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Human borna disease virus infection impacts host proteome and histone lysine acetylation in human oligodendroglia cells

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ABSTRACT

Background: Borna disease virus (BDV) replicates in the nucleus and establishes persistent infections in mammalian hosts. A human BDV strain was used to address the first time, how BDV infection impacts the proteome and histone lysine acetylation (Kac) of human oligodendroglial (OL) cells, thus allowing a better understanding of infection-driven pathophysiology in vitro.
Methods: Proteome and histone lysine acetylation were profiled through stable isotope labeling for cell culture (SILAC)-based quantitative proteomics. The quantifiable proteome was annotated using bioinformatics. Histone acetylation changes were validated by biochemistry assays.
Results: Post BDV infection, 4383 quantifiable differential proteins were identified and functionally annotated to metabolism pathways, immune response, DNA replication, DNA repair, and transcriptional regulation. Sixteen of the thirty identified Kac sites in core histones presented altered acetylation levels post infection.
Conclusions: BDV infection using a human strain impacted the whole proteome and histone lysine acetylation in OL cells.

Introduction

Borna disease virus (BDV), a member of the family Bornaviridae in the order Mononegavirales, is a neurotropic, enveloped virus with a non-segmented, negative-strand (NNS) ribonucleic acid (RNA) genome (de la Torre, 1994; Schneemann et al., 1995). BDV replicates in the cell nucleus and persistently infects a wide variety of mammal species including humans (Bode and Ludwig, 2003; de La Torre et al., 1996a; Iwata et al., 1998; Kinnunen et al., 2013; Ludwig et al., 1988). One of the first human BDV strains, Hu-H1, was isolated from a bipolar patient's white blood cells and differs genetically and biologically from the laboratory reference BDV strains V and C6BV (Bode et al., 1996; de la Torre et al., 1996b).

Oligodendroglia (OL) cells, a cell line derived from human fetal oligodendrocytes, are a major component of the brain white matter that play a pivotal role in maintaining neurological function. OL cells support both natural and experimental infection with several neurotropic NNS RNA viruses including BDV (Ibrahim et al., 2002; Koster-Patzlaff et al., 2007), canine distemper virus (Muller et al., 1995), and measles virus (Baczko et al., 1988). Laboratory-adapted and wild-type (e.g. human) BDV strains have been shown to differentially affect various host cell types (Li et al., 2013; Poenisch et al., 2009; Williams et al., 2008; Wu et al., 2013). Using a natural human BDV strain appeared particularly advantageous to provide better insight into the pathological consequences of human OL cell infection than laboratory strains from animal origin. Moreover, using a virus which had been isolated during an acute depressive episode

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of a bipolar patient should eventually support our understanding of BDV's impact on neuropathogenesis.

BDV affects the expression of several host mRNA transcripts (Carbone et al., 2001), but the mechanisms remain unclear. The recent discovery of histone lysine acetylation (Kac) as a modulator of gene expression in response to virus infection has brought fresh insight into viral epigenetic regulation (Ferrari et al., 2012). BDV infection has been shown to affect site-specific histone acetylation in cortical neurons in vitro (Suberbielle et al., 2008); however, the histone Kac profile of BDV-infected OL cells remains unknown.

In this study, we hypothesized that BDV infection epigenetically impacts the OL cell proteome through histone Kac. Therefore, using an integrated quantitative proteomic approach assisted by bioinformatic analysis, we comprehensively investigated the proteome profile and constructed a histone Kac atlas of BDV-infected OL cells.

Results

BDV infection of OL/BDV cells

To confirm successful BDV infection of OL/BDV cells, RT-PCR, Western blotting, and immunofluorescence assays were performed to examine the major markers of successful BDV infection – p24 and p40 RNA and protein levels (de la Torre, 1994). Rapid spread of the virus infection was observed in tissue culture; 100% of the cells were infected 14 days post-infection, while 100% of control cells remained non-infected (Supplemental Fig. 1).

Proteomic profiling

We applied SILAC labeling-based proteomics to comparatively quantify the host proteome of OL/BDV cells and control cells. A scheme of the experimental workflow is shown in Fig. 1. In total, 4436 non-redundant proteins were identified, of which 4383 proteins displayed quantifiable differential expression levels in response to BDV infection (Supplemental Table 1). Among these, 1572 proteins displayed a greater than or equal to 1.5-fold increased expression and 165 proteins displayed a lesser than or equal to 1.5-fold decreased expression in response to BDV infection. The proteomic dataset was divided into four quantiles (Q1-Q4) based on the cumulative distribution of SILAC L/H ratios: Q1, less than 15%; Q2, 15–50%; Q3, 50–85%; and Q4, greater than 85%. Enrichment analyses were separately performed in each quantile, and the overrepresented annotations were clustered through one-way hierarchical clustering for comparative analysis.

BDV infection affects transcription factors

We found 201 transcription factors in the whole OL cell proteome (Supplemental Table 2), of which 84 transcription factors showed significantly increased expression and 11 showed significantly decreased expression in response to BDV infection. Only 30 of these significantly differentiated transcription factors were mapped onto the KEGG database. The highest-ranking canonical KEGG pathways are listed in Table 1.

Bioinformatic analysis

We analyzed the quantifiable proteome data set for three enrichment gene ontology (GO) categories: biological process, molecular function, and cellular compartment (Fig. 2A–C). Using Interpro domain enrichment analysis, we analyzed the domain features of those enriched proteins dysregulated by BDV infection (Fig. 2D). We performed a pathway clustering analysis through the

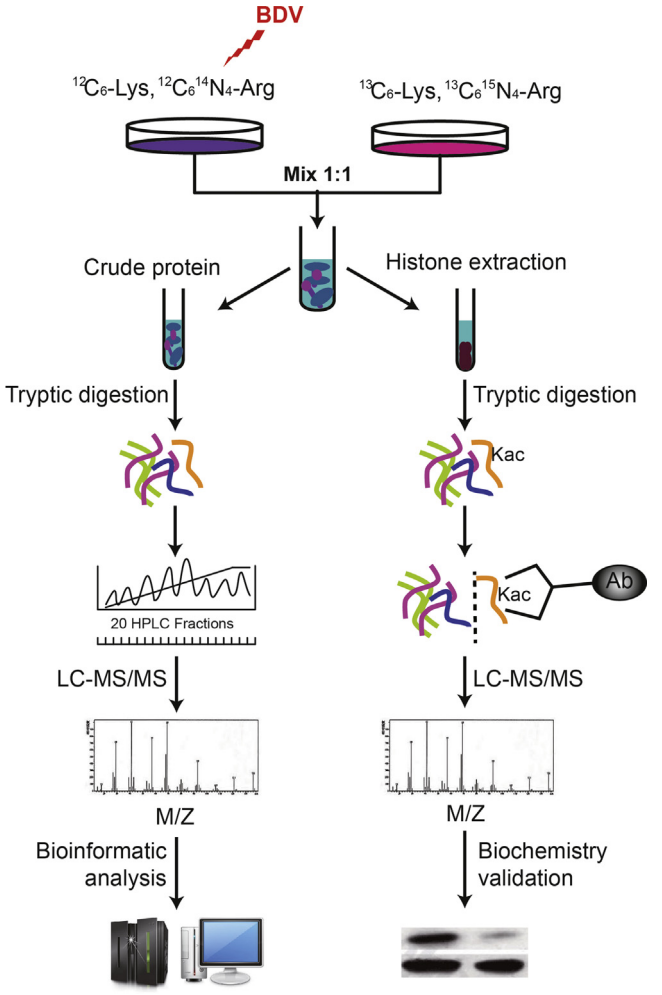

Fig. 1. Experimental workflow. OL/BDV cells and control cells were maintained in SILAC medium. An aliquot of crude proteins from the mixed cells was digested in solution by trypsin. Protein identification and quantification were performed using LC-MS/MS and data inquiry. In parallel, histones from the cell mixture were extracted, digested, and affinity enriched followed by LC-MS/MS analysis. Further biochemistry was applied to validate the MS analysis results.

Table 1
Canonical KEGG pathways associated with the differential transcription factors.

KEGG pathway	Mapping	*P*-value
hsa05202 Transcriptional misregulation in cancer	6	1.84E-06
hsa04380 Osteoclast differentiation	5	4.59E-05
hsa04917 Prolactin signaling pathway	3	2.04E-03
hsa04668 TNF signaling pathway	3	8.18E-03
hsa04630 Jak-STAT signaling pathway	2	1.07E-02
hsa04920 Adipocytokine signaling pathway	2	4.05E-02
hsa04062 Chemokine signaling pathway	3	4.14E-02

Kyoto Encyclopedia of Genes and Genomes (KEGG) (Fig. 2E). Using a manually curated CORUM database, we performed enrichment analysis on protein complexes and used k-means clustering to identify the specific subgroups that were most impacted by BDV infection (Fig. 2F). We obtained two protein complexes (the amyloid precursor protein mitochondrial translocase APP-TOMM40 and IFP35-NMI) enriched in Q4 that are primarily related to mitochondrial protein transport and interferon signaling, respectively (Lee et al., 2012; Zhou et al., 2000), and 17 protein