Quantitative Analysis of Lysine Acetylation in Vero Cells Infected With Peste Des Petits Ruminants Virus

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Research

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Abstract:

Background: Peste des petits ruminants virus (PPRV) is a negative-stranded RNA virus belonging to the Paramyxoviridae family and causes acute, highly contagious disease in small ruminants. Lysine acetylation plays central role in regulating gene expression. However, the extent and function of lysine acetylation in host cells during PPRV infection remains unknown.

Methods: Lysine acetylation of PPRV-infected Vero cells was tested and differentially expressed lysine acetylation was found. The acetylated peptides were enriched using specific antibody and labeled with demethylation. Proteins with acetylation sites were identified. Subsequently, intensive bioinformatics analysis of succinylome of PPRV-infected Vero cells was performed.

Results: We identified 4729 cellular proteins and 1068 proteins with 2641 modification sites quantifiable detected by mass spectrometry, of which 304 proteins with 410 acetylation sites were significantly acetylated in response to PPRV infection. Bioinformatics analyses revealed that the

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differentially acetylated proteins mainly participated in carbohydrate catabolic and DNA metabolic process, and were associated with multifarious functions, suggesting that intracellular activities were extensively changed after PPRV infection. Protein-protein interaction (PPI) network of the identified proteins further indicated that a variety of chaperone and ribosome processes were modulated by acetylation.

**Conclusions:** To our knowledge, this is the first study on acetylome in host cell infected with PPRV. It provides an important baseline to future study the roles of acetylation in the host response to PPRV replication.

**Keywords:** Post-translational modification, Acetylation, Protein-protein interaction; Peste des petits ruminants

**Background**

Peste des petits ruminants virus (PPRV), the causative agent of Peste des petits ruminants (PPR), belongs to the genus *Morbillivirus* in the family *Paramyxoviridae*, which also includes measles virus (MV), rinderpest virus (RPV), canine distemper virus (CDV), marine mammalian morbilliviruses (phocine distemper virus (PDV), porpoise morbillivirus (PMV), dolphin morbillivirus (DMV)) and feline morbillivirus (FeMV) [1]. PPRV has a negative, non-segmented single-stranded genomic RNA, encoding six structural proteins (nucleocapsid protein, N; phosphoprotein, P; matrix protein, M; fusion protein, F; haemagglutinin protein, H; and large polymerase protein, L) in the 3’ to 5’ direction and two non-structural proteins (V and C proteins).

PPRV was first reported in the Ivory Coast, West Africa, in 1942 and is currently endemic in Africa, the Middle East and Asia affecting global trade and causing significant economic losses [2]. Following the successful eradication of rinderpest, the World Organization of Animal Health (OIE) and the Food and Agriculture Organization (FAO) have proposed a plan for global eradication of PPR by 2030 [3].
The interaction between host and virus is a complex dynamic competitive process. As a kind of obligate intracellular parasites, viruses depend on their ability to "hijack" host cellular functions to facilitate their replication and inhibit host antiviral defenses. On the contrary, in order to maintain normal physiological functions, the host utilizes the nonspecific and specific immune based antiviral responses to resist viral invasion, inhibit virus replications, or eliminate virus particles. In previous studies, host cellular response was deciphered by clustered regularly interspaced short palindromic repeat (CRISPR) small interfering RNA (siRNA) and transcriptomic and proteomic analyses.

Today, it is well known that protein post-translational modifications (PTMs) affect significant diverse functions of proteins via modulating biological processes, protein activity, cellular location and protein-protein interaction (PPI) by transferring modified groups to one or more amino acid residues. To date, more than 450 protein modifications including over 200 PTMs have been identified to be dynamic and reversible protein processing events and play key roles in the response to the pathogenesis and development of diseases. PTMs have become a hot topic in viral infection. Some PTMs including phosphorylation, acetylation and succinylation have been shown to potently regulate innate immunity and inflammation in response to viruses infection.

Of the 20 amino acid residues, lysine is one of the most frequent targets of covalent modifications because it can accept different types of chemical groups. Among the lysine PTMs, lysine acetylation is widespread and one of the most well-studied PTM in both prokaryotes and eukaryotes. Lysine acetylation is highly conserved in organisms ranging from bacteria to human and is particularly important. Lysine acetylation impacts protein functions in multiple cellular processes including enzyme activity, chromatin structure, localization and PPI. Accumulating evidence highlights that lysine acetylation is an important molecular toggle of protein function and is a key regulatory point in mechanisms of both host antiviral response and virus replication. However, the extent and function of lysine acetylation
in host cells during PPRV infection have not yet been reported.

In this study, we investigated the acetylome in Vero cells (an African green monkey kidney cell line) infected with PPRV. By combining dimethylation labeling, HPLC fractionation and antibody-affinity enrichment with LC-MS/MS analysis, we systematically analyzed the quantitative comparison of the global proteome and acetylome in Vero cells with or without PPRV infection, and calculated the regularity of sequence features around the acetylated sites. We successfully quantified 4729 proteins and 2641 lysine acetylation sites in 1068 proteins with diverse molecular functions, biological processes and subcellular localizations. Altogether, the results provided the first extensive dataset on lysine acetylation in Vero cells infected with PPRV, and novel insights into the infection mechanism of PPRV.

Methods

Cell, virus and infection

Vero cells were maintained in authors’ laboratory, and cultured in DMEM medium (Sigma Aldrich, St Louis, MO, USA) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin and 100 μg/ml streptomycin at 37 °C in 5% CO₂ incubator. The PPRV vaccine strain Nigeria 75/1 was cultured in our laboratory and passaged in Vero cells as previously described [34]. Vero cells were infected with PPRV at 1 MOI of or mock-infected with phosphate-buffered saline (PBS, 0.01M, pH7.4) at 37°C for 1 h. The MOI was confirmed according to the viral titers of Vero cell line. After adsorption, the virus inoculum was removed, and the fresh medium was added to wells and incubated. In order to determine the sampling time point, the cells were harvested at 24, 48, 72 h post infection (p.i) and analysed by western blotting and pan anti-acetyllysine antibody (PTM-104, PTM Biolabs, Chicago, USA). Three biological replicates of independent (three parallel) experiments were performed.

Protein extraction

The harvested cell samples were washed twice with cold phosphate-buffered saline (PBS). Then,
each sample was sonicated on ice in lysis buffer (8 M urea, 1% Protease Inhibitor Cocktail, 3 μM TSA, 50 mM NAM and 2 mM EDTA). The resulting supernatants were centrifuged with 12,000 rpm for 10 min at 4 °C to remove the cell debris. The protein concentration was determined with BCA kit according to the manufacturer’s instructions.

**Trypsin digestion and dimethylation labeling**

The protein solution was reduced with 5 mM dithiothreitol for 30 min at 56 °C and subsequently alkylated with 11 mM iodoacetamide for 15 min at room temperature in dark. For tryptic digestion, the protein samples were diluted with urea concentration of less than 2M by adding 100 mM NH₄CO₃. Finally, trypsin was added at 1:50 trypsin-to-protein mass ratio for the first digestion overnight and 1:100 trypsin-to-protein mass ratio for a second 4 h-digestion at 37 °C. Then, peptide was desalted by Strata X C18 SPE column (Phenomenex) and vacuum-dried. Peptide samples were resuspended in 0.1 M TEAB and labeled in parallel in different tubes by adding CH₂O or CD₂O to the control and infected samples, respectively. The reactions were mixed and further treated with NaBH₃CN (sodium cyanoborohydride), incubated for 2 h at room temperature, then desalted adding formic acid and vacuum dried.

**Enrichment of acetylated peptides**

Lysine-acetylated peptides were enriched using agarose-conjugated pan anti-acetyllysine antibody (PTM Biolabs, Hangzhou, China). In brief, dried tryptic peptides re-dissolved in NETN buffer (100 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, 0.5% NP-40, pH 8.0) were incubated with pre-washed anti-acetyllysine pan antibody-conjugated agarose beads (PTM-104, PTM Biolabs, Hangzhou, China) and incubated at 4°C overnight with gentle oscillation. Then the beads were washed four times with NETN buffer and twice with ice-cold ddH₂O. The bound peptides were eluted 3 times from the beads with 0.1% trifluoroacetic acid (TFA; Sigma-Aldrich, Saint Louis, USA). The eluted fractions were pooled together and vacuum-dried. The resulting peptides were desalted with C18 ZipTips (Merck Millipore, Billerica, USA) according to the manufacturer’s instructions and dried by vacuum
centrifugation, followed by LC-MS/MS analysis.

**LC-MS/MS analysis**

The enriched peptides were dissolved in solvent A (0.1% Formic Acid in 2% acetonitrile (ACN)), directly loaded onto a home-made reversed-phase analytical column (1.9 μm particles, 120 Å pore, 15-cm length, 75 μm i.d.). The gradient included an increase from 9% to 25% solvent B (0.1% Formic Acid in 90% ACN) for 24 min, followed from 25% to 40% for 10 min, and reaching to 80% in 3 min, then maintained at 80% for the last 3 min on an EASY-nLC 1000 UPLC (Ultra Performance Liquid Chromatography) system at a constant flow rate of 700 nL/min.

The resulting peptides were ionized and subjected to tandem mass spectrometry (MS/MS) in Q Exactive™ Plus (Thermo Scientific) coupled online to the UPLC using NanoSpray Ionization (NSI) source. The electrospray voltage applied was 2.0 kV. Intact peptides were detected at a resolution of 70,000 with scan range of 350–1800 m/z for full MS scans in the Orbitrap. Peptides were then selected for MS/MS using NCE setting of 28 and ion fragments were detected at a resolution of 17,500. A data-dependent acquisition (DDA) that alternated between one MS scan followed by 20 MS/MS scans was applied for the top 20 precursor ions, with 15 s dynamic exclusion. Automatic gain control (AGC) was used to prevent overfilling of the ion trap and set at 5E4, with a fixed first mass of 100 m/z.

**Database searches**

The protein acetylation sites identification and quantification were processed using MaxQuant search engine (v.1.5.2.8) against *Chlorocebus sabaeus* database (19,228 sequences), concatenated with reverse database and common contaminants. Trypsin/P was specified as cleavage enzyme allowing up to 4 missing cleavages. The mass tolerance for precursor ions was set as 20 ppm in first search and 5 ppm in main search, and the mass tolerance for fragment ions was set as 0.02 Da. Carbamido-methylation of cysteine (Cys) was specified as fixed modification, and oxidation on methionine, acetylation on protein N-terminal and acetylation on lysine were specified as variable
modifications. The false discovery rate (FDR) and the minimum score for modified peptide were set at <1% and >40, respectively. The minimum peptide length was set at 7. All other parameters in MaxQuant were set to default values.

**Bioinformatics analysis**

Gene Ontology (GO) annotation was derived from the UniProt-GOA database ([http://www.ebi.ac.uk/GOA](http://www.ebi.ac.uk/GOA)). Proteins were classified based on three categories: biological process, cellular component and molecular function. Protein domain annotation was performed using the InterProScan ([http://www.ebi.ac.uk/InterProScan/](http://www.ebi.ac.uk/InterProScan/)) based on protein sequence alignment method, and the InterPro domain database ([http://www.ebi.ac.uk/interpro/](http://www.ebi.ac.uk/interpro/)) was used. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database ([http://www.genome.jp/kegg/](http://www.genome.jp/kegg/)) was used to annotate and map the pathways. GO, protein domain and KEGG pathway enrichment analysis were performed using the DAVID bioinformatics resources 6.8. Wolfpsort ([https://wolfpsort.hgc.jp/](https://wolfpsort.hgc.jp/)), a subcellular localization prediction software was used to predict subcellular localization. Amino acid sequence motifs (within ± 10 residues of the acetylated or succinylated sites) were analyzed by motif-X. Motif-based clustering analyses were also performed, and cluster membership was visualized using a heat map. Functional interaction network analysis was performed using the STRING database (v.11.0), with a high confidence threshold of 0.7, and visualized by Cytoscape 3.7.1.

**Results**

**Basic information on quantitative proteomic analysis of PPRV-infected Vero cells**

To explore and identify the acetylated host proteins or pathways involved in the PPRV replication process, we chose 24 h p.i. as the time point for quantitative proteomic analysis according the result of western blotting (Additional file 1: Figure S1), and conducted a comprehensive proteome analysis to detect acetylated proteins between the infected and control samples. To assess repeatability among the 3 biological replicates, Pearson’s correlation coefficients were generated and calculated between two samples, separately, and repeatability ranged from 0.78 to 0.81 for the global proteome (Fig.
The distribution of mass error was close to zero and most of them were less than 5 ppm (Fig. 1B). Consistent with the characteristics of tryptic peptides, most peptides were in the size range from 7 to 21 amino acids (Fig. 1C). In total, 5596 proteins were identified by LC–MS/MS, of which 4729 could be quantified. Based on the infection/control ratio, differential expression analysis revealed that 65 proteins were found up-regulated and 619 were down-regulated in infected group respectively (fold-change >1.5, P <0.05) (Fig. 2, Additional file 2: Table S1). To elucidate the function and distribution of differentially abundant proteins (80%) in Vero cells response to PPRV infection, GO classification, protein domain, protein annotation, and subcellular localization analyses were performed. The results showed that most proteins located in the cytoplasm and nucleus, and mainly involved in binding, catalytic activity, cellular and metabolic processes. Detail information was shown in Fig. 3, Figure S2 and Table S2.
Fig. 1 Pearson’s correlation analysis for quantitative proteome and QC validation of MS data. A Pearson’s correlation of protein quantitation. B Mass error of all identified peptides. C Length distribution of all identified peptides.
**Fig. 2** The number of differentially abundant proteins in different comparisons

**Fig. 3** Enrichment analysis of proteins related to PPRV in Vero cell.

A GO enrichment analysis. B KEGG pathway enrichment analysis. C Domain enrichment analysis

**Global detection of lysine acetylated sites on PPRV-infected Vero cellular proteins**

Lysine acetylation can alter the structure or function of proteins involved in diverse biological processes. To obtain a comprehensive view of the level of protein acetylation in PPRV-infected Vero...
cell, enriched analysis of acetylated peptides was performed to identify the proteins with differential acetylated sites using LC-MS/MS. The near-zero distribution of mass error and that the errors were predominantly < 0.02 Da, indicated a high degree of accuracy for identification of the modified peptides (Additional file 1: Figure S3A). Most of the enriched lysine-acetylated peptide lengths were in the range of 7-21 segments, which are consistent with cutting by trypsin at lysine residue sites (Additional file 1: Figure S2B). Repeatability among the 3 biological replicates ranged from 0.56 to 0.61 for the acetyl-proteome (Additional file 1: Figure S3C).

In total, 3229 lysine-acetylated sites belonging to 1315 proteins were identified, of which 2641 modification sites in 1068 proteins were quantifiable (Additional file 2: Table S3). Among these proteins, about 686 (52.16%) included a single acetylation site, 267 (20.30%) included two sites, 124 (9.43%) included three sites, 72 (5.48%) included four sites, and 166 (12.62%) included five or more than five sites (Fig. 4). 107 lysine acetylation sites were found on histone proteins, including 13 sites on H1, 12 sites on H2A, 13 sites on H2B, 9 sites on H3 and 8 sites on H4. These results provide a comprehensive overview of the acetylation events in PPRV-infected Vero cell.

![Fig. 4 Profile of identified acetylated sites and proteins in PPRV-infected Vero cell](image)

Based on a threshold of 1.2-fold changes and t test p< 0.05 as standards, differentially acetylated proteins (DAcPs) in different comparison were obtained as shown in Table 1. 410 acetylated sites in 304 proteins can respond to the PPRV infection (Additional file 1: Table S4). 126 acetylated sites of 109 proteins were up-regulated, and 284 acetylated sites of 195 proteins were down-regulated. Most of these proteins were modified at single acetylation site. 79 of these proteins were modified at
multiple lysine acetylation sites, including PDIA4 (7 sites), vimentin (6 sites), plectin (5 sites),
nucleolin (NCL, 4 sites) and molecular chaperones. Heat shock proteins (Hsps), including HspA8,
HspA5, HspA9, Hsp90AB1 and Hsp90B1, were acetylated at 8, 3, 2, 3 and 3 sites, respectively. Two
different sites were acetylated in six paralogous subunits of chaperonin TRiC (also called CCT).

Table 1 Summary of differentially quantified acetylated sites and proteins in different comparisons

| Compared group       | Up-regulated (>1.2) | Down-regulated (<1/1.2) |
|----------------------|----------------------|-------------------------|
| Infected-1/control-1 | Sites: 501           | Proteins: 351           |
|                      |                      |                         |
|                      | Sites: 445           | Proteins: 311           |
| Infected-2/control-2 |                      |                         |
|                      | Sites: 460           | Proteins: 330           |
|                      |                      |                         |
|                      | Sites: 126           | Proteins: 109           |
| Infected/control*    |                      |                         |

* The differentially acetylated sites and proteins were quantified in the 3 biological replicates.

Functional, subcellular localization and COG classification of differential acetylated proteins

To better understand the potential functions of lysine acetylation in PPRV-infected cells, all DAcPs
were classified by GO functional classification analysis based on their biological process, molecular
function, subcellular localization and COG/KOG categories (Fig. 5, Additional file 2: Table S5).
Fig. 5 Functional classification of the identified acetylated proteins in PPRV-infected Vero cell. A GO classification of the identified acetylated proteins in three categories: biological process, cellular component and molecular function. B Subcellular localization. C COG/KOG classification.

The result of GO classification is shown in Fig. 5A. In the classification of biological processes, two major classes of acetyl-proteins are associated with cellular and metabolism process, accounting for 34% and 30% of the total DAcPs, respectively. The classification result for molecular function showed that the acetylated proteins were mostly related to binding and catalytic activity, and the percentage of proteins in the two classes were 58% and 30%, respectively. Most of the DAcPs were distributed in the cytoplasm (43%), nucleus (25%), mitochondria (15%) and extracellular (7%) (Fig. 5B), respectively. Furthermore, from results of COG/KOG classification, a total of 306 DAcPs were successfully annotated to 4 categories (Fig. 5C). 117 DAcPs (33%) were involved in cellular processes and signaling, and 42% of these proteins played roles in posttranslational modification, protein turnover and chaperones. 99 DAcPs (28%) were related to information storage and processing, and 64% of these proteins acted on translation, ribosomal structure and biogenesis (32%), RNA processing and modification (32%), respectively. All classification results of the
up-regulated acetylated proteins were similar to that of the down-regulated acetylated proteins.

**Motifs of acetylated peptides**

Amino acid residues surrounding central lysine acetylation residues have specific patterns and preferences in both eukaryotes and prokaryotes [35]. Thus, to determine the characteristics of the acetylated lysine in PPRV-infected Vero cell, the sequence motif surrounding the specific acetylated sites was investigated using the Motif-X program with a significance threshold of p<0.000001. The amino acids flanking the acetylated sites were matched to the whole size, and the motif enrichment was illustrated in the form of a heat map.

Of all acetylated peptides, thirteen significantly enriched lysine acetylation site motifs from 2489 modified sites were identified among the amino acids from the −10 to +10 positions flanking the acetylated lysine site (Fig. 6). These motifs are KacK, KacS, KacF, KacH, KacR, KacT, KacN, KacL, KacV, KacG, KacI, KacD and Kac***K (Kac: acetylated lysine; *: residue of a random amino acid; Figure 6A). The 2489 modified sites accounted for 86.6% of the sites identified according to the criteria of specific amino acid sequence. According to the position and other properties of the residues around the acetylated lysine, there was a significant enrichment of lysine (K), serine (S), phenylalanine (F), histidine (H), arginine (R), threonine (T), asparagine (N), leucine(L), valine (V), glycine (G), or isoleucine (I) at position +1 (75.4%, Fig. 6C).
Fig. 6 Characterization of acetylated peptides. A Probability sequence motifs of acetylation sites consisting of 20 residues surrounding the targeted lysine residue using Motif-X. Thirteen significantly enriched acetylation site motifs were identified. B Heat map showing upstream (red) or downstream (green) of amino acid compositions around the acetylated lysine site (10 amino acids upstream and downstream of the acetylated lysine site). C Number of identified peptides possessing acetylated lysine in each motif.

Enrichment analysis of differential acetylated proteins

In order to further explore the functions of the acetylated proteins under PPRV infection, GO enrichment (cellular component, biological process and molecular function), KEGG and protein domain analysis were performed for all the identified proteins with differential acetylated sites (Fig. 7, Additional file 2: Table S6).
The GO enrichment result of acetylated proteins was showed in Fig. 7A. The cellular components mainly enriched in MCM complex. In the molecular function category, unfolded protein binding and helicase activity were found to be significantly enriched (Fig. 7A). The KEGG database was used to identify the pathway involved in these DAcPs. Interestingly, there were only two identified enriched processes (single-organism carbohydrate catabolic process and DNA metabolic process) (Fig. 7A). These acetylated proteins were involved in protein processing in endoplasmic reticulum (ER) (Fig. 7B). 17 of the DAcPs were involved in protein processing in ER, and 12 of the 17 proteins were down-regulated. The protein domain analysis showed that a large proportion of succinylated proteins were mainly associated with GroEL-like domain, TCP-1-like chaperonin intermediate domain, minichromosome maintenance (MCM) domain and DEAD/DEAH box helicase domain (Fig. 7C).

Protein–protein interaction network analysis of differentially acetylated proteins

To better understand how lysine acetylation regulates diverse metabolic processes and cellular functions, we assembled the PPI networks of the identified modified proteins. In total, 147 DAcPs were mapped to the protein network database. The global network graph of these interactions was shown in Fig. 8 and Table S7. As indicated in Fig. 8, five highly connected subnetworks, ribosome, proteasome, spliceosome, protein processing in ER and DNA replication of DAcPs were enriched. In
the first subnetwork, 19 proteins with 96 Kac sites and 484 direct physical interactions participated in the ribosome, suggesting that they play key roles in protein synthesis. The second subnetwork is related to 26S proteasome and chaperone, and comprised 14 proteins with 75 Kac sites and 184 direct physical interactions. Among the 14 proteins, six subunits of 26S proteasome and five paralogous subunits of chaperonin containing T-complex polypeptide-1 (CCT, also called TRiC) were identified as DAcPs for PPRV infection: proteasome 20S subunit (PSMA4, PSMA5, and PSMB3), proteasome 19S subunits (PSMC5, PSMD11 and PSMD13) and CCT subunit (CCT3, CCT4, CCT5, CCT6A and CCT7) indicated in Figure 8, many proteins were involved in multiple interactions. Moreover, in the ribosome and proteasome subnetworks, a tight protein–protein interaction network including 8 up-regulated proteins and 25 down-regulated proteins, were significantly enriched. Most of the proteins in the PPI network contained more than two acetylated sites. Overall, these results suggest a complicated interaction among acetylated proteins that might control the disease response or resistance during PPRV infection.
Discussion

Pathogen infection can regulate biological processes of the host at various levels that can lead to major changes in the proteome, involved at transcriptional, post-transcriptional, translational, and post-translational levels. As a widespread PTM, lysine acetylation plays central roles in regulating multiple biological processes, especially for metabolism [12]. In recent years, more and more protein acetylation on lysine residues have been identified. Lysine acetylation has diverse biological functions in regulating virus replication, transcription and immune evasion [32, 33, 36]. Up to date, although comparative transcriptome and proteomic profiling was analyzed in bone marrow-derived dendritic cells (BMDCs) or peripheral blood mononuclear cells (PBMCs) stimulated with PPRV [37-39], little is known about lysine acetylation in cells infected by PPRV. In the present study, combined proteomics and acetylome analysis approaches based on affinity purification and
LC–MS/MS were performed to elucidate the effects of PPRV infection on the protein lysine acetylation profiles of Vero cells in order to gain a great insight into PPRV pathogenesis. This is the first systematic analysis of lysine acetylation in Vero cells infected with PPRV, providing an important starting point for the functional studies on the acetylated proteins responding to PPRV infection.

Totally, we identified 3229 acetylation sites in 1315 proteins, and 2641 quantifiable modification sites in 1068 proteins regulated in viral infected cells. Statistics of proteins with differentially acetylated sites showed that 304 proteins with 410 acetylation sites were significant acetylated in response to PPRV infection. Of these DAcPs, PDIA4, vimentin, plectin, NCL, molecular chaperones and Heat shock proteins (Hsps) were modified at multiple lysine acetylation sites. PDIA4 is one of the largest PDI members comprising 645 amino acids and is important for protein homeostasis. siRNAs specific to PDIA4 significantly reduced the replication of influenza viruses [40]. All acetylation sites of PDIA were significantly down-regulated in this study, suggesting that widespread processes were regulated and the cellular homeostasis might be broken down due to PPRV replication. Vimentin, one of the most widely expressed intermediate filament proteins, has been reported to play important roles in viral infection by interacting with viral proteins. The binding of vimentin to porcine reproductive and respiratory syndrome virus (PRRSV), Japanese encephalitis virus, parvovirus, enterovirus 71 and SARS-CoV were shown to facilitate viral internalization and entry [41-44]. The interaction of viral proteins of Bluetongue virus, dengue virus and transmissible gastroenteritis virus with vimentin are necessary for virus replication [45-49]. Rearrangement of cytosolic vimentin and formation of vimentin cages around the viral factories during African swine fever virus and vaccinia virus infection were beneficial to prevent movement of viral components into the cytoplasm and contributes to the assembly efficiency and virus replication [50, 51]. NCL is an important multifunctional protein in eukaryotic cells. It plays important roles in the entry, replication and intracellular transport of many viruses. The localization of nucleolin is changed in the
virus infected cells. NCL interacts with PPRV N protein and indirectly inhibits the growth of PPRV by stimulating the interferon (IFN) pathway [52]. By contrast, NCL facilitates the entry of influenza A viruses and enterovirus (EV)71 [53, 54]. Acetylated NCL is present in speckle structures in the nucleoplasm and co-localized with the splicing factor SC35, this suggests that NCL may be involved in pre-mRNA synthesis or metabolism [55]. These results suggested that the acetylated proteins with an extensive range of biological functions participate in PPRV entry and replication.

We found the acetylation sites identified display a similar sequence motif to that of the previous reports by assessing the enriched motifs of acetylated peptides [33, 56, 57]. We also observed that residues flanking acetylation sites are highly enriched in lysine at the +1 position, and frequently contain lysine (K), serine (S), phenylalanine (F), histidine (H) and arginine (R). The GO functional classification and subcellular localization analysis showed that the identified acetylated proteins in PPRV-infected cells are mainly involved in cellular, metabolism and single-organism processes, and related to binding and catalytic activity. In addition, proteins related to MCM complex were highly enriched, suggesting the acetylation level of these proteins was widely altered by viral infection. This is in agreement with the molecular function category and protein domain enrichment results. MCM complex, a cellular DNA replication licensing factor is required for successful viral genome replication [58]. The complex composing MCM2 to MCM7 (MCM2–7), is a part of the viral chromatin at the replication origin in the terminal repeat region, unwinds DNA to initiate replication and acts as a helicase on elongating DNA [59, 60]. However, the acetylated MCM3 inhibits the initiation of DNA replication and cell cycle progression [61]. ER is critical for protein synthesis and maturation and resides on many molecular chaperones that assist protein folding and assembly. Virus infection can alter ER and activate the unfolded-protein response to facilitate viral replication [62, 63]. The KEGG pathway enrichment analysis indicated that differentially down-regulated proteins might play a major role in PPRV infection. Based on the results in this study, the acetylation of chaperonins and cytoskeleton proteins potentially play vital roles in virus infection, and the
relationship of PPRV infection and differentially acetylated proteins requires further investigations.

PPIs are critical for various biological processes. Our study provided the first global PPI network of acetylated proteins induced by PPRV infection. A variety of interactions in cells were modulated upon viral infection at the acetylation level. The subnetworks of ribosome and proteasome were enriched in this study, indicating the critical role of these acetylated proteins in response to PPRV infection. The ubiquitin-proteasome system (UPS) regulates the expression levels of cellular proteins by ubiquitination of protein substrates followed by their degradation via the proteasome. Viruses subvert or manipulate this cellular machinery to favor viral propagation and to evade host immune response. The UPS participates in viral propagation and acts as a double-edged sword in viral pathogenesis [64-66]. 26S proteasome is the major molecular machine that is responsible for protein degradation in eukaryotes. Six UPS-linked proteins were identified as DAcPs following PPRV infection: proteasome 20S subunit (PSMA4, PSMA5, and PSMB3), proteasome 19S subunit (PSMC5, PSMD11 and PSMD13). Among these subunits, four acetylated subunits were up-regulated, suggesting viral proteins might be degraded by UPS to limit PPRV infection. The CCT chaperonin mediates protein folding and is essential for the assembly of functional viral replicons. CCT acts as molecular chaperonin [67, 68] and involves in synergistic immunity [69], apoptosis [70, 71] and cell-cycle regulation[72]. With the increasing studies, it is found that molecular chaperones play an important role in the life cycles of viruses, including virus entry, replication, transcription, translation, virion assembly and even viral cell-cell movement[73-77]. In the present study, two different sites were acetylated in six paralogous subunits of CCT, the acetylation level of these CCT subunits were decreased in PPRV-infected cells, suggesting that an alteration of transcriptional response triggered by chaperones has happened, which might be able to alter PPRV infection. The proteasome subnetwork is consistent with KEGG analysis. Mutual corroboration between the results of subnetwork proteasome and KEGG pathway enrichment confirmed the reliability of analysis. The comprehensive study of the relationship between PPRV and these chaperones is required and will
open a new way for the understanding of pathogenesis, prevention and control of PPR.

Histone modification is one typical way of epigenetic modifications. Increasing evidence indicates that histone acetylation modification has an important role in virus infection. Several previous studies have reported virus induced changes in histone lysine acetylation sites, such as adenovirus [78], borna disease virus [31, 79], Influenza virus [30, 80], HIV [81], Zaire Ebolavirus [82], bovine herpesvirus [83], parvovirus [84]. In this study, nine histone lysine acetylation sites were significantly regulated, of which eight sites were down-regulated. This result hints that histone acetylation modification might also exert certain functions in Vero cells upon PPRV infection. It is worthy to make an intensive investigation to explore the precise biological function of these acetylated proteins in PPRV infection.

**Conclusion**

Quantitative proteomics was carried out to profile the acetylome of PPRV-infected Vero cells. We identified 304 proteins with 410 acetylation sites, which were significantly acetylated in response to PPRV infection. The differentially acetylated proteins primarily participated in carbohydrate catabolic and DNA metabolic process, suggesting that intracellular activities were extensively changed after PPRV infection. PPI network further indicated that a variety of chaperone and ribosome processes were modulated by acetylation. To our knowledge, this is the first study on acetylome in Vero cell with PPRV infection. It provides an important starting point for future studies for the acetylated proteins involved in the host response to PPRV replication.

**Abbreviations**

PPRV: Peste des petits ruminants virus; PTM: Post-translational modification; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; MS: Mass spectrometry; DAcPs: the differentially acetylated proteins.

**Supplementary Information**: Data are available via ProteomeXchange with identifier PXD025081.

**Additional file 1**: Figure S1. Western blot with anti-pan acetyllysine antibody in response to PPRV infection in Vero cell. Figure S2. GO Classification and Subcellular locations of all the identified proteins. a. Biological process; b. Cellular component; c. Molecular function; d. Subcellular locations. Figure S3. Pearson’s correlation
analysis for acetyl-proteome and QC validation of MS data.

Additional file 1: Tables S1. Differentially expressed protein. Tables S2. Protein annotation. Tables S3. Quantitative acetylated sites on proteins. Tables S4. Summary of differentially quantified acetylated sites and proteins in different comparable group. Tables S5. Functional classification of differentially acetylated proteins. Tables S6. Functional enrichment of differentially acetylated proteins. Tables S7. The identified acetylated proteins for PPI.

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Authors’ contributions:

Study design, Meng XL and Zhang ZD. Sample preparation, Zhang R and Zhu XL. Data analysis, Meng XL and Zhu XL. Drafting manuscript, Meng XL. Revising manuscript content, Zhang ZD. Approving final version of manuscript, Meng XL, Zhu XL, Zhang R and Zhang ZD. Meng XL, Zhu XL and Zhang ZD takes responsibility for the integrity of the data analysis.

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Availability of data and materials

All data generated and analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Yes.

Competing interests

The authors declare that there are no conflicts of interest.

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References
1. Baron MD, Diallo A, Lancelot R, Libeau G. Peste des Petits Ruminants Virus. Adv Virus Res. 2016; 95:1-42.

2. Survey on updating OIE World Animal Health Information System. Vet Rec. 2017; 180:386.

3. Fine AE, Pruvot M, Benfield CTO, Caron A, Cattoli G, Chardonnet P, Dioli M, Dulu T, et al. Eradication of Peste des Petits Ruminants Virus and the Wildlife-Livestock Interface. Front Vet Sci. 2020; 7:50.

4. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. Nat Protoc 2013; 8:2281-2308.

5. Marceau CD, Puschnik AS, Majzoub K, Ooi YS, Brewer SM, Fuchs G, Swaminathan K, Mata MA, et al. Genetic dissection of Flaviviridae host factors through genome-scale CRISPR screens. Nature. 2016; 535:159-163.

6. Koujah L, Shukla D, Naqvi AR: CRISPR-Cas based targeting of host and viral genes as an antiviral strategy. Semin Cell Dev Bio 2019; 96:53-64.

7. Liu F, Wu X, Zou Y, Li L, Liu S, Chi T, Wang Z. Small interfering RNAs targeting peste des petits ruminants virus M mRNA increase virus-mediated fusogenicity and inhibit viral replication in vitro. Antivir Res 2015; 123:22-26.

8. Zhang LK, Chai F, Li HY, Xiao G, Guo L. Identification of host proteins involved in Japanese encephalitis virus infection by quantitative proteomics analysis. J Proteome Res. 2013; 12:2666-2678.

9. Zhen S, Deng X, Wang J, Zhu G, Cao H, Yuan L, Yan Y: First Comprehensive Proteome Analyses of Lysine Acetylation and Succinylation in Seedling Leaves of Brachypodium distachyon L. Sci Rep 2016; 6:31576.

10. Zhang Z, Tan M, Xie Z, Dai L, Chen Y, Zhao Y. Identification of lysine succinylation as a new post-translational modification. Nat Chem Biol 2011; 7:58-63.

11. Chen L, Keppeler OT, Scholz C. Post-translational Modification-Based Regulation of HIV Replication. Front Microbiol. 2018; 9:2131.

12. Choudhary C, Kumar C, Gnadt F, Nielsen ML, Rehman M, Walther TC, Olsen JV, Mann M. Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science. 2009; 325:834-840.

13. Yang F. Post-translational Modification Control of HBV Biological Processes. Front Microbiol. 2018,
1. Liu J, Qian C, Cao X. Post-Translational Modification Control of Innate Immunity. Immunity. 2016; 45:15-30.

2. Zhou Y, He C, Wang L, Ge B. Post-translational regulation of antiviral innate signaling. Eur J Immunol. 2017; 47:1414-1426.

3. Zhang K, Chen Y, Zhang Z, Zhao Y. Identification and verification of lysine propionylation and butyrylation in yeast core histones using PTMap software. J Proteome Res. 2009; 8:900-906.

4. Zhang J, Sprung R, Pei J, Tan X, Kim S, Zhu H, Liu CF, Grishin NV, Zhao Y. Lysine acetylation is a highly abundant and evolutionarily conserved modification in Escherichia coli. Mol Cell proteomics. 2009; 8:215-225.

5. Peng C, Lu Z, Xie Z, Cheng Z, Chen Y, Tan M, Luo H, Zhang Y, et al. The first identification of lysine malonylation substrates and its regulatory enzyme. Mol Cell proteomics. 2011; 10:M111 012658.

6. Mattiroli F, Sixma TK. Lysine-targeting specificity in ubiquitin and ubiquitin-like modification pathways. Nat Struct Mol Biol. 2014; 21:308-316.

7. Tan M, Luo H, Lee S, Jin F, Yang JS, Montellier E, Buchou T, Cheng Z, et al. Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. Cell. 2011; 146:1016-1028.

8. Hirschey MD, Zhao Y. Metabolic Regulation by Lysine Malonylation, Succinylation, and Glutarylation. Mol Cell proteomics. 2015; 14:2308-2315.

9. Kim GW, Yang XJ. Comprehensive lysine acetylomes emerging from bacteria to humans. Trends Biochem Sci. 2011; 36:211-220.

10. Kwon OK, Kim S, Lee S. Global proteomic analysis of lysine acetylation in zebrafish (Danio rerio) embryos. Electrophoresis. 2016; 37:3137-3145.

11. Meng Q, Liu P, Wang J, Wang Y, Hou L, Gu W, Wang W. Systematic analysis of the lysine acetylome of the pathogenic bacterium Spiroplasma eriocheiris reveals acetylated proteins related to metabolism and helical structure. J proteomics. 2016; 148:159-169.

12. Zhou H, Finkemeier I, Guan W, Tossounian MA, Wei B, Young D, Huang J, Messens J, et al. Oxidative
stress-triggered interactions between the succinyl- and acetyl-proteomes of rice leaves. Plant Cell Environ. 2018; 41:1139-1153.

26. Liu Z, Cao J, Gao X, Zhou Y, Wen L, Yang X, Yao X, Ren J, Xue Y. CPLA 1.0: an integrated database of protein lysine acetylation. Nucleic Acids Res. 2011; 39:D1029-1034.

27. Kwon OK, Sim J, Kim SJ, Oh HR, Nam DH, Lee S. Global proteomic analysis of protein acetylation affecting metabolic regulation in Daphnia pulex. Biochimie. 2016; 121:219-227.

28. Cheng J, Yang H, Fang J, Ma L, Gong R, Wang P, Li Z, Xu Y. Molecular mechanism for USP7-mediated DNMT1 stabilization by acetylation. Nat Commun. 2015; 6:7023.

29. Feng Q, Su Z, Song S, Chiu H, Zhang B, Yi L, Tian M, Wang H. Histone deacetylase inhibitors suppress RSV infection and alleviate virus-induced airway inflammation. Int J Mol Med. 2016; 38:812-822.

30. Zhao D, Fukuyama S, Sakai-Tagawa Y, Takashita E, Shoemaker JE, Kawaoka Y. C646, a Novel p300/CREB-Binding Protein-Specific Inhibitor of Histone Acetyltransferase, Attenuates Influenza A Virus Infection. Antimicrob Agents Ch 2015; 60:1902-1906.

31. Liu X, Zhao L, Yang Y, Bode L, Huang H, Liu C, Huang R, Zhang L, et al. Human borna disease virus infection impacts host proteome and histone lysine acetylation in human oligodendroglia cells. Virology. 2014; 464-465:196-205.

32. Giese S, Ciminski K, Bolte H, Moreira EA, Lakdawala S, Hu Z, David Q, Kolesnikova L, et al. Role of influenza A virus NP acetylation on viral growth and replication. Nat Commun. 2017; 8:1259.

33. Murray LA, Sheng X, Cristea IM. Orchestration of protein acetylation as a toggle for cellular defense and virus replication. Nat Commun. 2018; 9:4967.

34. Meng X, Dou Y, Cai X. Ultrastructural features of PPRV infection in Vero cells. Virol Sin. 2014; 29:311-313.

35. Zhou X, Qian G, Yi X, Li X, Liu W. Systematic Analysis of the Lysine Acetylome in Candida albicans. J Proteome Res. 2016; 15:2525-2536.

36. Wei ZQ, Zhang YH, Ke CZ, Chen HX, Ren P, He YL, Hu P, Ma DQ, et al. Curcumin inhibits hepatitis B virus infection by down-regulating cccDNA-bound histone acetylation. World J Gastroentero. 2017; 23:6252-6260.
37. Manjunath S, Mishra BP, Mishra B, Sahoo AP, Tiwari AK, Rajak KK, Muthuchelvan D, Saxena S, et al. Comparative and temporal transcriptome analysis of peste des petits ruminants virus infected goat peripheral blood mononuclear cells. Virus Res. 2017; 229:28-40.

38. Li L, Wu J, Cao X, Zhou J, Yin S, Yang S, Feng Q, Du P, Liu Y, Shang Y et al. Proteomic analysis of murine bone marrow derived dendritic cells in response to peste des petits ruminants virus. Res Vet Sci. 2019; 125:195-204.

39. Li L, Wu J, Liu D, Du G, Liu Y, Shang Y, Liu X. Transcriptional Profiles of Murine Bone Marrow-Derived Dendritic Cells in Response to Peste des Petits Ruminants Virus. Vet Sci. 2019, 6:95.

40. Kim Y, Chang KO. Protein disulfide isomerases as potential therapeutic targets for influenza A and B viruses. Virus Res. 2018; 247:26-33.

41. Kim JK, Fahad AM, Shanmukhappa K, Kapil S. Defining the cellular target(s) of porcine reproductive and respiratory syndrome virus blocking monoclonal antibody 7G10. J Virol. 2006; 80:689-696.

42. Das S, Ravi V, Desai A. Japanese encephalitis virus interacts with vimentin to facilitate its entry into porcine kidney cell line. Virus Res. 2011; 160:404-408.

43. Du N, Cong H, Tian H, Zhang H, Zhang W, Song L, Tien P. Cell surface vimentin is an attachment receptor for enterovirus 71. J Virol. 2014; 88:5816-5833.

44. Yu YT, Chien SC, Chen IY, Lai CT, Tsay YG, Chang SC, Chang MF. Surface vimentin is critical for the cell entry of SARS-CoV. J Biomed Sci. 2016; 23:14.

45. Bhattacharya B, Noad RJ, Roy P. Interaction between Bluetongue virus outer capsid protein VP2 and vimentin is necessary for virus egress. Virol J. 2007; 4:7.

46. Chen W, Gao N, Wang JL, Tian YP, Chen ZT, An J. Vimentin is required for dengue virus serotype 2 infection but microtubules are not necessary for this process. Arch Virol. 2008; 153:1777-1781.

47. Fay N, Pante N. The intermediate filament network protein, vimentin, is required for parvoviral infection. Virology. 2013; 444:181-190.

48. Teo CS, Chu JJ. Cellular vimentin regulates construction of dengue virus replication complexes through interaction with NS4A protein. J Virol. 2014; 88:1897-1913.
49. Zhang X, Shi H, Chen J, Shi D, Dong H, Feng L. Identification of the interaction between vimentin and nucleocapsid protein of transmissible gastroenteritis virus. Virus Res. 2015; 200:56-63.

50. Risco C, Rodriguez JR, Lopez-Iglesias C, Carrascosa JL, Esteban M, Rodriguez D. Endoplasmic reticulum-Golgi intermediate compartment membranes and vimentin filaments participate in vaccinia virus assembly. J Virol. 2002; 76:1839-1855.

51. Stefanovic S, Windsor M, Nagata KI, Inagaki M, Wileman T. Vimentin rearrangement during African swine fever virus infection involves retrograde transport along microtubules and phosphorylation of vimentin by calcium calmodulin kinase II. J Virol. 2005; 79:11766-11775.

52. Dong D, Zhu S, Miao Q, Zhu J, Tang A, Qi R, Liu T, Yin D, Liu G. Nucleolin (NCL) inhibits the growth of peste des petits ruminants virus. J Gen Virol. 2020; 101:33-43.

53. Su PY, Wang YF, Huang SW, Lo YC, Wang YH, Wu SR, Shieh DB, Chen SH, et al. Cell surface nucleolin facilitates enterovirus 71 binding and infection. J Virol. 2015; 89:4527-4538.

54. Yan Y, Du Y, Wang G, Li K. Non-structural protein 1 of H3N2 influenza A virus induces nucleolar stress via interaction with nucleolin. Sci Rep. 2017; 7:17761.

55. Das S, Cong R, Shandilya J, Senapati P, Moindrot B, Monier K, Delage H, Mongelard F, et al. Characterization of nucleolin K88 acetylation defines a new pool of nucleolin colocalizing with pre-mRNA splicing factors. FEBS Lett. 2013; 587:417-424.

56. Svinkina T, Gu H, Silva JC, Mertins P, Qiao J, Fereshetian S, Jaffe JD, Kuhn E, et al. Deep, Quantitative Coverage of the Lysine Acetylome Using Novel Anti-acetyl-lysine Antibodies and an Optimized Proteomic Workflow. Mol Cell proteomics. 2015; 14:2429-2440.

57. Pehar M, Ball LE, Sharma DR, Harlan BA, Comte-Walters S, Neely BA, Vargas MR. Changes in Protein Expression and Lysine Acetylation Induced by Decreased Glutathione Levels in Astrocytes. Mol Cell proteomics. 2016; 15:493-505.

58. Kawaguchi A. [Dynamics of the influenza virus genome regulated by cellular host factors]. Virology. 2017; 67:59-68.

59. Ganaie SS, Zou W, Xu P, Deng X, Kleiboeker S, Qiu J. Phosphorylated STAT5 directly facilitates parvovirus
B19 DNA replication in human erythroid progenitors through interaction with the MCM complex. PLoS Pathog. 2017; 13:e1006370.

Dabral P, Uppal T, Rossetto CC, Verma SC. Minichromosome Maintenance Proteins Cooperate with LANA during the G1/S Phase of the Cell Cycle To Support Viral DNA Replication. J Virol. 2019; 93: e02256.

Takei Y, Assenberg M, Tsujimoto G, Laskey R. The MCM3 acetylase MCM3AP inhibits initiation, but not elongation, of DNA replication via interaction with MCM3. J Biol Chem. 2002; 277:43121-43125.

Jheng JR, Wang SC, Jheng CR, Horng JT. Enterovirus 71 induces dsRNA/PKR-dependent cytoplasmic redistribution of GRP78/BiP to promote viral replication. Emerg Microbes Infec. 2016; 5:e23.

Montalbano R, Honrath B, Wissniowski TT, Elxnat M, Roth S, Ocker M, Quint K, Churin Y, et al. Exogenous hepatitis B virus envelope proteins induce endoplasmic reticulum stress: involvement of cannabinoid axis in liver cancer cells. Oncotarget. 2016; 7:20312-20323.

Luo H. Interplay between the virus and the ubiquitin-proteasome system: molecular mechanism of viral pathogenesis. Curr Opin Virol. 2016; 17:1-10.

Kong F, You H, Kong D, Zheng K, Tang R. The interaction of hepatitis B virus with the ubiquitin proteasome system in viral replication and associated pathogenesis. Virol J. 2019; 16;17:73.

Yeom S, Jeong H, Kim SS, Jang KL: Hepatitis B virus X protein activates proteosomal activator 28 gamma expression via upregulation of p53 levels to stimulate virus replication. J Gen Virol. 2018; 99:655-666.

Dekker C, Stirling PC, McCormack EA, Filmore H, Paul A, Brost RL, Costanzo M, Boone C, et al. The interaction network of the chaperonin CCT. EMBO J. 2008; 27:1827-1839.

Yam AY, Xia Y, Lin HT, Burlingame A, Gerstein M, Frydman J. Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. Nat Struct Mol Biol.2008; 15:1255-1262.

Pejanovic N, Hochrainer K, Liu T, Aerne BL, Soares MP, Anrather J. Regulation of nuclear factor kappaB (NF-kappaB) transcriptional activity via p65 acetylation by the chaperonin containing TCP1 (CCT). PloS One. 2012; 7:e42020.

Lin YF, Lee YF, Liang PH. Targeting beta-tubulin:CCT-beta complexes incurs Hsp90- and VCP-related
protein degradation and induces ER stress-associated apoptosis by triggering capacitative Ca2+ entry, mitochondrial perturbation and caspase overactivation. Cell Death Dis. 2012; 3:e434.

71. Trinidad AG, Muller PA, Cuellar J, Klejnot M, Nobis M, Valpuesta JM, Vousden KH. Interaction of p53 with the CCT complex promotes protein folding and wild-type p53 activity. Mol Cell. 2013; 50:805-817.

72. Yokota S, Yanagi H, Yura T, Kubota H. Cytosolic chaperonin is up-regulated during cell growth. Preferential expression and binding to tubulin at G(1)/S transition through early S phase. J Biol Chem. 1999; 274:37070-37078.

73. Inoue Y, Aizaki H, Hara H, Matsuda M, Ando T, Shimoji T, Murakami K, Masaki T, et al. Chaperonin TRiC/CCT participates in replication of hepatitis C virus genome via interaction with the viral NS5B protein. Virology. 2011; 410:38-47.

74. Zhang J, Wu X, Zan J, Wu Y, Ye C, Ruan X, Zhou J. Cellular chaperonin CCTgamma contributes to rabies virus replication during infection. J Virol. 2013; 87:7608-7621.

75. Hafirassou ML, Meertens L, Umana-Diaz C, Labeau A, Dejarnac O, Bonnet-Madin L, Kummerer BM, Delaugerre C, et al. A Global Interactome Map of the Dengue Virus NS1 Identifies Virus Restriction and Dependency Host Factors. Cell Rep. 2018; 22:1364.

76. Knowlton JJ, Fernandez de Castro I, Ashbrook AW, Gestaut DR, Zamora PF, Bauer JA, Forrest JC, Frydman J, et al. The TRiC chaperonin controls reovirus replication through outer-capsid folding. Nat Microbiol. 2018; 3:481-493.

77. Wang Q, Huang WR, Chih WY, Chuang KP, Chang CD, Wu Y, Huang Y, Liu HJ. Cdc20 and molecular chaperone CCT2 and CCT5 are required for the Muscovy duck reovirus p10.8-induced cell cycle arrest and apoptosis. Vet Microbiol. 2019; 235:151-163.

78. Horwitz GA, Zhang K, McBryan MA, Grunstein M, Kurdistani SK, Berk AJ. Adenovirus small e1a alters global patterns of histone modification. Science. 2008; 321:1084-1085.

79. Suberbielle E, Stella A, Pont F, Monnet C, Mouton E, Lamouroux L, Monsarrat B, Gonzalez-Dunia D. Proteomic analysis reveals selective impediment of neuronal remodeling upon Borna disease virus infection. J Virol. 2008; 82:12265-12279.
80. Hatakeyama D, Shoji M, Yamayoshi S, Yoh R, Ohmi N, Takenaka S, Sai toh A, Arakaki Y, et al. Influenza A virus nucleoprotein is acetylated by histone acetyltransferases PCAF and GCN5. J Biol Chem. 2018; 293:7126-7138.

81. Jiang G, Nguyen D, Archin NM, Yukl SA, Mendez-Lagares G, Tang Y, Elsheikh MM, Thompson GR, 3rd, et al. HIV latency is reversed by ACSS2-driven histone crotonylation. J Clin Invest. 2018; 128:1190-1198.

82. Hatakeyama D, Ohmi N, Sai toh A, Makiyama K, Morioka M, Okazaki H, Kuzuhara T. Acetylation of lysine residues in the recombinant nucleoprotein and VP40 matrix protein of Zaire Eb olavirus by eukaryotic histone acetyltransferases. Biochem Bioph Res Co. 2018; 504:635-640.

83. Zhu L, Jiang X, Fu X, Qi Y, Zhu G. The Involvement of Histone H3 Acetylation in Bovine Herpesvirus 1 Replication in MDBK Cells. Viruses. 2018; 10:525.

84. Mantyla E, Salokas K, Oittinen M, Aho V, Mantysaari P, Palmujoki L, Kalliolinna O, Ihalainen TO, et al. Promoter-Targeted Histone Acetylation of Chromatinized Parvoviral Genome Is Essential for the Progress of Infection. J Virol. 2016; 90:4059-4066.
Figures

A

![Pearson's correlation analysis for quantitative proteome and QC validation of MS data.](image)

B

![Mass error of all identified peptides.](image)

C

![Length distribution of all identified peptides.](image)

Figure 1

Pearson's correlation analysis for quantitative proteome and QC validation of MS data. A Pearson's correlation of protein quantitation. B Mass error of all identified peptides. C Length distribution of all identified peptides.
Figure 2

The number of differentially abundant proteins in different comparisons
**Figure 3**

Enrichment analysis of proteins related to PPRV in Vero cell.

**Figure 4**
Figure 5

Functional classification of the identified acetylated proteins in PPRV-infected Vero cell. A GO classification of the identified acetylated proteins in three categories: biological process, cellular component and molecular function. B Subcellular localization. C COG/KOG classification.
Characterization of acetylated peptides. A Probability sequence motifs of acetylation sites consisting of 20 residues surrounding the targeted lysine residue using Motif-X. Thirteen significantly enriched acetylation site motifs were identified. B Heat map showing upstream (red) or downstream (green) of amino acid compositions around the acetylated lysine site (10 amino acids upstream and downstream of the acetylated lysine site). C Number of identified peptides possessing acetylated lysine in each motif.
Figure 7

Enrichment of the acetylated proteins related to PPRV in Vero cell. A GO enrichment. B KEGG pathway enrichment. C Domain enrichment.
Figure 8

Representative PPI networks of acetylated proteins