold of 5,000 ions/s, a maximum injection time of 200 ms and a dynamic exclusion time of 30 s for the tandem mass spectrum scanning. The aim was to improve the efficiency of the mass spectrometry and avoid repeated scanning of the parent ions.

Database Search

The secondary mass spectrometry data were determined by Maxquant (v1.5.2.8). The SwissProt Human database (20,130 sequences) was used. Considering the problem of the false positive rate (FDR), an inverse library was added during retrieval. In addition, to eliminate the influence of

contaminant proteins affecting the identification results, a common contamination database was added to the analysis. Trypsin/P was set as the enzyme digestion mode. The number of missing cut positions was set to 4. The minimum length of each peptide should be restricted to 7 amino acid residues. The maximum number of modifications for a peptide was set at 5. The tolerance of the quality error for the primary parent ion of the first search was set to 20 PPM, that of the primary parent ion for the main search was set to 5 PPM and that of the secondary fragment ion was set to 0.02 Da. Carbamidomethyl on Cysteine was specified as a fixed modification, and the K_{hib} modification and oxidation on Met were specified as variable modifications. The FDR was adjusted to < 1%, and the minimum score for the modified peptides was set to > 40.

Bioinformatics Methods

1) We used WoLFPSORT, subcellular localization predication software, to predict the subcellular localization. WoLFPSORT is an updated version of PSORT/PSORT II that is used for the prediction of eukaryotic sequences.

2) The Gene Ontology (GO) annotation proteome was derived from the UniProt-GOA database (www. http://www.ebi.ac.uk/GOA/). Through the UniProt-GOA database (), the proteomics level is reported as GO analysis comments. First, the system converts the protein ID into the UniProt ID that is detected by UniProt-GOA, and then, the UniProt ID is used to match the GO ID to be analyzed, and finally, the annotation information of the corresponding protein is retrieved in the database according to the GO ID. InterProScan can be used to predict the GO function of a protein that is not retrieved from the UniProt-GOA database. The retrieved protein information was classified into three categories: cell composition, molecular function and physiological process. For each category, two-tailed Fisher's exact test was employed to test the enrichment of the differentially modified proteins against all the identified proteins. GO data with corrected p-values < 0.05 are considered significant.

3) The KEGG pathway annotation tools were used. First, KEGG online KAAS service tools were used to annotate the KEGG database description. Then, the annotation results from the KEGG pathway database were mapped using the KEGG online service tool, KEGG mapper. The KEGG database was used to identify enriched pathways by a two-tailed Fisher's exact test, which is used to determine the enrichment of the differentially modified proteins against all the identified proteins. The pathway with a corrected p-value < 0.05 was considered significant. These pathways were classified into hierarchical categories according to the KEGG website.

4) The functional descriptions of the identified protein domains were annotated by InterProScan (a sequence analysis application) based on the protein sequence alignment method, and the InterPro domain database was used. InterPro (http://www.ebi.ac.uk/interpro/) is a database that integrates diverse information about protein families, domains and functional sites, and it is made freely available to the public via web-based interfaces and services. The diagnostic models, known as signatures, are central to the database and can be used to search protein sequences to determine their potential function. InterPro has utility in the large-scale analysis of whole genomes and meta-genomes, as well as in characterization of individual protein sequences. For each category of proteins, InterPro database (a resource that provides functional analysis of protein sequences by classifying them into families and predicting the presence of domains and important sites) was searched, and a two-tailed Fisher's exact test was employed to determine the enrichment of the differentially modified proteins against all identified proteins. Protein domains with corrected p-values < 0.05 were considered significant.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE(Perez-Riverol et al. 2019) partner repository with the dataset identifier PXD015351.

Results

K_{hib} protein detection and identification

Utilizing antibody-based affinity enrichment and nano-HPLC/MS/MS analyses of the K_{hib} peptides. Based on the criteria, p value < 0.05, we identified that 156 upregulated proteins(fold change > 1.5),124 downregulated proteins(fold change < 1/1.5), including 220 K_{hib} sites that were upregulated and 187 K_{hib} sites that were downregulated. The proteins with higher fold change and multiple upregulated modifications were hemoglobin subunit gamma-2 (P69892), 40S ribosomal protein S9 (P46781), and 60S ribosomal protein L6 (Q02878). The proteins with higher fold change and multiple downregulated modifications were GRIP1-associated protein 1 (Q4V328), heterogeneous nuclear ribonucleoprotein A/B (Q99729), and heterogeneous nuclear ribonucleoproteins A2/B1 (P22626), the differentially expressed protein summary in Table2. The K_{hib} modification sites with > 7 of proteins were Spectrin alpha chain, nonerythrocytic 1 (Q13183), Talin-1 (Q9Y490), Spectrin beta chain, erythrocytic (P11277) (Fig. 1a). Of the 897 2-hydroxyisobutylated proteins, most contain 1 ~ 2 modification sites, some have 3 ~ 7 modification sites, and a few contain 8 or more modification sites. Most peptides vary in length from 8 to 20 amino acids, consistent with trypsin digestion(Fig. 1b). To investigate the presence of K_{hib} in SLE, western blotting with K_{hib} antibody was carried out in the total protein, we can saw a large number of protein bands, then a wide protein mass range was observed, revealing that K_{hib} is highly abundant with the protein (Fig. 2a). To identify K_{hib} sites in SLE,we randomly selected H3K79$_{hib}$ site and H3K14$_{hib}$ loci for western blotting analysis, and detected the expression of H3K79$_{hib}$ site and H3K14$_{hib}$ antibodies in SLE with H3 as a reference(Fig. 2b).

Table 2
The proteins K$_{hib}$ modifications with higher fold change upregulated and downregulated.

Protein accession	Protein description	Gene name	SLE/NC Ratio	Up/Down
P69892	Hemoglobin subunit gamma-2	HBG2	7.226	Up
P69892	Hemoglobin subunit gamma-2	HBG2	6.69	Up
P46781	40S ribosomal protein S9	RPS9	6.044	Up
P69892	Hemoglobin subunit gamma-2	HBG2	4.994	Up
Q02878	60S ribosomal protein L6	RPL6	3.702	Up
P61254	60S ribosomal protein L26	RPL26	3.215	Up
P16949	Stathmin	STMN1	3.145	Up
P08865	40S ribosomal protein SA	RPSA	2.751	Up
P62249	40S ribosomal protein S16	RPS16	2.748	Up
Q16851	UTP−glucose-1-phosphate uridylyltransferase	UGP2	2.688	Up
Q4V328	GRIP1-associated protein 1	GRIPAP1	0.666	Down
Q99729	Heterogeneous nuclear ribonucleoprotein A/B	HNRNPAB	0.665	Down
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1	HNRNPA2B1	0.664	Down
Q14978	Nucleolar and coiled-body phosphoprotein 1	NOLC1	0.662	Down
Q01813	ATP-dependent 6-phosphofructokinase, platelet type	PFKP	0.662	Down
P52272	Heterogeneous nuclear ribonucleoprotein M	HNRNPM	0.662	Down
P02671	Fibrinogen alpha chain	FGA	0.662	Down
P01009	Alpha-1-antitrypsin	SERPINA1	0.662	Down
P62753	40S ribosomal protein S6	RPS6	0.661	Down
P11277	Spectrin beta chain, erythrocytic	SPTB	0.661	Down
P01011	Alpha-1-antichymotrypsin	SERPINA3	0.661	Down
O60925	Prefoldin subunit 1	PFDN1	0.661	Down

Subcellular localization information and GO classifications of proteins with K $_{hib}$

According to the subcellular localization analysis, the largest percentage of proteins with K$_{hib}$ was in the cytoplasm, and proteins with higher fold change modifications were localized in the cytoplasm. For the upregulated proteins with K$_{hib}$ shown in Fig. 3(a), 49% were located in the cytoplasm 49%, 19% in the nucleus, and 10% in the mitochondria. Among the downregulated proteins in Fig. 3(b), 42% were located in the cytosol, 30% in the nucleus, and 14% in the extracellular fraction. The GO classification indicated that the identified proteins with K$_{hib}$ fell into three categorizations (the cellular component, molecular function, and biological process). Of the upregulated proteins in the cellular component category, as shown in Fig. 4(a), 20% were in the cell, 19% were in the organelle, 15% were in the extracellular region, and 13% were in the membrane. Among the downregulated proteins shown in Fig. 4(b), 20% were in the cell, 20% were in the organelles; 14% were in the membrane and 14% were in the membrane-enclosed lumen. Based on their molecular function, of the upregulated proteins shown Fig. 4(c) and the downregulated proteins shown Fig. 3(d), 51% and 55%, respectively, were in the largest category: binding. Based on their biological process, of the upregulated proteins shown in Fig. 4(e), 13% were involved in cellular processes, 12% were involved in biological regulation, and 11% were involved in single-organism processes. The percentages of the downregulated proteins were involved in the following biological processes: cellular processes, 14%; biological regulation, 12%; metabolic processes, 11%; and single-organism processes, 11% Fig. 4(f).

Functional Enrichment Of Proteins With K

Functional enrichment analysis revealed that the proteins with K$_{hib}$ were broadly involved in biological processes. Figure 4 shows the functional enrichment description based on the GO analysis (cellular component, molecular function, biological process), the Fisher exact test p-value was determined after the logarithmic transformation. The longer the bar in the graph, the more significant the enrichment of differentially expressed

proteins in this classification or function. Based on the upregulated proteins with the red bar of Fig. 5(a), the proteins were enriched for the adherens junctions, anchoring junctions, and cell-substrate junctions in the cellular components category. The proteins were enriched for the domain-specific binding, structural constituent of ribosome, and structural molecule activity in the molecular function categories. As determined by the biological process categories, the proteins localized at the endoplasmic reticulum, for actin cytoskeleton reorganization, and for the regulation of the viral life cycle were enriched. Based on the downregulated proteins, signified by the blue bar in Fig. 5(b), for the cellular component category, the proteins were enriched in the cytosolic ribosomes, ribosomal subunits, and the cytosol; for the molecular function category, these proteins were enriched in the structural constituents of ribosomes for structural molecule activity and DNA binding; and for the biological process category, these proteins were enriched for ribosomal large subunit biogenesis, viral gene expression, and protein localization to the endoplasmic reticulum.

The KEGG pathways mainly include metabolism, genetic information, environmental information, cellular processes, rat diseases, and drug development. Of the 9 different signaling pathways revealed by the KEGG pathway enrichment analysis, the hsa03010 Ribosome pathway was the most relevant, as shown in Fig. 6. The multiple modifications with an fold change > 2.0 for the proteins with the upregulated proteins of the has03010 Ribosome pathway analysis were 40S ribosomal protein S9(P46781), 60S ribosomal protein L6(Q02878), 60S ribosomal protein L26(P61254), 40S ribosomal protein SA(P08865), 40S ribosomal protein S16(P62249), 40S ribosomal protein S3a(P61247). The 40S ribosomal protein S9(P46781) K_{hib} modification sits locus was predicted by STRING (Fig. 7), the 40S ribosomal protein S9(P46781) had 6 K_{hib} modification sits with K121,K139,K93,K52,K91and K180 .

The protein domain functional descriptions identified for K_{hib} were annotated by InterProScan (Fig. 8). Upregulated proteins with K_{hib} were significantly enriched for the Calponin homology domain, the Nucleotide-binding alpha-beta plait domain and FERM, and the N-terminal. The downregulated proteins with K_{hib} were enriched for Globin-like, Globin/Protoglobin, Globin domains and so on.

Discussion

Since the discovery of phosphorylation, many new PTMs have been identified over the years, such as crotonylation, propionylation, malonylation, butyrylation, succinylation, glutarylation, β-hydroxybutyrylation and 2-hydroxyisobutyrylation(Wu et al. 2018). PTMs have been found in different pathologic conditions in SLE(Lu et al. 2016; Zavala-Cerna et al. 2014). Lu's studies showed that FOXO3a was a downregulated molecule in SLE patients, while FOXO3 was upregulated by glucocorticoids (GCs), which relied on the suppression of pI3K/AKT-mediated FOXO3a phosphorylation. In one of their other studies, FOXO3a plays an important role in the GC-mediated inhibition of NF-B activity, which might involve FOXO3a interaction with NF-B p65 protein(Lu et al. 2016). The study reported that 2 – 1, an altered isoform, showed lower levels of phosphorylation in SLE patients, which may be related to the pathogenesis of SLE; it also demonstrated that higher concentrations of phosphoproteins are found in the cell membrane and nucleus and that 50 nucleic acid metabolic pathways are modified during the pathogenesis of SLE, specifically the MAPK signaling pathway, which leads to abnormal intracellular signaling(Routsias and Tzioufas 2010). Zieve(Zieve and Khusial 2003) studied the methylation of SmD1 protein (sDMA) in SLE patients. H2B and H4 of nucleosomes were shown to undergo apoptosis-induced acetylation in mice with lupus and patients with SLE(van Bavel et al. 2009). K_{hib} is one of the newly discovered PTM types that influences protein properties, including biological processes, such as cellular localization, protein interaction, structure stability, and enzymatic activity. Dai identified K_{hib} on histones in HeLa cells and mouse embryonic fibroblast cells, which were the first to be reported in the world, and the research shows that histone Kac and Kcr is involved during male germ cell differentiation in distinct genomic distributions.

Currently, there are no reports of K_{hib} PTMs in SLE. In our study, we identified 156 upregulated proteins(fold change > 1.5),124 downregulated proteins(fold change < 1/1.5), which included 220 K_{hib} sites that were upregulated and 187 K_{hib} sites that were downregulated. Hemoglobin subunit gamma-2 (P69892) is an upregulated protein with higher fold and multiple change modifications that has 3 K_{hib} sites and a subcellular localization in the cytoplasm. The 40S ribosomal protein S9(P46781) has 3 K_{hib} sites and subcellular localization in the cytoplasm. As both an upregulated and downregulated protein, the 40S ribosomal protein S9(P46781) regulates ribosomal function and the protein production mechanism in the hsa03010 ribosome pathway.

Ribosomes are remarkable ribonucleoprotein complexes that are responsible for protein synthesis in all forms of life(Noller et al. 2017). Our results showed that proteins with K_{hib} were enriched in the hsa03010 ribosome, and research on venous thromboembolism (VTE) showed that module genes were mainly enriched in the pathways of the hsa03010 ribosome(Zhou et al. 2015). It has been reported that the number or activity of ribosomes could be increased upon exposure to cytokines released at the site of inflammation(Yewdell et al. 1996). The immune response of the human body is related to cell necrosis. Cell apoptosis and necrosis can induce an immune response. Therefore, appropriate treatment of inflammation is conducive to the occurrence and development of autoimmune diseases. SLE is an autoimmune multisystem injury disease in which thrombosis is a likely symptom, and its mechanism in the endothelium is caused by the activation of a variety of immune inflammatory factors where there is cellular damage and vasculitis. Inflammation is an important factor in SLE pathogenesis. Vasculitis prevalence in patients with SLE is reported to be between 11% and 36%(Barile-Fabris et al. 2014). The presence of antibodies against vascular inflammation, which causes cell destruction, has been documented in SLE(Cieslik et al. 2008; Guilpain and Mouthon 2008). SLE patients with inflammatory cytokines IL-1, IL-6 IL-8 and IL-18 and a high concentration of TNF-α and its related disease activity, which causes blood coagulation in patients, except in the fibrinolytic system, form thrombosis that is enhanced by the immunity-induced inflammation(Sabry et al. 2006). Interferon (IFN)-α and type III IFNs (IFN-λ) have

also recently been associated with SLE. The levels of circulating IFN-λ1 and IFN-α define subsets of patients with SLE with different characteristics(Oke et al. 2017). The increased release of factors in the inflammatory network during the pathogenesis of SLE is an important factor involved in the pathogenesis of SLE immunological thrombosis, which can promote the occurrence and exacerbation of the clinical symptoms of SLE.

In conclusion, bioinformatics analysis based on a proteome analysis of K_{hib} in the peripheral blood of patients with SLE indicated that K_{hib}-modified proteins were distributed in various cellular compartments and were involved in a broad spectrum of processes. In addition, proteins with K_{hib} were enriched in the hsa03010 ribosome, and ribosomal proteins can regulate inflammatory cytokines to affect the pathogenesis of SLE. Our results provide novel insights into the functions of proteins with K_{hib} in SLE. However, the clinical applicability of K_{hib}-modified ribosomal proteins should be verified in the future.

Declarations

Ethics approval and consent to participate

It is verbal agreement. Guangxi Key Laboratory of Metabolic Diseases Research Ethics Committee

Consent for publication

All presentations of case reports must have consent for publication.

Availability of data and material

The datasets generated and/or analysed during the current study are available in the PRIDE repository. PXD015351, Username: reviewer73483@ebi.ac.uk, Password: 2qd6MgH1.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by Project Plan Document of Guangxi Key Laboratory Construction (17-259-57).The Science and Technology Plan of Shenzhen (No. JCYJ20170307095606266), The Shenzhen Science and Technology Research Foundation(JCYJ20160422154407256)

Acknowledgements

Not applicable

Endnotes

Full name :Yong Dai

Institutions: Nephrology Department of Guilin No.924 Hospital, Guangxi Key Laboratory of Metabolic Diseases Research, Guilin Key laboratory of Kidney Diseases Research; Clinical medical research center, The Second Clinical Medical College of Jinan University (Shenzhen People's Hospital)

Tel.: +86-0755-22942780

Fax: +86-0755-22942107

E-mail: daiyong22@aliyun.com

Full name: Donge Tang

Institutions: Clinical medical research center, The Second Clinical Medical College of Jinan University (Shenzhen People's Hospital)

Street Name & Number: No. 1017, North of Dongmen Road

Shenzhen City, Guangdong Province 518020, China

Tel.: +86-0755-22942106

Fax: +86-0755-22942107

E-mail: donge66@126.com

References

Barile-Fabris, L., M. F. Hernandez-Cabrera, and J. A. Barragan-Garfias（2014） Vasculitis in systemic lupus erythematosus. Curr Rheumatol Rep **16:** 440.

Cieslik, P., A. Hrycek, and P. Klucinski（2008） Vasculopathy and vasculitis in systemic lupus erythematosus. Pol Arch Med Wewn **118:** 57-63.

Dai, L., C. Peng, E. Montellier, Z. Lu and Y. Chen *et al.*（2014） Lysine 2-hydroxyisobutyrylation is a widely distributed active histone mark. Nat Chem Biol **10:** 365-370.

Dong, H., Z. Guo, W. Feng, T. Zhang and G. Zhai *et al.*（2018） Systematic Identification of Lysine 2-hydroxyisobutyrylated Proteins in Proteus mirabilis. Mol Cell Proteomics **17:** 482-494.

Guilpain, P., and L. Mouthon（2008） Antiendothelial cells autoantibodies in vasculitis-associated systemic diseases. Clin Rev Allergy Immunol **35:** 59-65.

He, H., T. Shuang, J. Ming, T. Zhanyun and S. Miho *et al.* p300-Mediated Lysine 2-Hydroxyisobutyrylation Regulates Glycolysis. Molecular Cell.

Huang, J., Z. Luo, W. Ying, Q. Cao and H. Huang *et al.*（2017） 2-Hydroxyisobutyrylation on histone H4K8 is regulated by glucose homeostasis in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A **114:** 8782-8787.

Lu, M., W. Xu, B. Gao and S. Xiong（2016） Blunting Autoantigen-induced FOXO3a Protein Phosphorylation and Degradation Is a Novel Pathway of Glucocorticoids for the Treatment of Systemic Lupus Erythematosus. J Biol Chem **291:** 19900-19912.

Marcelli, S., M. Corbo, F. Iannuzzi, L. Negri and F. Blandini *et al.*（2018） The Involvement of Post-Translational Modifications in Alzheimer's Disease. Curr Alzheimer Res **15:** 313-335.

Meng, X., S. Xing, L. M. Perez, X. Peng and Q. Zhao *et al.*（2017） Proteome-wide Analysis of Lysine 2-hydroxyisobutyrylation in Developing Rice (Oryza sativa) Seeds. Sci Rep **7:** 17486.

Noller, H. F., L. Lancaster, S. Mohan and J. Zhou（2017） Ribosome structural dynamics in translocation: yet another functional role for ribosomal RNA. Q Rev Biophys **50:** e12.

Oke, V., S. Brauner, A. Larsson, J. Gustafsson and A. Zickert *et al.*（2017） IFN-lambda1 with Th17 axis cytokines and IFN-alpha define different subsets in systemic lupus erythematosus (SLE). Arthritis Res Ther **19:** 139.

Perez-Riverol, Y., A. Csordas, J. Bai, M. Bernal-Llinares and S. Hewapathirana *et al.*（2019） The PRIDE database and related tools and resources in 2019: improving support for quantification data. Nucleic Acids Res **47:** D442-D450.

Regunathan-Shenk, R., and J. Radhakrishnan（2018） Pathogenesis of SLE Nephritis in the Era of Precision Medicine. Curr Rheumatol Rev **14:** 140-144.

Routsias, J. G., and A. G. Tzioufas（2010） B-cell epitopes of the intracellular autoantigens Ro/SSA and La/SSB: Tools to study the regulation of the autoimmune response. Journal of Autoimmunity **35:** 256-264.

Sabry, A., H. Sheashaa, A. El-Husseini, K. Mahmoud and K. F. Eldahshan *et al.*（2006） Proinflammatory cytokines (TNF-alpha and IL-6) in Egyptian patients with SLE: its correlation with disease activity. Cytokine **35:** 148-153.

van Bavel, C. C., J. Dieker, S. Muller, J. P. Briand and M. Monestier *et al.*（2009） Apoptosis-associated acetylation on histone H2B is an epitope for lupus autoantibodies. Mol Immunol **47:** 511-516.

Wu, Q., L. Ke, C. Wang, P. Fan and Z. Wu *et al.*（2018） Global Analysis of Lysine 2-Hydroxyisobutyrylome upon SAHA Treatment and Its Relationship with Acetylation and Crotonylation. J Proteome Res **17:** 3176-3183.

Yewdell, J. W., L. C. Antón, and J. R. Bennink（1996） Defective ribosomal products (DRiPs): a major source of antigenic peptides for MHC class I molecules? Journal of Immunology **157:** 1823-1826.

Yu, Z., J. Ni, W. Sheng, Z. Wang and Y. Wu（2017） Proteome-wide identification of lysine 2-hydroxyisobutyrylation reveals conserved and novel histone modifications in Physcomitrella patens. Sci Rep **7:** 15553.

Zavala-Cerna, M. G., E. A. Martinez-Garcia, O. Torres-Bugarin, B. Rubio-Jurado and C. Riebeling *et al.*（2014） The clinical significance of posttranslational modification of autoantigens. Clin Rev Allergy Immunol **47:** 73-90.

Zhou, T., Y. Zhang, P. Wu, Q. Sun and Y. Guo（2015）Screening Feature Genes of Venous Thromboembolism with DNA Microarray. Chem Biol Drug Des **86:** 821-828.

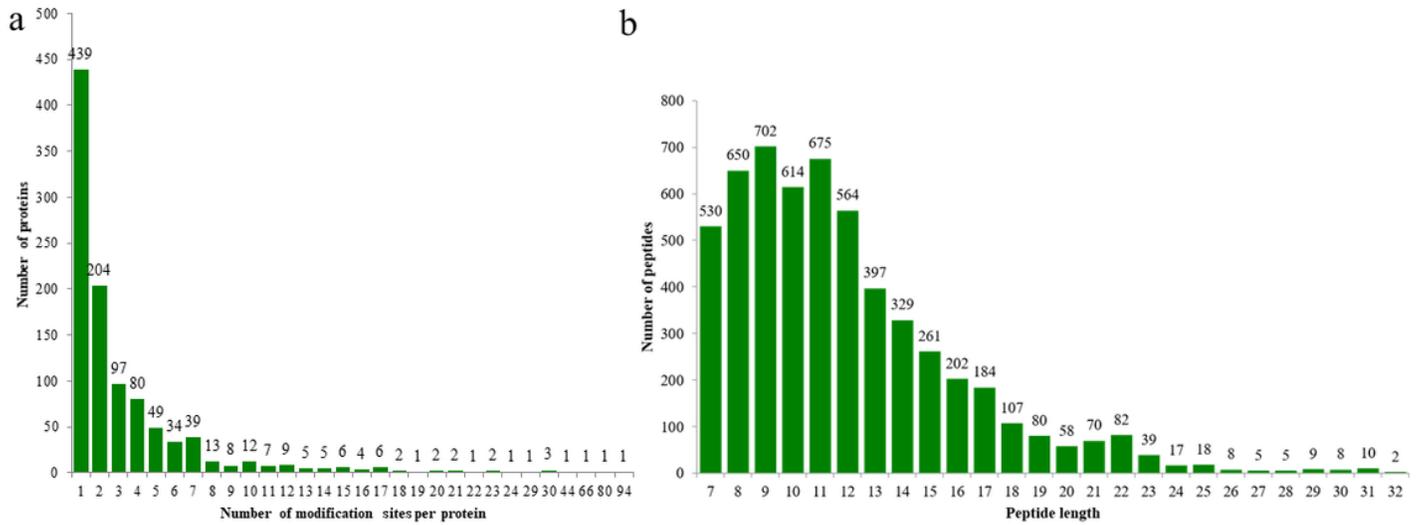
Zieve, G. W., and P. R. Khusial（2003）The anti-Sm immune response in autoimmunity and cell biology. Autoimmun Rev **2:** 235-240.

Figures

Figure 1

Comparatively analysis of whole proteome and lysine crotonylation between SLE and NC. (a) Distribution of lysine crotonylation in one protein. (b) Distribution of lysine crotonylation peptides based on their length.

Figure 2

Western blotting analysis of Khib modifed proteins and sites in SLE and NC. (a)Coomassie brilliant blue staining to investigate the existence of Khib modifed proteins in SLE and NC.(b) Western blotting with anti-H3K79hib,anti-H3K14hib antibody and anti-H3 antibody in SLE and NC.

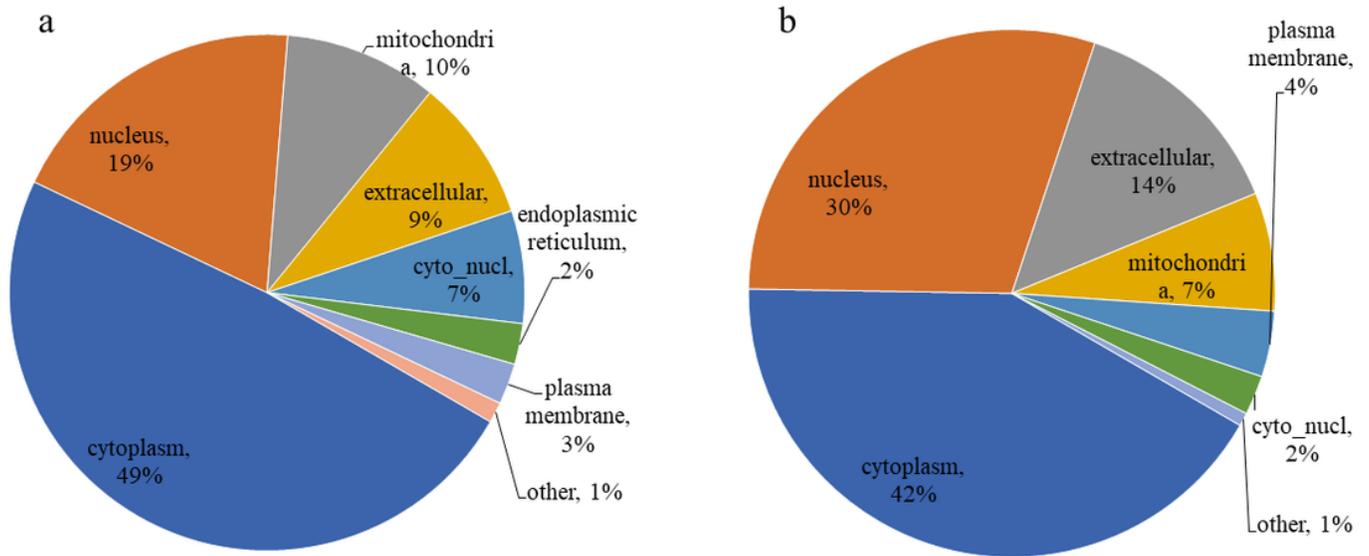
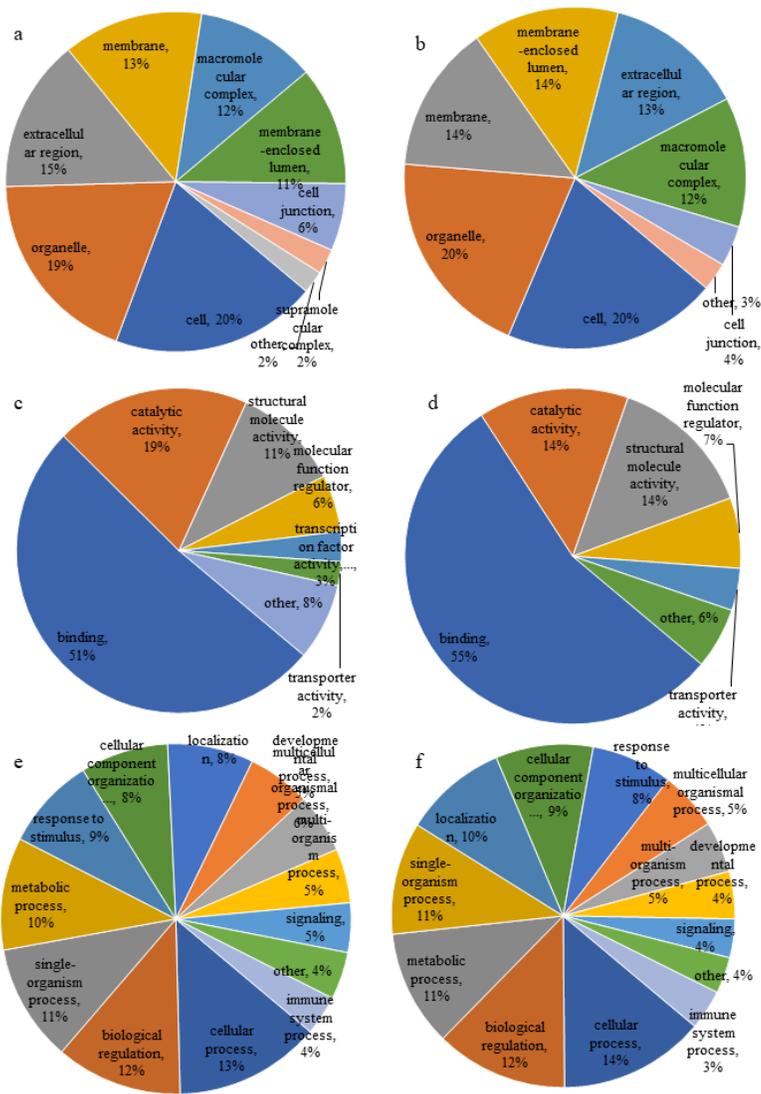



Figure 3

Functional classification of lysine 2-hydroxyisobutyrylation in subcellular. (a) Subcellular localization of upregulated Khib proteins (b) Subcellular localization of downpregulated Khib proteins.

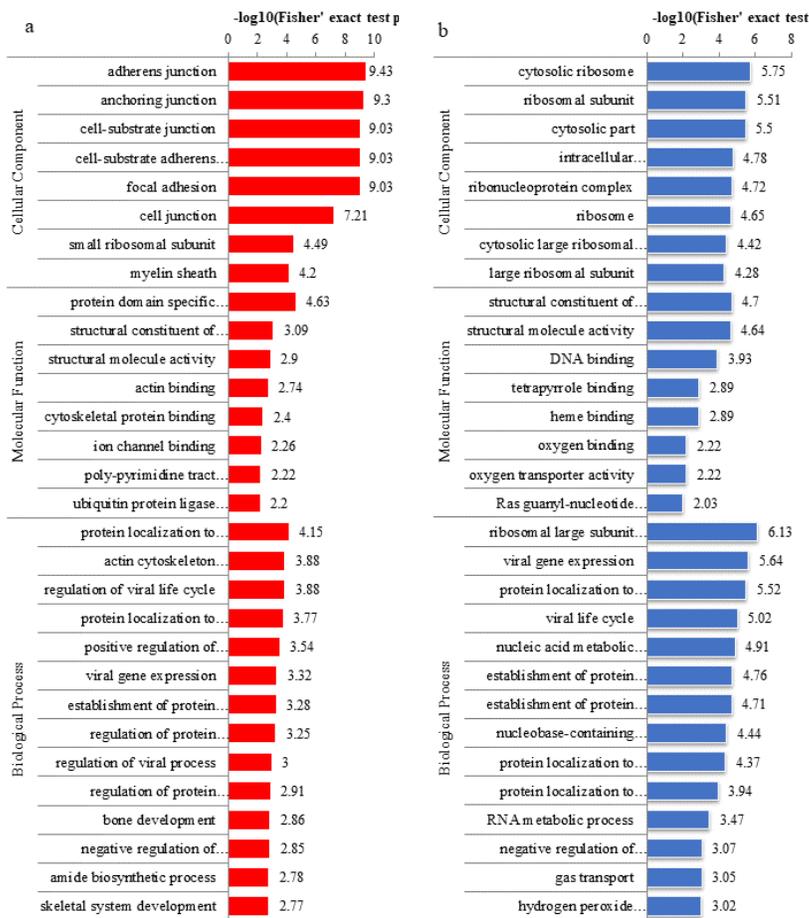



Figure 4

Functional classification of lysine 2-hydroxyisobutyrylation in GO. (a) GO analysis for upregulated Khib proteins in cellular component. (b) GO analysis for downregulated Khib proteins in cellular component. (c) GO analysis for upregulated Khib proteins in molecular function. (d) GO analysis for downregulated Khib proteins in molecular function. (e) GO analysis for upregulated Khib proteins in biological processes. (f) GO analysis for downregulated Khib proteins in biological processes.

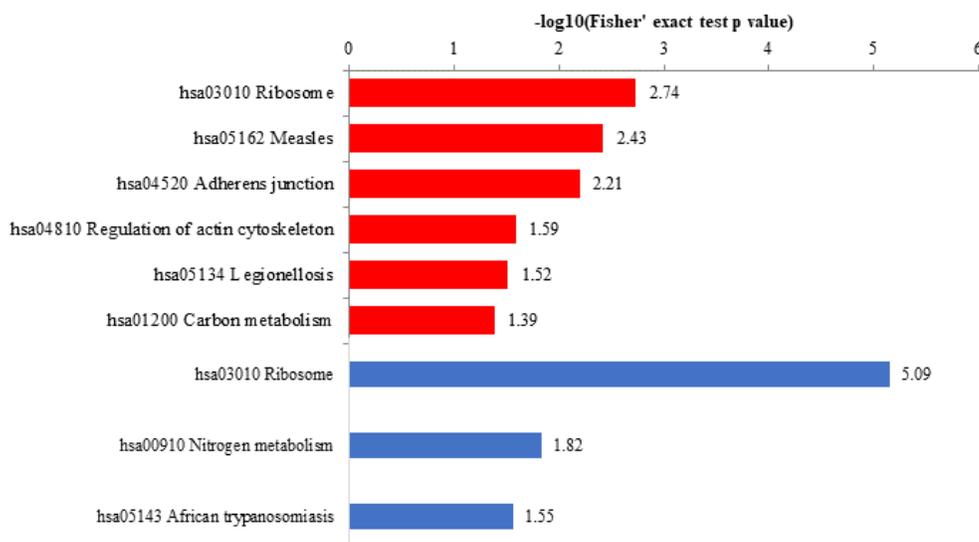



Figure 5

Functional enrichment analysis of lysine 2-hydroxyisobutyrylation in GO. And Upregulated Khib proteins (red bar), downregulated Khib proteins(blue).

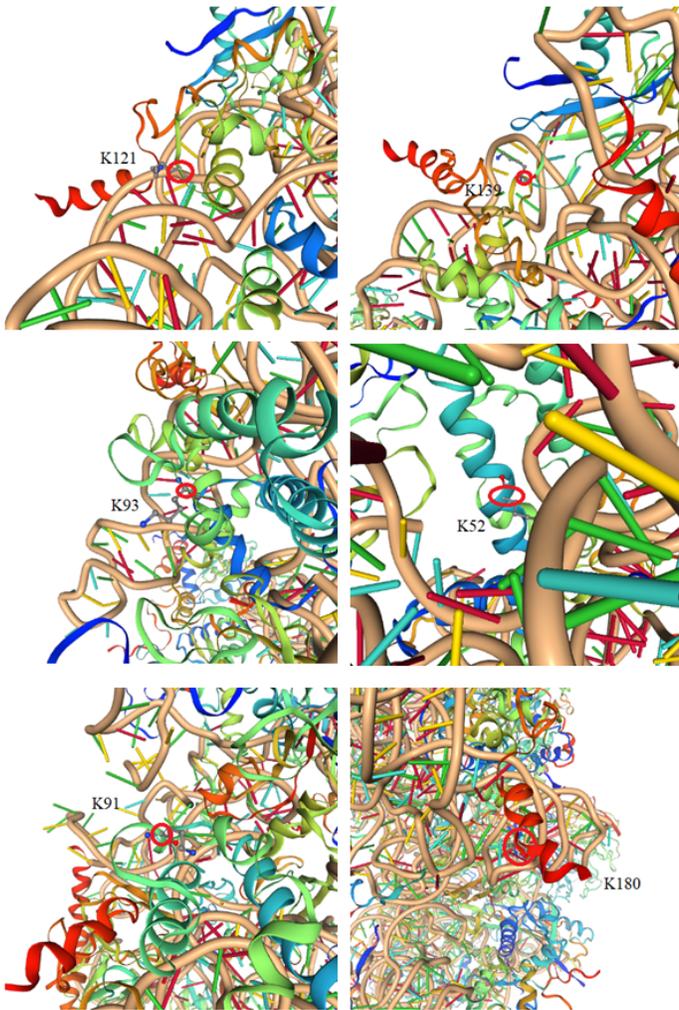



Figure 6

Functional enrichment analysis of lysine 2-hydroxyisobutyrylation in KEGG. And Upregulated Khib proteins (red bar), downregulated Khib proteins(blue).

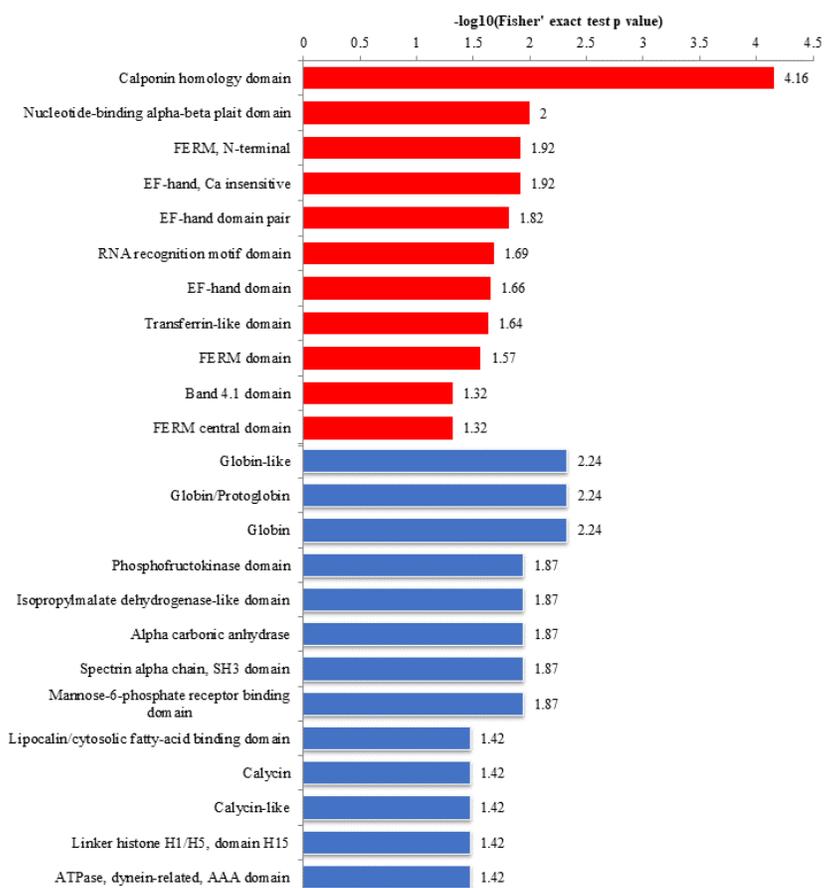



Figure 7

The 40S ribosomal protein S9(P46781) Khib modification sits (K121,K139,K93,K52,K91,K180).

Figure 8 not available with this version of the manuscript.

Figure 8

Functional enrichment analysis of lysine 2-hydroxyisobutyrylation in protein domain. And Upregulated Khib proteins (red bar), downregulated Khib proteins(blue).