

Comprehensive Analysis of Lysine Crotonylation Modification in Patients with Chronic Renal Failure

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Abstract

Background Post-translational modifications (PTMs) are at the heart of many cellular signaling events, which changes the function of protein. Crotonylation, one of the most important and common PTMs, plays a key role in the regulation of various biological processes. However, no study has evaluated the role of lysine crotonylation modification and chronic renal failure patients.

Methods: Here, we comparatively evaluated the crotonylation proteome of normal controls and chronic renal failure patients using liquid chromatography tandem mass spectrometry (LC-MS/MS) coupled with highly sensitive immune-affinity purification.

Results: A total of 1109 lysine modification sites were identified, of which 772 sites were up-regulated and 69 sites were down-regulated; this suggests that crotonylation modification maintains high levels in the patients' kidneys with chronic renal failure. Gene ontology enrichment analysis showed that the crotonylated proteins were significantly enriched in the platelet alpha granule lumen, platelet degradation, and cell adhesion molecule binding. In addition, KEGG-based gene and genomic functional enrichment analysis in Kyoto encyclopedia showed that battoacyl protein was enriched in CD36, which has an important relationship with renal failure.

Conclusion: This is the first report on the global crotonylation proteome of chronic renal failure patients. crotonylation of histone and non-histone may play an important role in delaying the continuous deterioration of renal function in patients with chronic renal failure.

1. Background

Chronic renal failure refers to the abnormal renal function structure or dysfunction for more than 3 months with or without the decrease of glomerular filtration rate, and its clinical manifestations vary from asymptomatic, laboratory abnormalities to uremia[1]. In recent years, the incidence of chronic renal failure, especially the incidence of terminal stage renal diseases, has increased significantly, which is a serious threat to human health. The prevalence rate of the global general population has reached 14.3% [1–2]. Renal interstitial fibrosis is a histological feature of chronic renal failure and an important predictor of renal function loss in patients [3]. It has been demonstrated that PTMs can form epigenetic layers that respond to environmental signals and external stimuli, thereby altering the expression of genes involved in chronic kidney disease [5]. Epigenetic changes, including the importance of PTM in fibrosis, inflammation and immunity related to various kidney diseases, and the development of kidney are becoming more important [6].

Post-translation modification (PTM) performs an essential part in the process of protein maturation, which changes the function of protein[7, 8]. The improvement of mass spectrometry (MS) technique increases the size of proteome research, and also contributes to the recognition of a rich list of PTMS [9]. Protein lysine acetylation is a ubiquitous and reversible PTM, the initial results were mainly through the catalytic regulation of gene transcription and expression in the nucleus by histone acetyltransferase and

histone deacetylase[10]. In chromatin biology, lysine acetylation was discovered in tubulin and mitochondrial proteins, which suggests that lysine acetylation acts an important part in cell biology [11]. Except for lysine acetylation, some new types of PTMs, such as malonylation and lysine succinylation, have been identified and interpret an important role in the regulation of a wide range of eukaryotic and prokaryotic physiological functions [12, 13].

Lysine crotonylation, a new protein PTM, was initially found in human cell lines and mouse sperm histone [19]. The discovery of Lysine crotonylation has attracted extensive attention in the scientific circle. Lysine crotonylation has been deeply studied in a short period of time. It is the first demonstration that Lysine crotonylation is a potent indicator of active promoters and may be a major signal for controlling male germ cell differentiation [19]. Wei believes that DNA replication may be affected by protein crotonylation, which may result in inhibition of DNA replication, thus affecting the cell cycle [25]. All studies suggest that lysine crotonylation can control the interpretation of genetic data at the chromatin level and acts a major role in gene expression and cell fate.

Histone acetylation has a good characteristic in this paradigm. So far, the study of histone acetylation has focused on tumors, neuropsychiatric disorders, lupus, cardiovascular disease, acute lymphoblastic leukemia, diabetic nephropathy, acute renal injury, chronic nephropathy [29–34, 1]. On this basis, the histone acetylation modification group is very similar to the Lysine crotonylation group in structure[19, 20]. In addition, histone acetylation shares the same enzyme system as histone crotonylation [20, 35]. Current studies have shown that HDAC inhibitors have protective effects on some experimental models of renal injury [36, 37]. Histone crotonylation has been observed in renal tissue at present, suggesting that histone crotonylation may play a role in epigenetic regulation of gene expression during renal injury [38]. The present study found that lysine-crotonylation in renal tissue increased during acute renal injury [39]. Therefore, we speculate that the Lysine crotonylation plays an important part in a wide scope of biological processes, and maybe closely related to the pathogenesis of the disease. However, there is little research on the relationship between disease and its pathogenesis remains unclear. Most of the studies are focused on the histone crotonylation and its function, but recent studies have shown that lysine crotonylation also occurs widely in non-histone proteins [40]. suggesting that the non-histone protein is also widely present in the human body. And play a related function, but this aspect of the research is still a blank. Therefore, we speculate that the Lysine crotonylation is closely related to the pathogenesis and changes of renal failure in non-histone proteins.

In this study, TMT labeling, crotonyl enrichment and high resolution liquid chromatography-mass spectrometry were used to study quantitative proteomics. A quantitative study on crotonyl proteomics was carried out in this project. In total, 1109 lysine crotonylation sites were identified out of 347 proteins. The identified crotonyl proteins are mainly located in cytoplasm, nucleus, mitochondria and extracellular. The identified crotonylated proteins were primarily localized in the cytoplasm, nucleus, mitochondria, and extracellular region. Bioinformatic analysis was performed to reveal the biological functions of crotonylated proteins; Further bioinformatics analysis showed that the biological function of crotonyl protein was significantly enriched on CD36 of platelets and blood cells. As far as we know, this study

describes lysine crotonylation for the first time in the global proteome of chronic renal failure, thus expanding the understanding of the role of crotonylation in the pathophysiological process of patients with chronic renal failure.

2. Materials And Methods

2.1 Sample collection

The peripheral blood samples of 6 patients with chronic renal failure and 6 healthy blood donors were tested. Six patients were diagnosed as chronic renal failure (CKD 5-stage GFR \leq 15ml/min) by Shenzhen People's Hospital. Healthy controls have no clinical signs of any diseases, cancer, allergies, immunocompromised diseases, diabetes, or other infectious diseases included. All patients and healthy blood donors in the studies were over 18 years old (Table 1), they had written informed consent, and this study was approved by the Ethics Committee of Clinical Research of Shenzhen People's Hospital. Peripheral venous blood was collected from patients with CKD and normal subjects, and peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation using lymphocyte separation reagent. PBMC was dissolved with TRIzol reagent and stored at -80°C .

Table 1
Clinical characteristics of patients and normal controls.

Group	number	sex		age
		man	woman	
CRF	6	4	2	39.78 \pm 11.07
NC	6	4	2	42.22 \pm 12.41

2.2 Protein extraction

After sampling from the hydrolytic buffer, the hydrolytic buffer is cracked with high intensity ultrasound. After centrifugation at 12,000 \times g and 4°C for 10 min, the supernatant removing the cell debris was transferred to the new centrifuge tube and the protein concentration was measured with a dichondroic acid kit.

2.3 Trypsin digestion

Under the condition of 56°C , the solution was put into 5mm dithiothreitol for 30min. After reducing the protein solution, 11mm iodoacetamide was added and incubated in dark for 15min. The urea concentration of the sample was then diluted and the solution was added to the trypsin 1:50 trypsin-protein mass ratio (1:50) for digestion for the first time. Finally, the solution was added to 1:100 trypsin-protein for digestion for 4h.

2.4 TMT tag

The Strata X C18 SPE of trypsin digested peptide was desalted and dried under vacuum and reassembled in 0.5M Teab and processed in accordance with the instructions of the TMT kit /iTRAQ kit (AB Sciex, Foster City, CA).After thawing 1 unit of TMT/iTRAQ reagent, the recombination was performed in acetonitrile.Finally, the peptide was incubated at room temperature for 2h, concentrated, desalted, dried and labeled by vacuum centrifugation.

2.5 Affinity enrichment

The peptides were dissolved in IP buffer solution, the supernatant was transferred to the pasteurized resin, and shaken in the warm bath overnight.After several times of washing, the peptides bound with the resin were eluted, the eluent was collected and vacuum-frozen, and finally provided for liquid-mass analysis.

2.6 LC-MS/MS analysis

The peptides were dissolved in liquid chromatography (HPLC) mobile phase A for separation.Then ionization was performed, and then Q ExactiveTM Plus mass spectrometry analysis was performed. After the first scan, secondary mass spectrometry analysis was performed.

2.7 Database search

MaxQuant search engine is used to process MS/MS data obtained (V.1.5.2.8).The tandem mass spectrometry was searched to a database connected to the reverse decoy database.Trypsin /P is designated as a cutting enzyme, allowing up to four missing cuts.The first time, the tolerance of precursor ions was 20 PPM, the main search was 5 PPM, and the mass tolerance of fragment ions was 0.02Da.The carbonyl methylation on Cys was fixed and the oxidation on Met was variable.Adjust the error discovery rate (FDR) to & LT;1%, the minimum score of modified peptide was set as & GT;40 points.

2.8 Gene ontology (GO) annotation

GO analysis is the ability to combine all kinds of information about the process of genes and gene products organically, and it is a bioinformatics method to analyze statistical information.

2.9 Protein domain annotation

Protein domain is a special domain with conserved sequences and can function independently.Protein domains usually consist of 25 to 500 amino acids.

2.10 KEGG channel annotation

The protein pathways are annotated using the KEGG pathway database, and the submitted proteins are then matched to the corresponding pathways in the database using the KEGG mapper.

2.11 Subcellular localization

Proteins in eukaryotic cells are located in detail in various parts of the cell, depending on the membrane structure to which they bind.

2.12 Motif analysis

The software Motif- X was used to analyze the Motif characteristics of modification sites. When the number of peptide segments greater than 20 and the P value obtained in the statistical test is less than 0.000001, the characteristic sequence is considered to be a motif of modified peptide segments.

3. Results

3.1 Comparative analysis on complete protein and lysine crotonylation in patients with chronic renal failure and healthy controls

The whole process consists of nine steps (Fig. 1A). A total of 1209 lysine acylation sites were identified, of which 1109 of 347 proteins were quantifiable (Table S1). As fold changes over 1.2 as an up regulation and below 1/1.2 as a down regulation. There was a differential expression of 260 protein loci in chronic renal failure and healthy controls, of which 772 were from up-regulated 260 proteins and 51 from 69 down-regulated proteins. Another result showed that significantly differential expression lysine crotonylation between the CRF and NC (Table S2). In order to verify the validity of mass spectrum data, the mass errors of all identified peptides were evaluated. The quality error is centered at 0 and below 10 ppm, which shows that the quality accuracy of ms data meets the requirement (Fig. 1B). Among 347 crotonyl proteins, most proteins contain one or two crotonylation sites, while fewer proteins have 7 or more crotonylation sites (Fig. 1C). The length of most peptides varied from 8 to 20 amino acids, which was in accordance with the law of trypsin digestion peptide (Fig. 1D) for one day.

3.2 Analysis on the motifs of the pasteurization sites

In order to understand the sequence commonness around crotonyl site and compare it with acetylated site, the sequence motifs of all identified crotonyl peptides were studied by Motif-X program. In total, 7 conserved motifs (KK, KD, AK, EK, K.D, Ke, K.K) were retrieved (Table S3, Fig. 2A). Particularly the motifs of Ke and KD are very conservative. It is important that the amino acids that are significantly conserved among these motifs, E and D, are negatively charged and are rarely found in other PTMS. These motifs may represent a characteristic of crotonylation in chronic renal failure. Hierarchical clustering analysis is also worked out to further analyze these topics (Fig. 2B). At - 10 to - 5 and 10 to 5, the positively charged K residue is enriched, while the negatively charged residues D and E are significantly enriched at the - 1 to

4 position. A residues of short aliphatic group often appeared at the - 7 ~ 8 position, but sulfur-containing C residues were not found.

3.3 Functional classification of Kcr in GO and Kcr subcellular localization

Functional classification of GO and subcellular lysine crotonylation the subcellular localization characteristics of lysine crotonylation have been identified (Fig. 3A). It turned out that the up-regulated proteins were mainly distributed in cytoplasm (56%), nucleus (10%), mitochondria (10%) and extracellular (10%), while down-regulated proteins were mainly distributed in cytoplasm (57%), nucleus (7%), mitochondria (4%) and extracellular (17%) (Fig. 3B). It showed that there was no significant difference in protein localization between up-regulated and down-regulated proteins. In order to understand the general situation of crotonyl protein in chronic renal failure, on the basis of its biological process, molecular function and cell composition, the GO functional classification of all crotonyl proteins was studied (Table S4). In the category of biological process, most crotonyl proteins are associated with cellular processes, single organism processes, biological regulation and stimulation (Fig. 3C), and most down-regulated proteins are associated with cellular processes, single organism processes, biological regulation and stimulation (Fig. 3D). In cell components, most crotonyl proteins are associated with cells, organelles, extracellular domains, and the membrane of up-regulated proteins (Fig. 3E), while most of the down-regulated proteins are associated with cells, organelles, extracellular domains and membranes (Fig. 3F). In the molecular functional category, most crotonyl proteins are associated with up-regulated protein binding, catalytic activity, structural molecular activity and molecular function modulators (Fig. 3G), while most down-regulated proteins are concerned with binding, catalytic activity, molecular function modulators and structural molecular activity (Fig. 3H). There was no significant difference in GO functional classification between up-regulated and down-regulated proteins, suggesting that crotonylation of lysine might have a large scale of biological functions.

3.4 Functional enrichment of Kcr in GO, KEGG, and protein domain

The functional enrichment of GO, KEGG and protein domain lysine crotonylation based on GO was studied (Table S5). Highly expressed Croton protein is highly enriched on platelets and erythrocytes CD36 (Fig. 5A). However, there is no up-regulated crotonyl protein in GO, but down-regulation of crotonyl protein mainly includes (Fig. 4A). At the same time, the function enrichment analysis based on KEGG was carried out (Table S6). It was found, however, that there was no up-regulated Croton acylation protein in KEGG (Fig. 4B). In addition, the down-regulated crotonylation domain includes the S100/CaBP9K calcium binding subdomain, globin, globin-like, Globin/Protoglobin. The EF-hand domain pair and EF-hand

domain (Table S7, Fig. 4C) suggest an important part for crotonylation during these processes. Similarly, there was no up-regulated crotonylation of the protein domain in this study. Pentose phosphate pathway has an important relationship with complications of CRF. Search for crotonyl proteins involved in carbon metabolism, including dense protein interaction networks (Fig. 5B).

3.5 Cluster analyses in GO, KEGG, and protein domain

For the sake of understanding the function of lysine crotonylation in more detail, we performed GO, KEGG, and protein domain enrichment-based clustering analyses. All quantized crotonylation sites were separated into four quartiles according to the multiple changes of the Lysine crotonylation sites: Q1 ($0 < - < 0.77$), Q2 ($0.77 < 0.77$), Q3 ($1.2 < - < 1.3$), and Q4 (ratio > 1.3), P value < 0.05 . Q1, Q2, Q3 and Q4 have 54, 15, 98 and 647 crotonylation, respectively. Then, the four kinds of quantifiable proteins were analyzed by cluster analysis. Q1 and Q2 are considered down-regulated, while Q3 and Q4 are considered up-regulated (Fig. 5C).

For GO analysis, it was found that the crotonylated protein in Q1 was mainly enriched in cell secretion, and the crotonylated protein in Q2 was mainly enriched in cell substrates, neurons and cytoskeleton, the crotonylated protein in Q3 was mainly enriched in cell junctions, while the crotonylated protein in Q4 was mainly abundant in cell microcrystals composed of cell membranes and cells (Fig. 6A). In addition, the biological enrichment process of crotonylation was also carried out. It was found that the Croton acylated protein in Q1 was mainly concentrated in the cell response to pathophysiology, and that the crotonylated protein in Q2 was located in cell function, such as migration and development. However, the crotonyl protein in Q3 is mainly enriched in cell metabolism. In addition, the crotonyl protein in Q4 is mainly concentrated in the cell response to the outside world (Fig. 6B). For the molecular functional crotonyl proteins, they were found to be highly enriched in the activity of Q1 cells and highly increased in the cell-binding processes of Q3 and Q4 (Fig. 6C).

The enrichment of KEGG suggests that certain pathways of Q3 and Q2 are associated with diseases, for instance, pancreatic cancer, type II diabetes, cellular metabolism, and salmonella infection, while Q1 crotonylated acylated protein is abundant in cell signaling pathways and measles and influenza viruses, and Q4 crotonylated acylated protein is enriched in cell signaling pathways and germ cell division (Fig. 6D).

At the same time, the protein domain of Croton protein was studied. They were found to be highly enriched in Q2: Calponin homology domain, aldolase-Tim type barrel, globin/proton protein, globin, globin-like (Fig. 6E).

3.6 Protein-protein interaction network of the acylation of Croton acylation proteins

The protein-interaction network of acylation of Croton acylation protein was established by further identifying the cellular processes regulated by the acylation of Croton acylation protein (Fig. 7). A total of

888 pairs of protein-protein mapping to the protein interaction database, showing the different cellular function of Croton protein in CRF. The physiological interaction between these protein complexes may contribute to their synergy and coordination in CRF.

4. Discussion

In this study, a comparative study was made between the normal control group and the crotonyl proteome of chronic renal failure, and the Croton acylated peptide was identified by antibody affinity enrichment method. High resolution mass spectrometry was performed. The method identified 1209 crotonyl sites on 377 proteins, of which 1109 sites of 347 proteins contained quantitative information. The modification level of 772 loci was up-regulated and the modification level of 69 loci was down-regulated in CRF/ healthy control group. The roles of these differential proteins in cellular and molecular functions and the related signaling pathways were discussed by GO analysis and KEGG analysis, which laid a foundation for the study of the distribution of apparent lysine-crotonylation modification in higher biological eukaryotes.

As we've seen that our study was the first attempt to describe crotonylation in patients with chronic renal failure. In the past, there has been evidence of the role of PTM mutations in chronic renal failure. Fibrosis, cell cycle and abnormal expression of inflammatory genes are the key events in the progression of chronic renal failure and are related to the changes of PTM [41]. Non-histone crotonyl proteins exist widely in the human body and play a related role [43]. According to the above data, crotonylation levels of histone and non-histone increased among patients with chronic renal failure. A current study has found that renal Lysine crotonylation increases during acute renal injury. Lysine crotonylation may act a role in the repair of acute renal injury. Because Lysine crotonylation in renal tubular cells and kidneys in vitro increases the expression of PGC-1 α in cultured renal tubular cells and normal kidneys [44], and down-regulates genes involved in tissue damage, such as encoding MCP-1 Chemokines of CCL2. PGC-1 α is a factor that primarily regulates mitochondrial biogenesis and metabolism, and it is related to the extent of renal damage, and it is necessary for patients recovering from kidney-related diseases [45]. Crotonic acid has been shown to promote the development of crotonylation modification in cell experiments. Parenteral injection of crotonic acid increases the level of crotonylation in renal tissue and reduces markers of inflammation, mitochondrial stress, renal dysfunction and renal damage through excessive doses of experimental aldosterone protection [46]. These results suggest that Lysine crotonylation is beneficial to the recovery of acute renal injury. Therefore, the level of Lysine crotonylation increased during acute renal injury. Acute renal injury is the main reason of chronic renal failure. It has been found that the effect of histone crotonylation may be related to the background: histone crotonylation can promote the expression of genes in the range of increasing substrate availability, but under the conditions of promoting inflammation or cell stress, the effect of histone crotonylation may be related to the background [47-48]. The expression of some genes may be reduced by the histone crotonylation. In another study, data showed that crotonylation-modified proteins were reduced in hemodialysis patients, because crotonylation may contribute to the recovery of acute kidney damage. But patients who maintain hemodialysis are unlikely to restore renal function [49], combined with the role of crotonic modification

currently being studied in kidney cells. We can speculate that crotonylation can also alleviate the progression of the disease in patients with chronic kidney disease and may restore some of its functions, while in patients with chronic kidney disease histone and non-histone proteins are higher than those in healthy controls. It can be speculated that in some ways, the body can increase the modification of histone and non-histone protein to delay renal fibrosis, further restore renal function and delay the progress of chronic kidney disease.

The functional enrichment of lysine crotonylation in GO showed that crotonyl protein was related to many biological processes, including cellular structural components, cellular molecular binding and pathophysiological processes involved in these biological processes, suggesting that the various interactions that related to these biological processes may be regulated by protein modification. The functional enrichment of crotonylation of lysine in KEGG was studied. However, only the B-class scavenger receptor CD36, a KEGG pathway, is closely related to renal failure, it showed that crotonylated protein were enriched in CD36. CD36 can regulate inflammation in many ways [50]; the combination of TSP1 and CD36 also stimulates macrophage secretion of the inflammatory factor TNF- α . It also stimulates macrophage-like transformation of smooth muscle cells and exacerbates the inflammatory response process [51]. Current studies have shown that in patients with diabetes, hyperglycemia can induce increased expression of CD36, aggravate platelet-mediated inflammation [53], and cause apoptosis of renal tubular epithelial cells, accelerate renal tubular degeneration and renal interstitial fibrosis [54]. Under the condition of promoting inflammation or cell stress, histone Croton acylation may decrease the expression of some genes, which is mainly enriched in CD36. We can speculate that histone crotonylation may affect the expression of CD36-related genes.

At present, the etiology of chronic renal failure is complicated, the clinical manifestations are diversified, the treatment is difficult and the prognosis is of a sort. But the current treatment is very limited. Few drugs can put off the progression of chronic renal failure. By further studying the lysine crotonylation reaction in the global proteome of chronic renal failure, the function of crotonylation in the progression of chronic renal failure can be further understood. The present therapeutic drugs for post-translational modification of histone, even if they may affect the expression of multiple genes with different or even opposite functions, can also have certain positive effects under various pathological conditions, including renal injury. [59]. if the mechanism of lysine crotonylation modification in chronic renal disease was studied, By changing the crotonylation modification to achieve the purpose of preventing and treating diseases, it provides a new idea for the therapy of chronic renal failure.

Declarations

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Availability of data and materials

The data of the current study are available from the corresponding author on reasonable request.

Authors' contributions

Jiahuang,Huang carried out data collection, and measurement, statistical analysis, interpretation of results and wrote the manuscript. Donge,Tang participated in collecting data and reviewed medical records.Fengping,Zheng and Huixuan,Xu participated in the study design and coordination as well as managing the research project. Yong,Dai provided critical appraisal of the manuscript.All authors read and approved the final version prior to submission.

Ethics approval and consent to participate

The clinical trial was found to be in accordance to the ethical principles and the national norms and standards for conducting medical research in China. All patients and healthy blood donors in the studies were over 18 years old. This research was approved by the Ethics Committee of Shenzhen People's Hospital No:LL-KY-2015106,Approval Date: 2018-05-15. The purpose of the research was explained for participants in the study and written informed consent was obtained from them.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

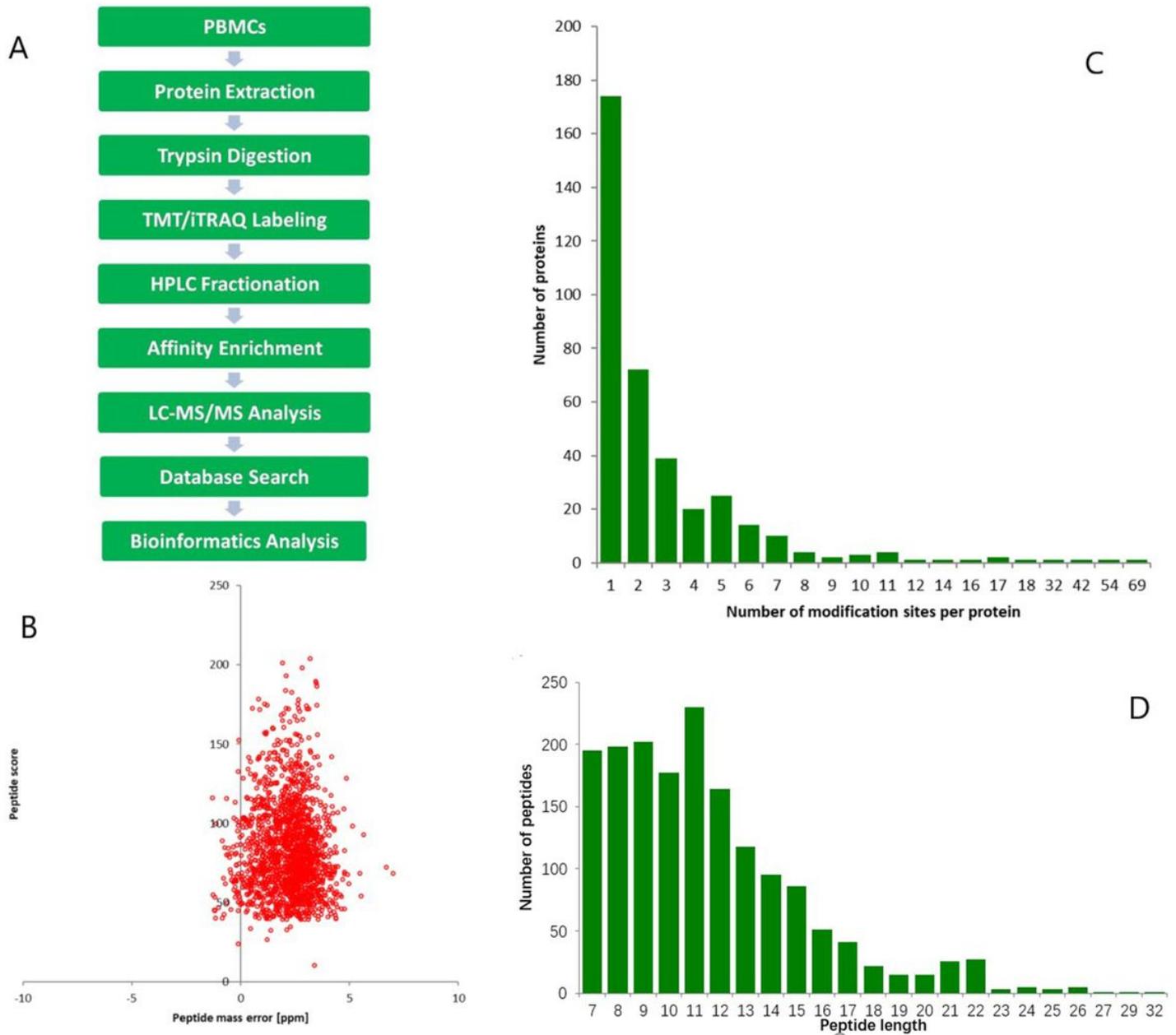


Figure 1

Comparatively analysis of whole proteome and lysine crotonylation between CKD and NC.(A) Overview of experimental procedures used in the presentstudy.(B) Mass error distribution of all crotonylated peptides. (C) Distribution of lysine crotonylation in one protein.(D) Distribution of lysine crotonylation peptides based on their length.

A	Motif Logo	Motif	Motif Score	Foreground		Background		Fold Increase
				Matches	Size	Matches	Size	
	KK.....	16	179	1169	47867	602450	1.93
	KD.....	16	141	990	32428	554583	2.44
	AK.....	15.48	113	849	30225	522155	2.3
	EK.....	9.32	132	736	51113	491930	1.73
	K.D.....	9.58	63	604	19163	440817	2.4
	KE.....	7.82	97	541	42246	421654	1.79
	K.....K..	6.97	63	444	26675	379408	2.02

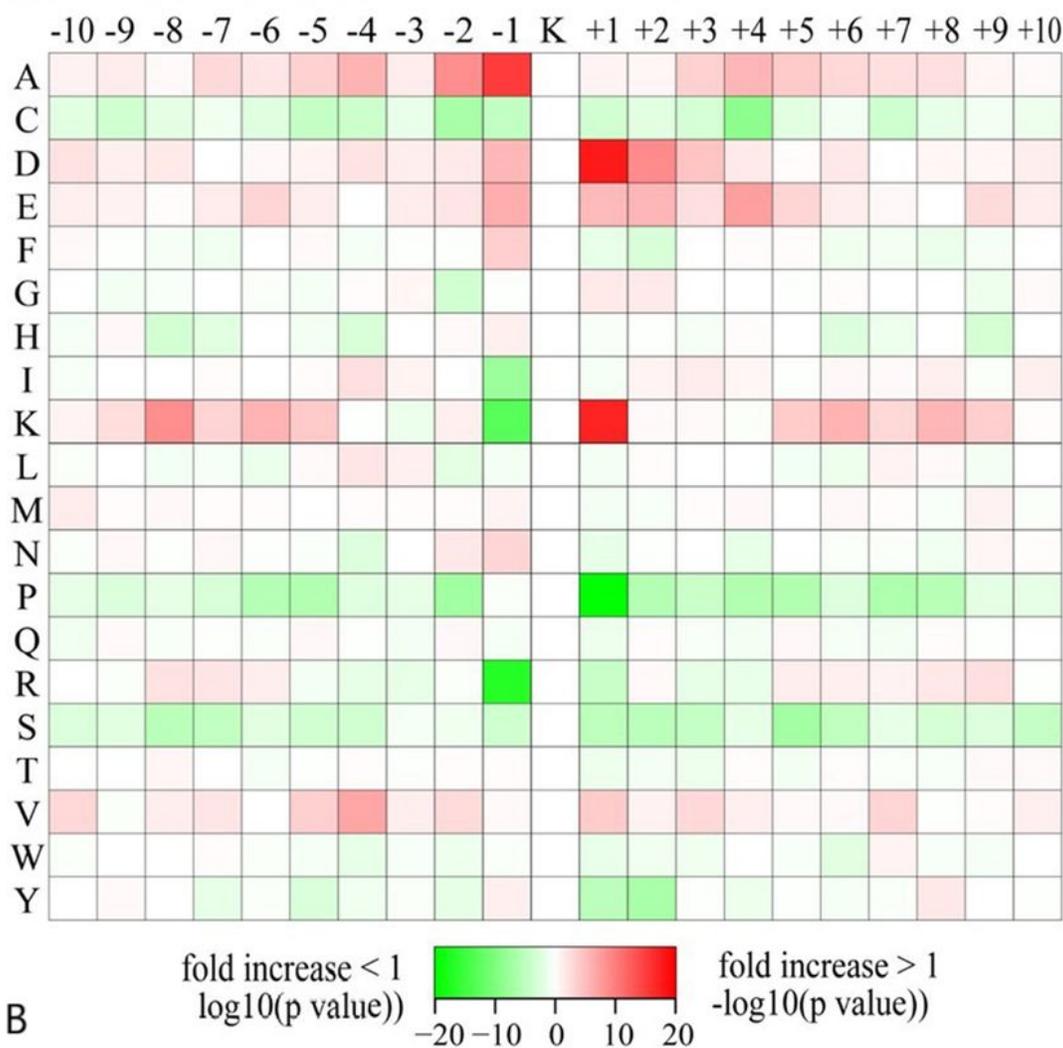


Figure 2

Analysis of crotonylation site motif. (A) Sequence probability logos of significantly enriched crotonylation site motifs around the lysine crotonylation sites. (B) Heat map of the amino acid compositions around the down-regulated lysine crotonylation sites showing the frequency of different types of amino acids around this residue. Red indicates enrichment and green indicates depletion. Table S3. The seven motifs retrieved from lysines crotonylated peptides.

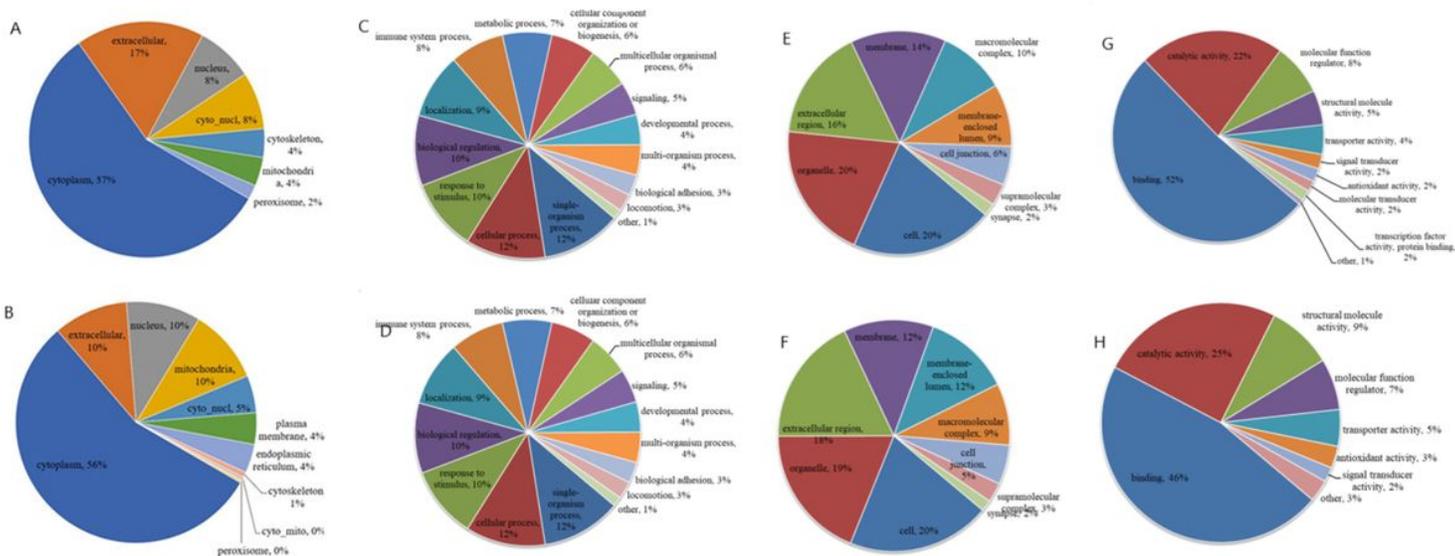


Figure 3

Functional classification of lysine crotonylation in GO and subcellular. (A) and (B) Subcellular localization of upregulated and downregulated crotonylated proteins. (C) and (D) GO-analysis for upregulated and downregulated crotonylated proteins in biological processes. (E) and (F) GO-analysis for upregulated and downregulated crotonylated proteins in cellular component. (G) and (H) GO-analysis for upregulated and downregulated crotonylated proteins in molecular function.

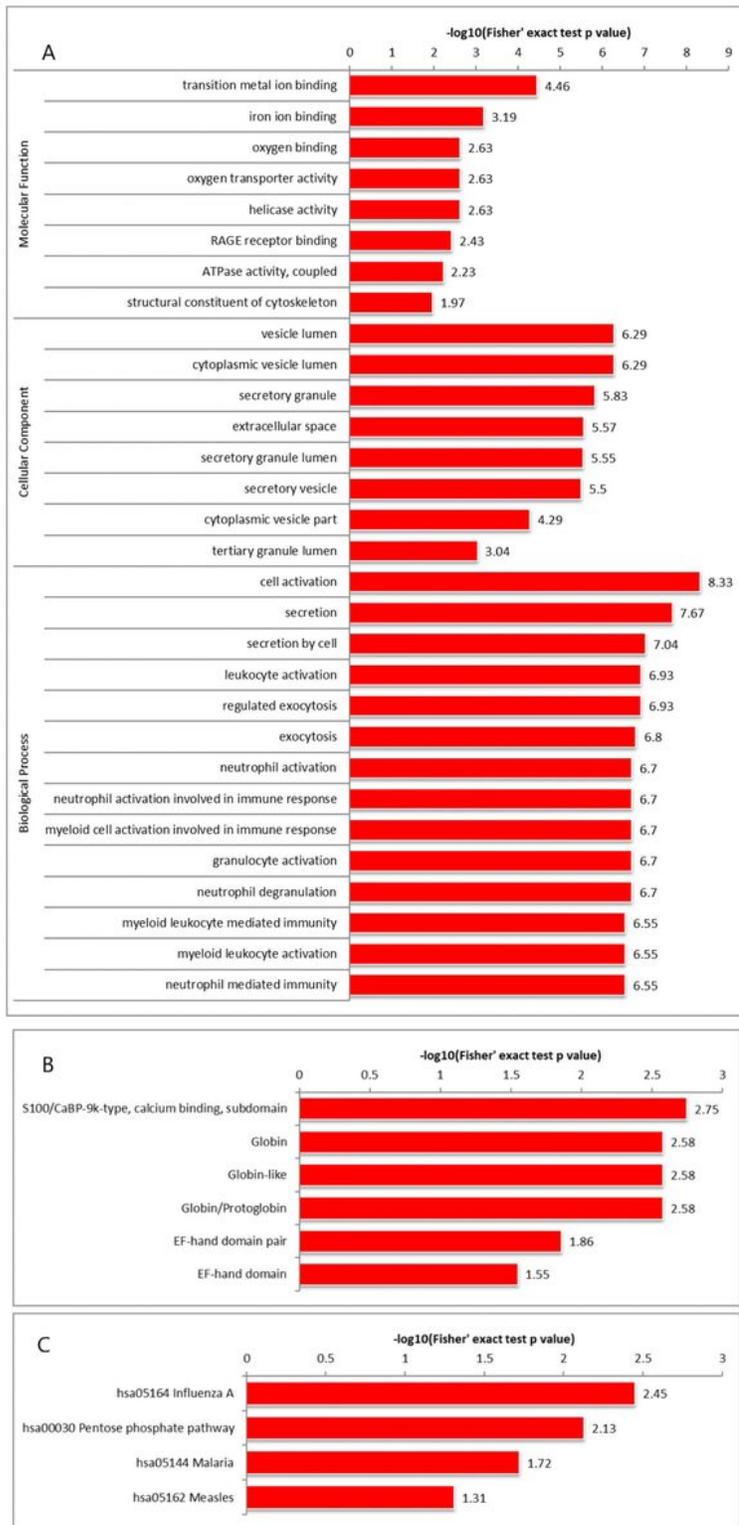


Figure 4

Functional enrichment of lysine crotonylation in GO, KEGG, and protein domain. (A) GO-based enrichment analysis of upregulated crotonylated proteins in terms of cellular component, molecular function, and biological process. (B) KEGG-based enrichment analysis of upregulated crotonylated proteins. (C) Protein domain-based enrichment analysis of upregulated crotonylated proteins.

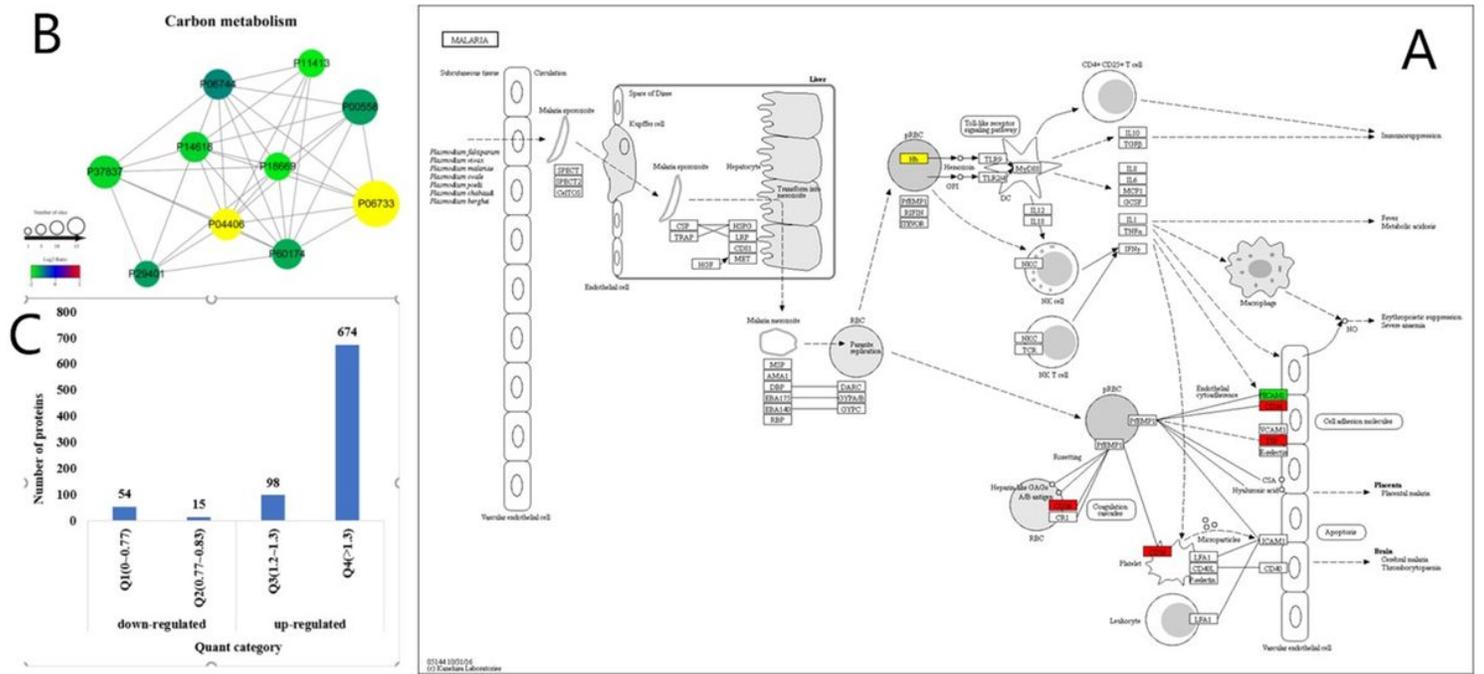


Figure 5

(A) Protein-protein interaction Network of carbon Metabolism Pathway .(B) Visual display of significant enrichment of proteins corresponding to differential modification sites in a KEGG pathway (CRF/NC). The modification level upregulation protein is indicated by red; the modification level down-regulation protein is expressed by bright green; and there are several proteins in the node expressed by yellow, which contain the up-regulated and down-regulated proteins at the modification level. (C) quantified crotonylated proteins were divided into four quantiles.

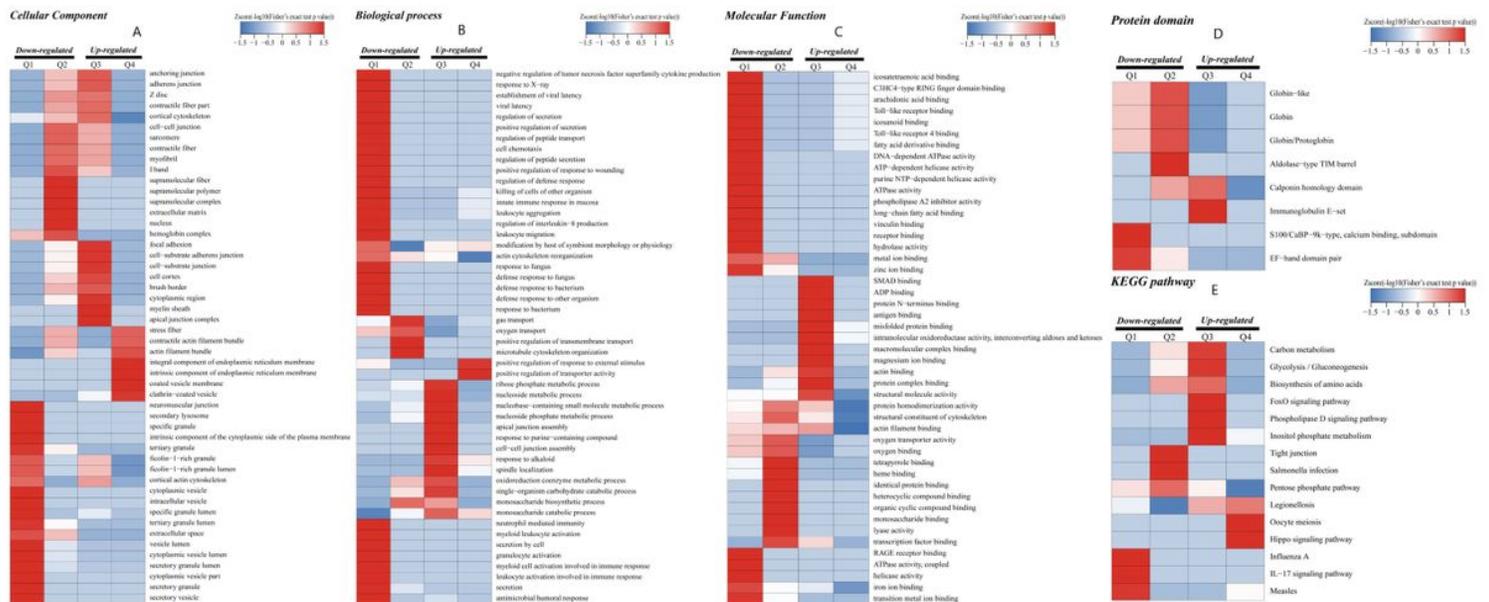


Figure 6

Clustering analysis in GO, KEGG and protein domain.(A)Cellularcomponent analysis.
(B)Biologicalprocessanalysis.(C) Molecular function analysis.(D)KEGGpathwayanalysis.(E) protein domain.

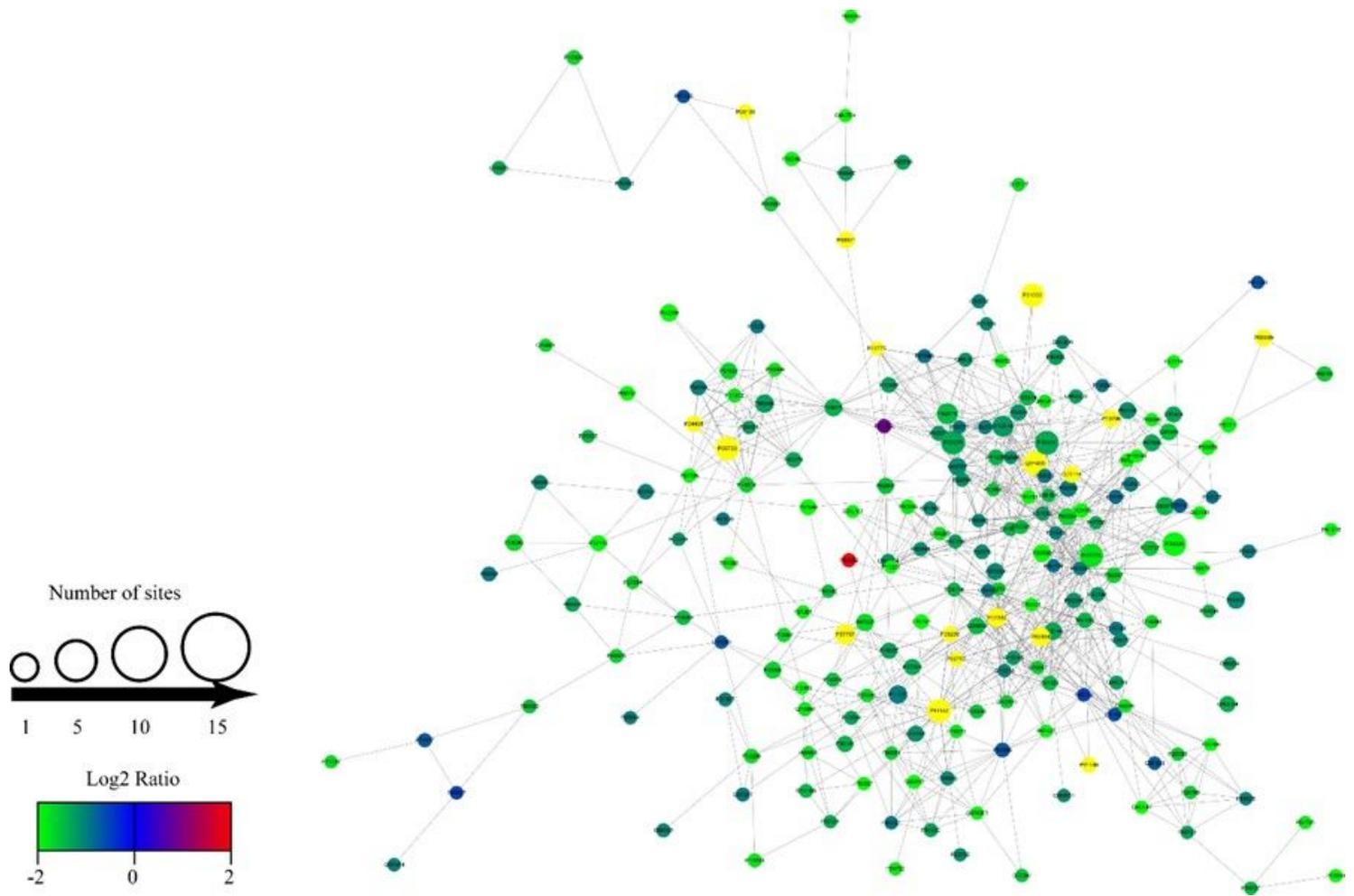


Figure 7

protein-protein interaction network based on KCR protein. KCR = acylation of Croton acylation.

Supplementary Files

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- [TableS6.xlsx](#)
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