

Quantitatively profiling acetylome of DNA repair proteins in early DNA damage

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Research

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Abstract

Background Lysine acetylation is a reversible regulated post-translational modification that can regulate the stability, localization, and function of proteins in multiple cellular processes. However, the regulative mechanism of acetylation on the repair proteins in the early DNA damage is not fully understood.

Methods We performed a global proteome and acetylome of DNA repair proteins in DNA damage in 1 h after treated with epirubicin by using high affinity enrichment and high-resolution liquid chromatography–tandem mass spectrometry approaches.

Results: 190 Kac sites in 50 repair proteins were identified in cells treated with epirubicin as compared to the control. 42 acetylated lysine sites and 24 deacetylated lysine sites were observed in 21 and 16 repair proteins, respectively. 7 repair proteins simultaneously contained both acetylated and deacetylated lysine sites. 11 acetylation sites were located in the function domains of 7 repair proteins that might reveal mechanisms by which acetylations alter DDR protein function. In 17 repair proteins, the induced acetylation changes were for the first time identified in the present study.

Conclusion: The proteome and acetylome results indicated that fast acetylation or deacetylation on these repair proteins might play a critical role in the early DNA damage repair process.

Background

A highly versatile DNA repair system in eukaryotes is dedicated to resolving types of DNA lesions, which ensures the genomic integrity and accuracy (1, 2). Accumulating evidence has revealed that the alterations of PTM on repair proteins frequently affected the repair efficiency, which potential to trigger the carcinogenesis, affect cancer proliferation and metastasis, and connect with therapeutic resistance (3–5). Various DNA repair inhibitors have been confirmed to increase the therapeutic response rate to anti-cancer agent in types of cancers (6–8).

Acetylation on lysine controlled by KATs and KDACs is a dynamic and reversible process of PTM that provides a sophisticated mechanism in affecting the protein translation, conformation, stability, subcellular localization, and substrate interaction. Previous quantities of studies have demonstrated the crucial role of the acetylation of histone proteins in DNA repair processes and tumorigenesis. Advances in proteomic technology, such as liquid chromatography coupled with LC-MS/MS, have facilitated high throughput characterization of endogenously Kac sites in histones as well as non-histone proteins (9). Accumulated evidence has revealed the Kac or Kdeac on repair proteins involved in the DNA damage response and repair. A mass spectrometry based study firstly reported the comprehensive survey of Kac and Kdeac sites in non-histones including repair proteins of CBP and p300 (10). Functional study indicated that p300/CBP acetylated Ku70, RAD51, and APE1 in the regulation of non-homologous end-joining, homologous recombination and base excision repair (11–13). Recently, KDACi targeting the acetylation of repair proteins have approved for treating various types of cancers. Using modified quantitative proteomic approach, Elia et. al. have profiled ubiquitination and acetylation of cellular

proteins in response to ionizing radiation, and obtained 33,500 ubiquitination and 16,740 acetylation sites, respectively (9). Focus on the acetylation of nuclear proteins, a profiling study identified 217 Kac sites and analyzed the dynamic change in response of DNA damage induced by irradiation(14). Pan-cancer analysis of TCGA data has discovered frequent mutations of acetylation and ubiquitination sites in cancer driver genes, suggesting the PTM at these sites as novel mechanisms of cancers (15). However, the global mapping of proteome and acetylome of repair proteins remains large unknown. Here, we investigated the acetylation-status change of repair proteins in the early response of DNA damage using high-resolution mass spectrometry analysis.

Materials And Methods

Cell culture and treatment.

The experimental design process was portrayed in Figure 1a. Human embryonic kidney HEK293T cells (CRL-11268) were purchased from American Type Culture Collection and maintained at 37°C and 5% CO₂ in Dulbecco's Modified Eagle Medium supplemented with 10% (v/v) FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin. The 293T cells in exponential state were treated with 0.8uM epirubicin (Sigma Aldrich) for 1h (EPI + group) as well as control group (EPI - group) without treatment in culture medium. Three biological replicates were performed for each group.

Protein extraction and digestion

The cell samples were sonicated three times on ice using a high-intensity ultrasonic processor (Scientz) in lysis buffer (8 M urea, 1% protease inhibitor cocktail, 3 μM TSA and 50 mM NAM). After centrifugation with 12000g at 4 °C for 10 min, the supernatant was collected. Protein concentration was determined with a BCA kit according to the manufacturer's instructions (Pierce). After recover and alkylation, the protein samples were digested twice in digestion buffer containing trypsin (1:50 and 1:100 trypsin-to-protein) at room temperature for overnight and 4h, respectively.

Affinity enrichment of lysine acetylation peptides

The peptides were dissolved in immunoprecipitation buffer solution (100 mM NaCl, 1 mM EDTA, 50 mM tris-hcl, 0.5% np-40, pH 8.0), and the supernatant was transferred to the pre-washed acetylated resin (antibody resin no. PTM-104, from Hangzhou jingjie PTM Bio), placed on a rotating shaker at 4 °C, gently shaken and incubated overnight. After incubation, the resin was washed with IP buffer solution 4 times and deionized water twice. Finally, 0.1% trifluoroacetic acid eluent was used to elute the resin-bound peptide for three times. After draining in vacuum, the eluted peptides were cleaned using C18 ZipTips (Millipore) according to the manufacturer's instructions for subsequent LC-MS/MS analysis.

LC-MS/MS Analysis

The tryptic peptides were dissolved in solvent A (0.1% formic acid in water), directly loaded onto a home-made reversed-phase analytical column (15 cm length, 75 μm inside diameter, Sigma Aldrich). For

proteomics analysis, peptides were separated with a gradient from 4% to 6% solvent B (0.1% formic acid in acetonitrile) in 2 min, 6% to 24% over 68 min, 24% to 32% in 14 min and climbing to 80% in 3 min then holding at 80% for the last 3 min, all at a constant flow rate of 300 nL/min on a nanoElute UHPLC system (Bruker Daltonics). For acetylomics analysis Peptides were separated with a gradient from 6% to 22% solvent B (0.1% formic acid in acetonitrile) over 43 min, 22% to 30% in 13min and climbing to 80% in 2 min then holding at 80% for the last 2 min, all at a constant flow rate of 400 nL/min on the nanoElute UHPLC system.

The peptides were subjected to capillary source followed by the timsTOF Pro mass spectrometry (Bruker Daltonics). The timsTOF Pro was operated in parallel accumulation serial fragmentation mode with an electrospray voltage 1.60 kV. Precursors and fragments were analyzed at the TOF detector with a MS/MS scan range from 100 to 1700 m/z. . Precursors with charge states 0 to 5 were selected for fragmentation, and 10 PASEF-MS/MS scans were acquired per cycle. The dynamic exclusion was set to 30 s.

Database Search

The resulting MS/MS data were processed using Maxquant search engine (version 1.6.6.0). Trypsin/P was specified as cleavage enzyme allowing up to 2 missing cleavages. The mass tolerance for precursor ions was set as 40 ppm in first search and 40 ppm in main search, and the mass tolerance for fragment ions was set as 0.04 Da. FDR was adjusted to < 1%.

Protein annotation

Acetylation on lysine sites detected in the EPI⁺ group while were not detected in all replicates of the EPI⁻ group were considered as acetylated lysine sites. Deacetylated sites were on the contrary. In case a protein ratio is not determined, normalization was done based on a logarithm-transformation algorithm as described (16). The cutoff for differently expressed proteins in EPI⁺ group compared to EPI⁻ group was strictly set in 1.5-fold. Comparisons between variables were tested by paired t-test. P values < 0.05 were considered to be statistically significant.

Bioinformatics analysis.

Gene Ontology (GO) annotation analysis (<http://www.ebi.ac.uk/GOA>) was derived from the UniProt-GOA database for functional classification of proteins. Kyoto Encyclopedia of Genes and Genomes (KEGG, <http://www.genome.jp/kegg>) database was used to annotate protein pathways.

To generate the PPI network, acetylated proteins were searched against the STRING database version 11.0 (<https://string-db.org/>) with interactive score ≥ 0.7 as high confidence. Subsequently, Cytoscape software version 3.7.2 (<http://www.cytoscape.org/index.html>) was used for visualization of the PPI network, in which nodes represented genes and edges represent interactions between genes. Helm (version 1.0.3.7 <https://helm.sh/>) software was used to make the heatmap.

Motif analysis

MeMe suite version 5.1.1 (<http://meme-suite.org/>) was used to analyze the model of sequences constituted with amino acids in specific positions of modifier-21-mers (10 amino acids upstream and down-stream of the site) in the sequences of proteins which contain acetylated or deacetylated lysine sites.

Results

Overview of proteome and acetylome in epirubicin-induced early DNA damage

We profiled the proteome and acetylome of DNA repair proteins in HEK293T cells in 1 h after treated with epirubicin using LC-MS/MS (Figure 1a). In total, 6291 proteins were detected and 5526 proteins were quantified with a label-free strategy. Among these quantified proteins, 106 repair proteins associated with NER, BER, MMR, HR, and NHEJ pathways were identified (Figure 1b, Table S1, S3).

The acetylated proteins and their modification sites were identified using a label-free strategy and anti-acetyl antibody affinity enrichment followed by high-resolution LC-MS/MS. The length of most peptides was distributed between 7 and 20, which agreed with the property of tryptic peptides (Figure 1d; Table S5). Among 6789 Kac sites in 2400 proteins identified, 4457 Kac sites in 1778 proteins were quantified, including 190 Kac sites in 50 repair proteins (Figure 1b; Table S2, S4). The overall acetylated proteins contained different numbers of acetylation sites from 1 to 29. 1132 acetylated proteins (47.2%) contained only one acetylation site. The proportions of proteins with two, three, four or more modification sites were 18.4, 10.3, and 24.1%, respectively (Figure 1c; Table S6). Of these 50 repair proteins, the acetylation changes were identified in 30 proteins. we next analyzing lysine sites on repair proteins, 42 acetylated and 24 deacetylated lysine sites were observed in 21 and 16 repair proteins, respectively, whereas, both acetylated and deacetylated lysine sites were detected in 7 repair proteins (Figure 1b, 2a). In 17 repair proteins, the epirubicin induced acetylation changes were identified for the first time in the present study. (Figure 5b). The number of Kac sites in repair proteins with acetylation modification ranged from 1 to 27 (Figure 1b; Table S7). Repair proteins with acetylated or deacetylated lysine sites were shown in Figure 2 b.

Sites that previously have identified to underwent acetylation modification in DNA damage and repair processes were also identified in our result, such as K120 and K164 in TP53 (17), K77 and K13 in PCNA (18). Of the 106 repair proteins analysed, 50 repair proteins were identified to contain acetylation and deacetylation modification. Analysing the expression of 106 proteins, only RAD23A was significantly up-regulated (1.76 fold) in cells treated with epirubicin (Figure 2c). The proteome and acetylome results indicated that rapid acetylation or deacetylation of lysine in DNA repair proteins were responsible for manipulating their functions to coordinate the repair progress earlier than the alteration of expression levels in the early stage of DNA damage repair process.

KEGG pathway classification

The 50 repair proteins with acetylation were able to be classified into six major pathways including NER, BER, MMR, HR, NHEJ, DNA replication, and other pathways related to DNA repair process (Figure 3a, Table S8). The NER pathway ranked the first place containing 21 in 34 repair proteins. Epirubicin can induce inter-chain cross-linking and DNA adduct, inhibit the activity of topoisomerase II, and release oxygen-free radicals resulting in DNA lesions and activating several repair pathways, which was consistent with our results.

Functional analysis of the repair proteins with acetylated or deacetylated lysine sites

Of 50 repair proteins containing acetylation modifications, we analysed the cellular component, molecular function, and biological process of 30 proteins with acetylated or deacetylated changes (Figure 3b). These repair proteins were mainly distributed in the nuclear including nuclear lumen organelles (96.7%), nucleoplasm (93.3%), and chromosome (60%), respectively. The top three molecular functions of these proteins were DNA binding; catalytic activity acting on DNA and ATPase activity. The foremost biological process that these repair proteins were involved in DNA metabolic process and DNA repair, chromosome organization, and DNA recombination.

Protein to protein interaction between repair proteins

The protein interaction of acetylated repair proteins was conducted with PPI network analysis. The PPI sources were originated from the STRING database and visualized through the Cytoscope. Interaction information came from experiments and databases resources, and the minimum required interaction score was set to the high level (0.700) to ensure the reliability of the relationship. The relationship of the 50 repair proteins was illustrated in Figure 4a (Table S10). The PPI network revealed the interacting partners of the 17 proteins with new identified acetylation lysine sites, suggesting possible molecular functions related to the effect of acetylations (Figure 4a, 4b).

Acetylation sites located in the functional domains of repair proteins.

The PTM on the domains of proteins can significantly regulate the protein functions. We subsequently explored the relationship between the acetylation sites and functional domain of 17 repair proteins. Of the 32 acetylation sites analysed, 9 acetylation sites were located in the functional domains of 7 repair proteins (Figure 5a). Rad23B simultaneously have 2 acetylated and 1 deacetylated lysine sites that respectively are AcK67, AcK36 and DeacK45 which were all located in UBL domain. AcK64 and DeacK380 on PRP19 were positioned in the U box and WD40-repeat regions. AcK171 and AcK313 on RECQL were both located in the 2 RecA-like domains. The other four proteins (RFC5, RFC3, XAB2 and RAD17) contained 1 acetylation site located in their main functional domain.

Analysis of Acetylated Lysine Motifs

To identify the possible specific motifs flanking acetylation lysine site, the amino acid sequence from the -10 to the +10 positions surrounding the 1090 acetylated peptides and 1047 deacetylated peptides were analyzed using the MeMe suite. Motifs K[Ac]Y, K[Ac]N, and K[Ac]T ranked the top three of acetylated-Lys

motifs, and among deacetylated-Lys motifs, the top three motifs were GK[Ac], K[Ac]S, and K[Ac]Y (Figure 6a, 6b). The matching peptides accounted for all peptides respectively were 27.3% and 24.1%. Among the 30 repair protein with acetylation sites, motifs K[Ac]K, K[Ac]H, and K[Ac]F were most enriched (Figure 6c).

Discussion

In this study, we utilized label-free LC-MS/MS strategy to acquire proteome and acetylome dataset of early DNA damage in 293T cell lines treated with epirubicin. A total of 5526 quantified proteins and 6789 Kac sites in 2400 proteins were identified, among which 4457 Kac sites in 1778 proteins were quantified. Up to now, the maximal quantitative proteomic atlas of acetylation in DNA damage response was reported in 2015 by Elia, A.E. and his colleges (9). With the combination of SILAC and FACET-IP strategy, 16740 Kac sites in 3361 proteins were identified in Hela cells that treated with 40 J/m² UV or 10 Gy IR for 1 hour via LC-MS/MS (9). Compared with the dataset of Elia, A.E.'s, we identified additional 3858 Kac sites in our acetylome result.

Among 106 quantitative repair proteins, a total of 190 Kac sites were identified in 50 of them, which were distributed in NER, BER, MMR, HR, NHEJ and other pathways closely related to DNA repair, possibly indicating that proteins in multiple repair pathways were regulated by acetylation and involved in restoring the lesions induced by epirubicin. Kac sites on GTF2H2C, RAD51C, and RAD17 were discovered for the first time. 66 acetylated or -deacetylated lysine sites induced by epirubicin were observed in 30 proteins. 7 repair proteins simultaneously contained acetylated and deacetylated lysine sites. According to the Go analysis, the 30 repair proteins were mainly equipped with DNA binding ability and ATPase activity to concentrate on the chromosome organization, regulation of DNA metabolic process, and DNA recombination.

Of the 50 repair proteins with acetylation, the regulation mechanisms of acetylation on 17 repair proteins still need further studied to illustrate., the molecular functions of them were mainly distributed in chromatin, DNA, ATP, nucleic, protein binding, and ligase, ATPase, DNA clamp loader activity according to the GO annotation. Increasing studies have revealed that acetylation within the domain region were capable of regulating the function of proteins (19, 20). For instance, acetylation of K1626 and K1628 in the Tudor-UDR domain of 53BP1 was dynamic regulated by CBP and KDAC2, which was associated with 53BP1 interaction with nucleosomes and the choice of DNA repair pathway (21). Hence, via analyzing the regions of these acetylation sites on the 50 repair protein, 9 acetylated or deacetylated Kac sites were observed to locate in the function domains of 7 repair proteins.

We have identified new acetylation changes in 17 repair protein including PRP19, RECQL, RFC5, e.g. and analysed the associations of acetylation sites with functional domain in these proteins. 7 proteins were observed to have acetylated or deacetylated lysine sites on their functional domains. RAD23B, as component of the XPC complex, is the first factor for recognizing DNA lesions involved in global genome nucleotide excision repair (22). Three lysine sites (acetylated K67 and K36 and deacetylated K45) detected in our study were positioned in the UBL of RAD23B that is responsible for mediating the

degradation of the ubiquitinated substrate in proteasome. Therefore, AcK67, AcK36, and DeacK45 in the early DDR are also highly possible to connect with protein degradation in DNA repair.

PRP19 is a ubiquitin ligase involved in DNA damage response. The U box and WD40-repeat regions of PRP19 are importance for recruiting E2 ubiquitin-conjugating enzyme and interacting E3 ubiquitin-protein ligase complex to catalyze the polyubiquitination of target proteins involved in DNA damage response (23) AcK64 and DeacK380 were located in the U box region and fourth WD40-repeat, respectively, suggesting these acetylation changes might regulate the ubiquitination on target protein of PRP19. RECQL. involved in DNA duplex helix in DNA repair (24). AcK171 and AcK313 were located in the RecA-like domains where harbored the ATP-dependent translocation activity and were sought to form a cleft to bind with nucleotide (25) Therefore, it is worthwhile to explore whether the acetylation in RECQL is able to affect the molecular conformational change or ATP-dependent translocation activity or other features.

RFC5, RFC3, XAB2 and RAD17 only have one acetylated lysine site identified in this study. AcK66 in RFC5 located in the AAA+ ATPase domain. ATPase activity within RFC couple the chemical energy of ATP hydrolysis to the assemble of PCNA onto the RNA-primed DNA (26, 27). Whether the acetylation on RFC5 is related to the DNA elongation still needs further elucidation. Mass spectrum result implicated that AcK590 located in the TPR motif 9-15 of XAB2. TPR motifs 11–12 have been validated that were essential for efficient HR (28). Hence, the acetylation of K590 is inferred to probably affect downstream combinations between XAB2 and other proteins during the repair process. K313 in RAD17 was the first identified acetyl-site. In the early DNA damage repair process, Rad17 was considered to involve in triggering the DNA damage checkpoint when combined with RFC2-5 complex forming an RFC-like complex and also were capable of coupling the hydrolysis of ATP to load PCNA onto DNA (29). Whether the AcK313 in RAD17 is related to the RFC complex conformation and PCNA loading still awaits elucidation.

According to the results of proteome and acetylome, 66 acetylated or deacetylated sites were discovered in 30 repair proteins, whereas, the majority of the repair proteins have no significant changes in the expression level. These results appeared to prompt that the fast acetylation and deacetylation on the repair proteins were responsible for mediating signaling transduction and participating in repair pathway activation in response to DNA damage, which was in line with the result of a previous study that investigated the acetylation dynamics of human nuclear proteins during the ionizing radiation-induced DNA damage response (14). Increasingly studies have informed that the abnormal acetylation status of repair proteins was capable of modulating the repair efficiency, which was closely related to cancer risk, progression, and therapeutic response (3-5). Aberrant mutations of lysine on proteins also influenced the precise acetylation on them and consequently affected their functions. Recently, various of KDAC inhibitors were applied to clinical praxis as effective anti-tumor drugs (30). Therefore, figuring out the signaling transduction mediated by acetylation on repair proteins in the early DNA damage response network is vital to develop new medicine targeted protein acetylation.

Conclusion

Our study comprehensively profiled the proteome and acetylome in early DNA damage and provided acetylation changes of DNA repair protein in different repair pathways. Furthermore, the potential regulative mechanism for acetylation on 17 proteins were deeply analysed for the first time. The results in this study might provide insight of new mechanisms for acetylation on regulating repair proteins in the early DNA damage.

Abbreviations

CBP: CREB-binding protein

53BP1: P53-binding protein

Kac: Lysine acetylation

KATs: Lysine acetyltransferases

KDAC2: Lysine deacetylase 2

KDACi: KDAC inhibitor

KDACs: Lysine Deacetylases

Kdeac: Lysine deacetylation

KEGG: Kyoto Encyclopedia of Genes and Genomes

LC-MS/MS: liquid chromatography–tandem mass spectrometry

PCNA: Proliferating Cell Nuclear Antigen

PPI: Protein-Protein Interaction

PRP19: Pre-mRNA Processing Factor 19

PTM: Post-Translational Modification

RAD17: Rad17-like protein

RAD23B: Homolog B of *Saccharomyces cerevisiae* Rad23

RECQL: RecQ like helicase

RFC3: Replication Factor C Subunit 3

RFC5: Replication Factor C Subunit 5

UBL: Ub-like

Declarations

Ethics declarations

The study was approved by the Research Ethics Committee of the Xiang An Hospital of Xiamen University

Consent for publication

Not applicable.

Availability of data and materials

The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests

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Author's Contributions

Dr. Hanxiang An conceived and designed the study. Shiqin Li collected samples, analyzed the data, and drafted the manuscript and figures. Dr. Hanxiang An revised the manuscript. Dr. Lin Zhou provide cell experimental guidance. All authors read and approved the final manuscript.

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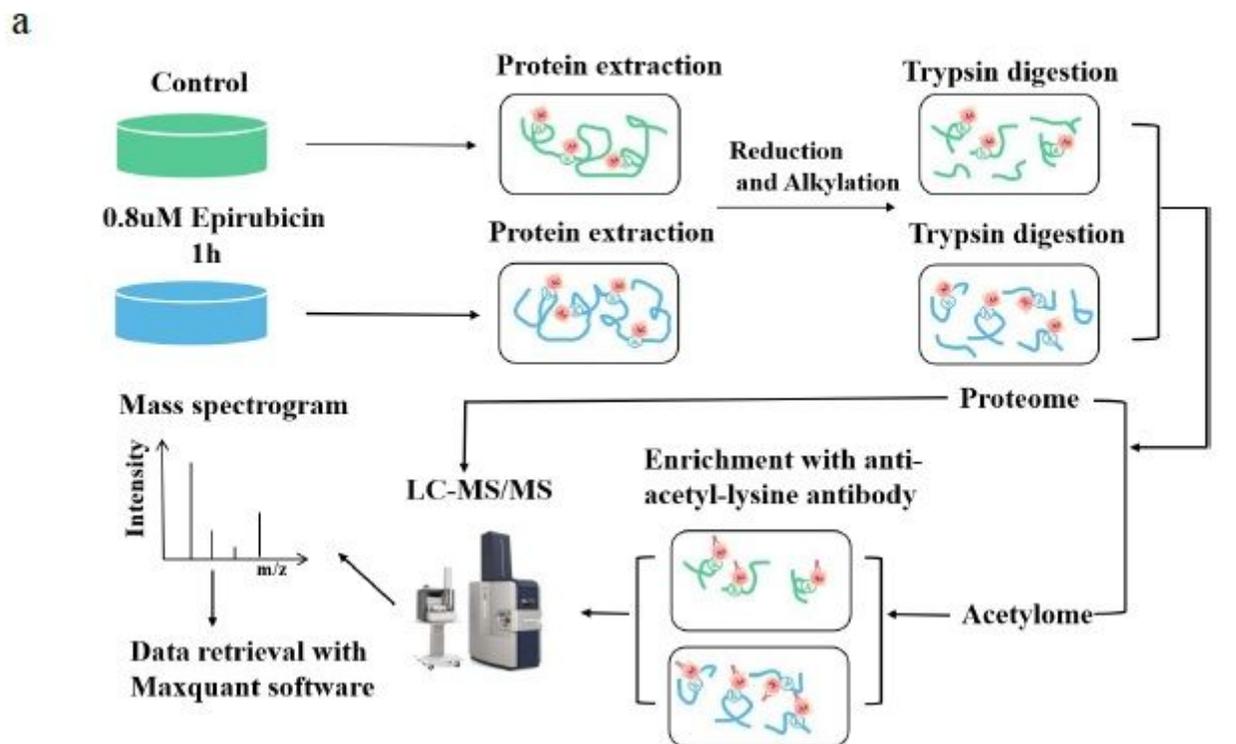
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Figures



b

All quantifiable proteins	All lysine acetylation sites	All quantified lysine acetylation sites	All quantifiable lysine acetylation sites in repair proteins	All -acetylated / -deacetylated sites in repair proteins
/	6789	4457	190	42/24
5526 (proteins)	2400 (protein)	1778 (protein)	50 (protein)	21/16 (protein)

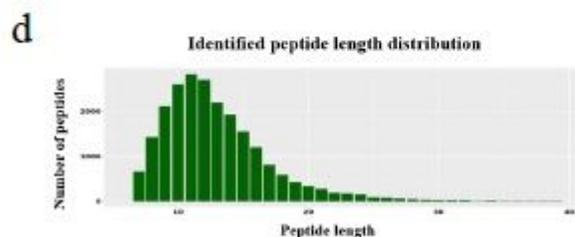
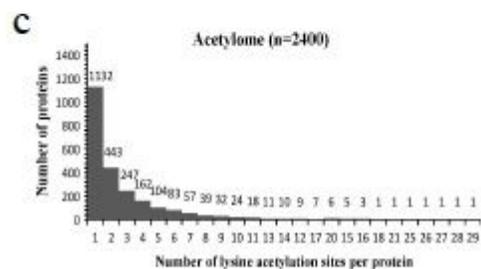


Figure 1

Global mapping of proteome and acetylome. (A) Experimental workflow for cell treatment and performing LC-MS/MS. (B) Table exhibiting the number of all quantifiable proteins (column 1), all lysine acetylation sites (column 2), all quantified lysine acetylation sites (column 3), quantified lysine acetylation sites in repair proteins (column 4), and acetylated or deacetylated sites in repair proteins (column 5). The number of proteins encompass all these acetylation sites was also shown (bottom lane). (C) The distribution of 2400 acetylated proteins with different number of lysine acetylation sites. (D) Peptide length distribution.

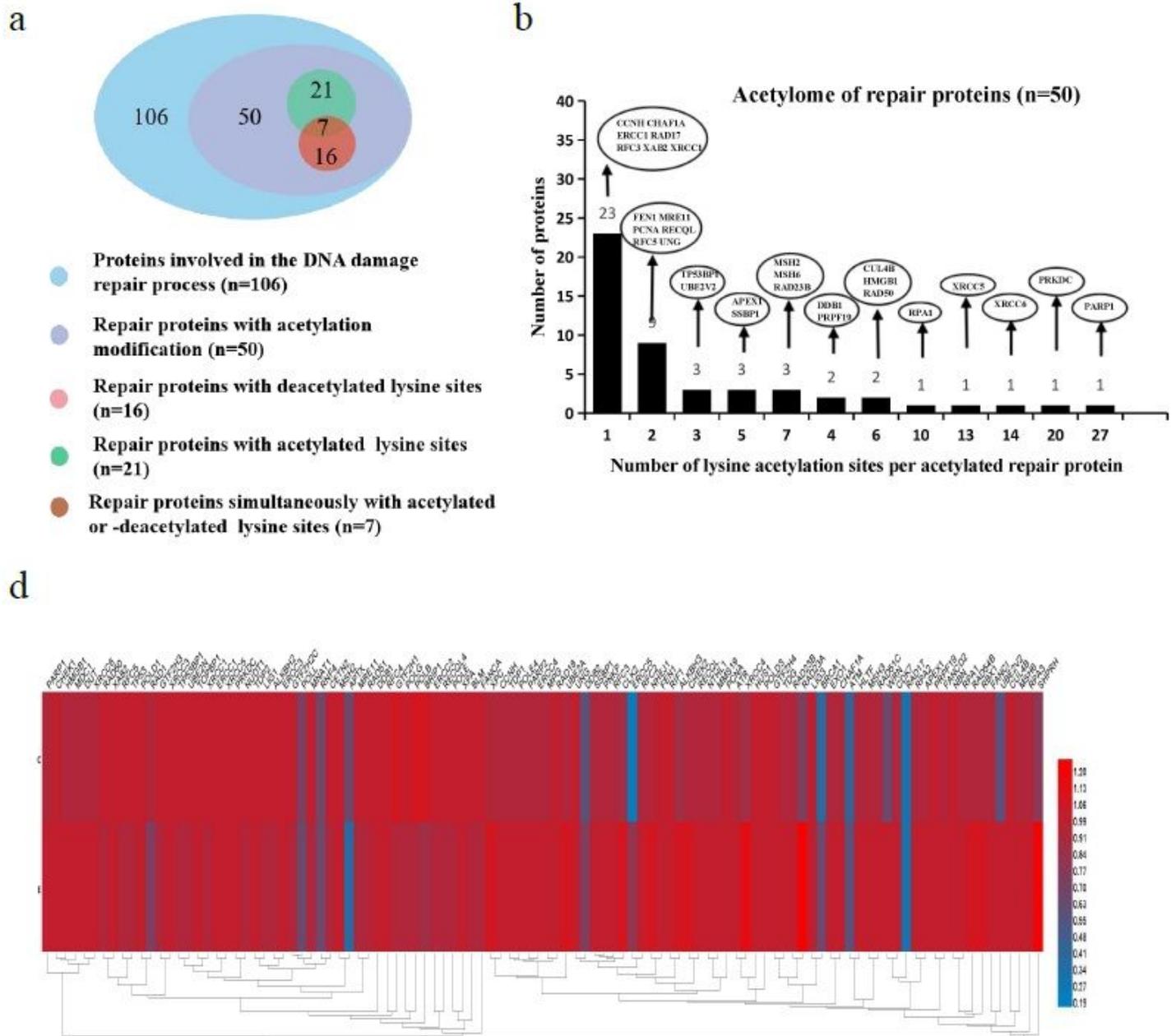


Figure 2

Profile of repair proteins with acetylation. (A) Of the 106 repair proteins, 50 repair proteins have acetylation modification. 21 repair proteins contained acetylated lysine sites. 16 repair proteins had deacetylated lysine sites. 7 proteins possessed acetylated and deacetylated lysine sites simultaneously.

(B) The distribution of 50 acetylated proteins with different number of lysine acetylation sites. All 30 repair proteins that contained acetylated or deacetylated lysine sites were shown. (C) Heat map of the relative expression of 106 repair proteins in EPI (-) group and EPI (+) group. RAD23A was significantly up-regulated at a threshold of 1.76 ($p \leq 0.05$).

a **KEGG pathway of 50 repair proteins with acetylation modification**

KEGG Pathway Classification	Proteins
NER	CCNH, <u>CUL4B</u> , <u>DDB1</u> , DDB2, ERCC1, ERCC4, GTF2H1, GTF2H2*, <u>LIG1</u> , <u>PCNA</u> , <u>POLD3</u> , <u>POLE4</u> , <u>RAD23B</u> , RBX1, RFC2, RFC3, RFC4, <u>RFC5</u> , <u>RPA1</u> , RPA2, <u>XPC</u>
MMR and DNA replication	FEN1*, <u>LIG1</u> , <u>MSH2</u> *, <u>MSH6</u> *, <u>PCNA</u> *, <u>POLD3</u> , <u>POLE4</u> , RFC2, RFC3, RFC4, <u>RFC5</u> *, <u>RPA1</u> , RPA2, <u>SSBP1</u>
BER	<u>APEX1</u> , FEN1, <u>HMGB1</u> , <u>LIG1</u> , <u>LIG3</u> , <u>PARP1</u> *, <u>PCNA</u> , <u>POLD3</u> , <u>POLE4</u> , <u>UNG</u> , <u>XRCC1</u> *
HR	<u>MRE11</u> , NBN, <u>POLD3</u> , <u>RAD50</u> *, <u>RAD51C</u> *, <u>RPA1</u> , RPA2, <u>SSBP1</u>
MHEJ	FEN1*, <u>MRE11</u> *, <u>PRKDC</u> *, <u>RAD50</u> *, <u>XRCC5</u> *, <u>XRCC6</u> *
Others related to DNA repair process	TP53, UBE2N, FAM179A, <u>RECQL</u> , <u>RAD17</u> *, <u>PRPF19</u> *, FANCD2, XAB2, RIF1, UBE2V2*, TP53BP1*, <u>CHAF1A</u> , <u>DUT</u>

* * repair proteins were discovered to have acetylation for the first time
 underline repair proteins have acetylated lysine sites
 * repair proteins have deacetylated lysine sites

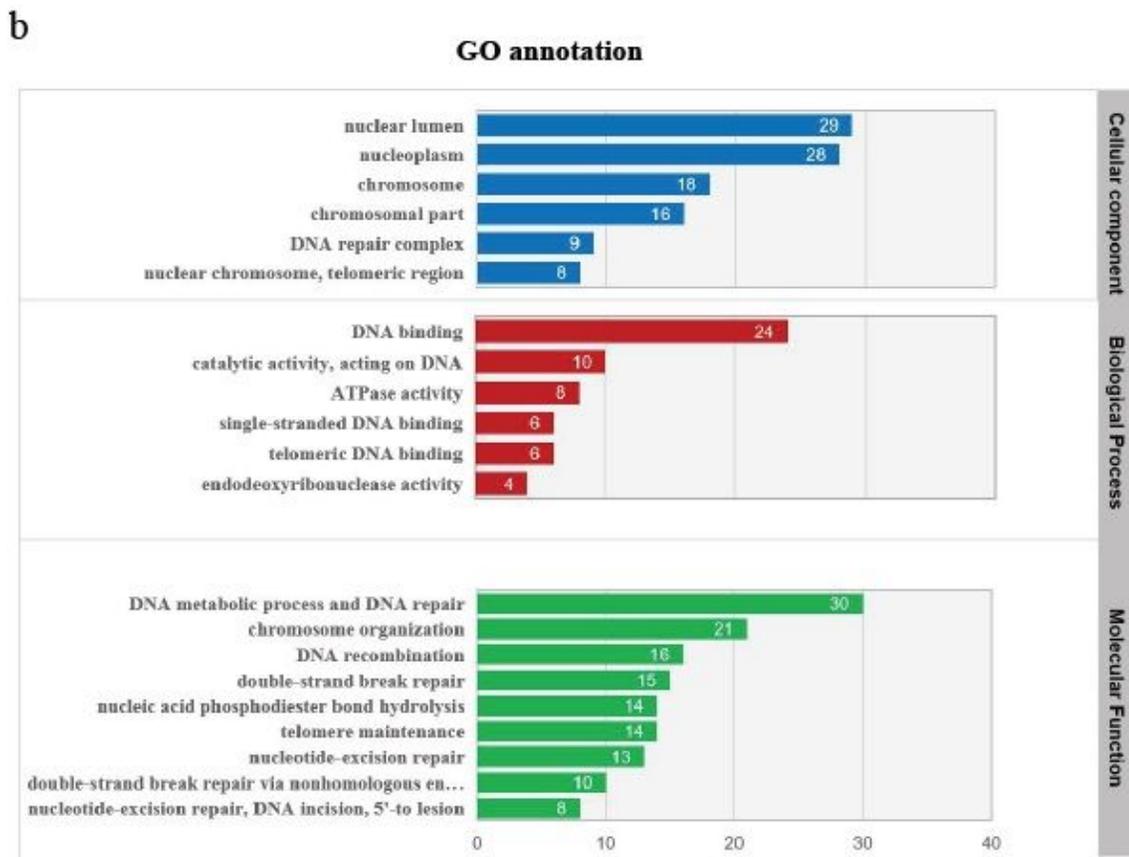


Figure 3

Kegg pathway classification and Go annotation (A) Table showing that 50 repair proteins with acetylation were classified into five repair pathways and other pathways involved in the repair process. 3 repair proteins marked with “*” were discovered to have acetylation for the first time. Protein included acetylated or deacetylated sites were also marked with underline and “✖”, respectively. (B) Go annotation of 30 acetylated or deacetylated repair proteins.

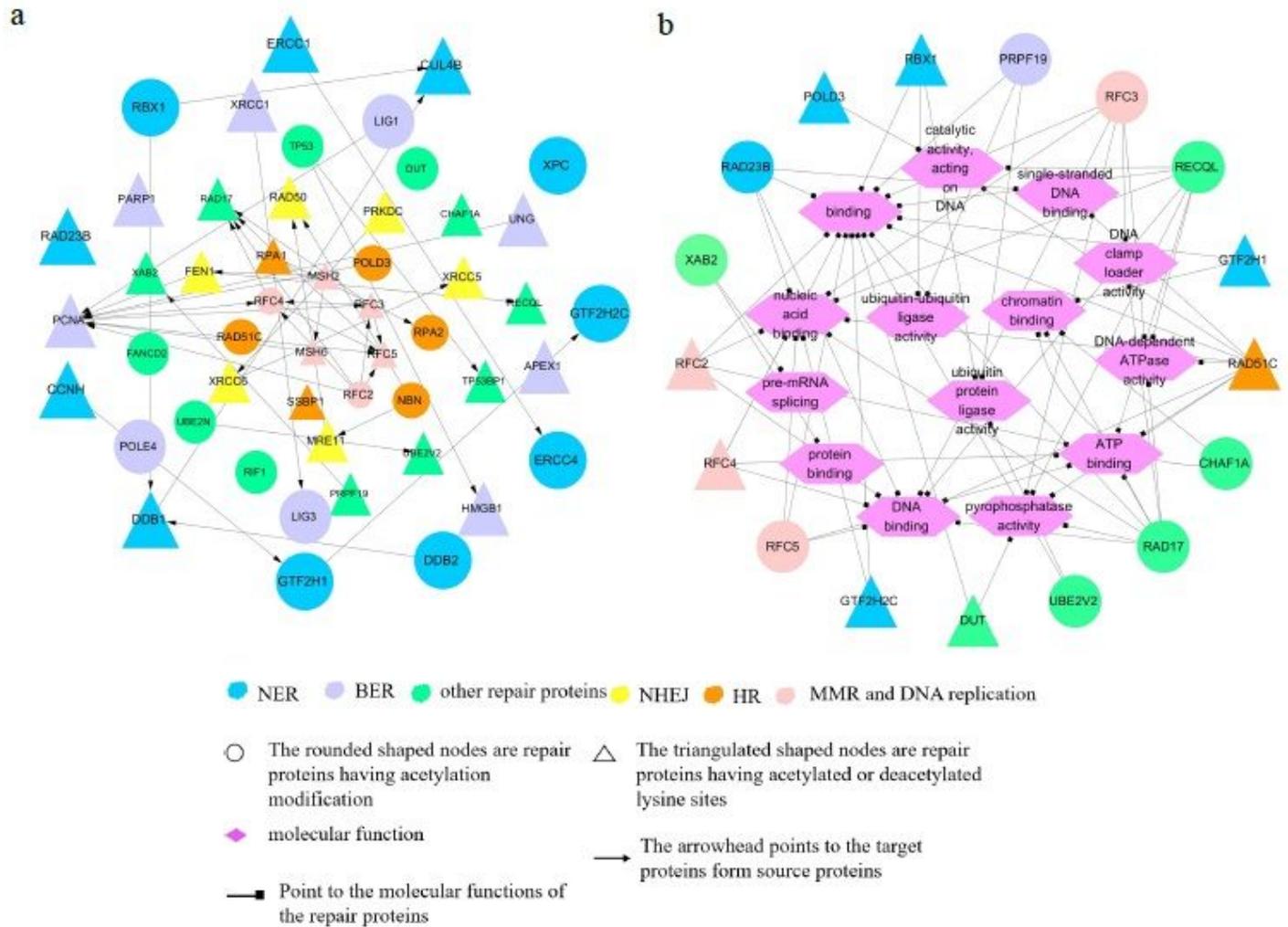


Figure 4

Interaction networks of all repair proteins revealed by Cytoscape software and molecular function of 17 repair proteins. (A) PPI network of 50 repair proteins with acetylation modification. Proteins in red font were discovered to have acetylation for the first time. Proteins in triangulated shaped nodes included acetylated or deacetylated sites. (B) Molecular functional annotation of 17 repair proteins whose effects of acetylation on their function were unclear.

a

The domain where the acetylated sites are located and the functions of the domain

Proteins	Acetylated lysine sites	Domains	Summary of function or the biological process involved in the domain
PRPF19	K64	U box region	① E2 ubiquitin-conjugating enzyme recruitment domain ② catalyze the polyubiquitination modification of target proteins
RAD23B	K67, K36	Ub-like domain	① degradation of the ubiquitinated substrate in proteasome
RFC5	K66	AAA+ ATPase domain	① couple the chemical energy of ATP hydrolysis to the installment of PCNA
RFC3	K154	AAA+ ATPase domain	① ATPase activity ② DNA elongation
XAB2	K590	tetratricopeptide repeats	① efficient HR
RAD17	K313	Rad17 combines with the RFC multisubunit complex to form an RFC-like complex	① combine with the RFC2-5 complex ② load PCNA onto DNA
RECQL	K171, K313	RecA-like domains	① ATP-dependent translocation activity ② form a cleft to bind with nucleotide

b

The domain where the deacetylated sites are located and the functions of the domain

Proteins	Deacetylated lysine sites	Domain	Summary of function or biological process involved in the domain
PRPF19	K380	fourth WD40-repeat	① protein-interaction-domain ② bind for WD40 repeats containing proteins
RAD23B	K 45	Ub-like domain	① degradation of the ubiquitinated substrate in proteasome
RFC5	/	/	/
RFC3	/	/	/
XAB2	/	/	/
RAD17	/	/	/
RECQL	/	/	/

Figure 5

Summary of proteins that the acetylated or deacetylated sites were located in the protein function domain. (A) Domain where acetylated sites were located in. (B) Domain where deacetylated sites were located in.

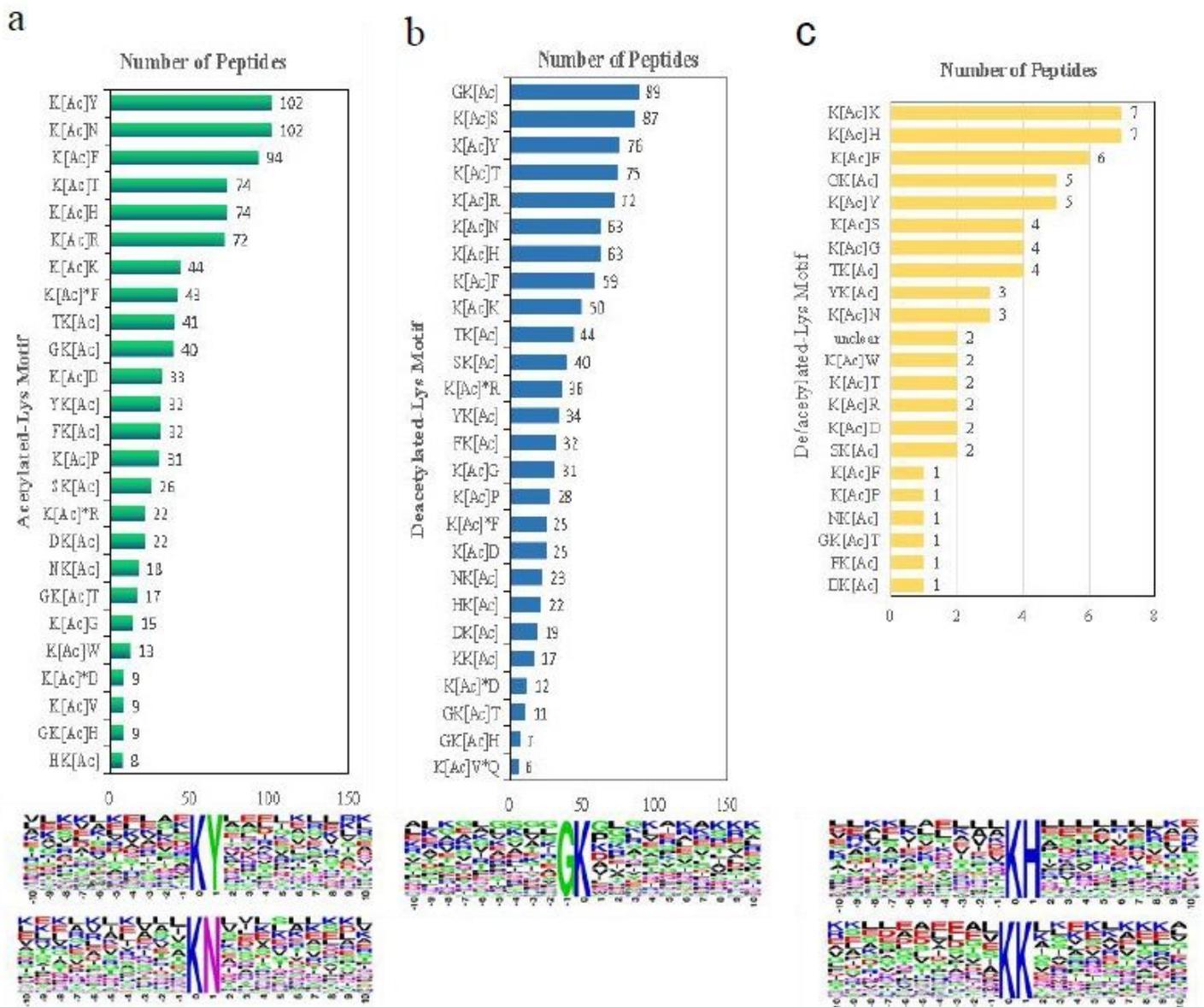


Figure 6

Properties of the acetylated and deacetylated peptides. (A) The number of identified peptides containing acetylated lysine in the motif, and sequence logos of motif that matched maximum peptides. (B) The number of identified peptides containing deacetylated lysine in the motif, and sequence logos of motif that matched maximum peptides. (C) The number of identified peptides containing acetylated or deacetylated lysine in the motif of repair proteins, and sequence logos of motif that matched maximum peptides.

Supplementary Files

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