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Herpes Simplex Virus 1 Infection Alters the mRNA Translation Processing in L-02 Cells*

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Abstract: HSV-1 infection-mediated regulation of mRNA translation in host cells is a systematic and complicated process. Investigation of the details of this mechanism will facilitate understanding of biological variations in the viral replication process and host cells. In this study, a comparative proteomics technology platform was applied by two-dimension electrophoresis of HSV-1 infected normal human L-02 cell and control cell lysates. The observed protein spots were analyzed qualitatively and quantitatively by the PDQuest software package. A number of the different observed protein spots closely associated with cellular protein synthesis were identified by matrix-assisted laser-desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS). The expression levels of the RPLP1 protein, which is required for mRNA translation, and KHSRP protein, which is involved in rapid decay of mRNA, were up-regulated, whereas the expression level of RNP H2, which is involved in positive regulation on the mRNA splicing process, was down-regulated. All of these results suggest that HSV-1 infection can influence cellular protein synthesis via modulation of cellular regulatory proteins involved in RNA splicing, translation and decay, resulting in optimisation of viral protein synthesis when cellular protein synthesis is shut off. Although there is need for further investigations regarding the detailed mechanisms of cellular protein control, our studies provide new insight into the targeting of varied virus signaling pathways involved in host cellular protein synthesis.

Key words: HSV-1; L-02 cell; Comparative proteomics

As a DNA virus with complicated genomic structures (13), Herpes Simplex Virus1 (HSV-1) can interact specifically with host cells in different infectious phases via multifunctional viral proteins, resulting in modulation of the biological characteristics (17) of cells and establishment of latent and lytic infections (15). Although the mechanistic details of these two types of HSV-1 infection remain unclear, some data demonstrate that the monitoring of and switch from host cell to viral protein synthesis during HSV-1 infection are thought to be involved in the process (5). HSV-1 infection appears to functionally

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terminate translation and synthesis of many mRNAs and the linkage between degradation of an entire mRNA and protein synthesis (16). Of course, such common characteristics, which are required by many infectious viruses, have been extensively recognized. However, as far as HSV-1 infection is concerned, the mechanisms of redirecting infected cells to preferentially synthesize viral proteins during the lytic infectious phases are not completely clear. Previous studies have demonstrated that HSV-1 could redirect cellular viral protein synthesis via viral specific proteins, such as the ability of virion host shutoff protein to inhibit normal translation and mRNA synthesis (10). Further investigations of the molecular biology of HSV-1 will likely help elucidate the influences of HSV-1 or other viruses on host cellular functions in the context of multiple pathways and systems. Therefore, it seems wise to use more systematic approaches to study cellular protein translation and mRNA synthesis during HSV-1 infections.

Among investigations of the interaction between HSV-1 and host cells, an analysis based upon proteomics technology has been highlighted for a particular viral protein followed by investigation of other associated and interacting proteins (6, 19). The optimal pathway to systematically analyze mRNA synthesis and translation into proteins in HSV-1 infected cells should be based upon a comparative proteomic technology to analyze global variations in host cells with certain timing and spacing extensions, so as to facilitate phasic and integrated observations. Two-dimension electrophoresis (2-DE), which is currently well developed, is the basis for such a comparative technology. In our work, we performed preliminary analysis of the synthesis variation in HSV-1 infected normal human liver L-02 cells by using 2-DE. The synthesis variation associated with cellular mRNA decay and translation was identified in the process of HSV-1 infection of L-02 cells by 2-DE and matrix-assisted laser-desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) assays. The potential significance of this method is discussed.

MATERIALS AND METHODS

Virus and cells

HSV-1 HF strain was isolated and provided by this lab; normal human liver L-02 cells were used.

Main reagents and instruments

96-well cell culture plates; DMEM media was purchased from Gibco. Urea, thiourea, CHAPS, DTT, SDS, Tris-base, agarose, iodoacetamide, bromophenol blue, glycine, acrylamide, ammonium peroxydisulfate, TEMED, glycerin, and Coomassie brilliant blue G-250 were purchased from Ameresco; Bio-Lyte IPG prepared gel strips (pH3-pH10, 17 cm) and mineral oil were purchased from BIO-RAD; protein marker was obtained from Takara; RNase and DNase were purchased from Sigma.

PROTEAN IEF SYSTEM, PROTEAN II XL Cell, Powerpac HC Power Supply, and PDQUEST 2-D gel analysis software were purchased from BIO-RAD; Millipore ultra filter, MilliQ ultra purified water system and Centrifuge 5804R were purchased from Eppendorf.

Preparation of cell culture and protein samples

L-02 cells in DMEM-10 cell culture media (containing 10% bovine sera, glutamine) were incubated in a 5% CO_2 incubator at 37℃. HSV-1 was inoculated onto L-02 cells at an MOI of 1. Cultures were aspirated at 24 h post-infection and were subjected to

digestion by trypsin enzyme followed by the addition of PBS. The cell pellets were then centrifuged at 1 000 ×g for 10 min and the supernatant was discarded; this was repeated a total of 3 times. The resultant pellet harvested in 1.5mL microtubes was subjected to 3 freeze/thaw cycles in liquid nitrogen and a 37℃ water bath. 60 ~ 100μL lysis buffer (7mol/L Urea, 2 mol/L thiourea, 4% CHAPS, 100mmol/L DTT, 0.2% pH3/10 Bio-Lyte) was added to each lysate of 1×10^6 cells, followed by the addition of 50 μg/mL RNase and 200 μg/mL DNase. The samples were mixed completely and incubated at 4℃ for 30 min. The samples were then centrifuged at 12 500 r/min at 4℃ for 60 min and the resultant supernatant was collected.

1 volume of ice cold acetone was added to 3 volumes of protein extract and incubated at –20℃ for 2 h. The samples were centrifuged at 12 500r/min at 4℃ for 10 min and the supernatant was discarded. 1 mL ice cold acetone was added to each tube for precipitation of protein. The tubes were then subject to two cycles of centrifugation at 4℃ for 10 min. Lysis buffer was added to dissolve the precipitate and the samples were stored at –20℃. Meanwhile, protein samples of normal L-02 cells were similarly prepared as controls.

Determination of protein concentration

The maximum absorption value (λmax) was changed from 465nm to 595 nm by the protein binding to Coomassie Blue G-250. The absorption value at 595nm (A_{595}) was found to be in proportion to the protein concentrations. A protein normalization curve was made by using BSA protein references with a series of concentrations on the X-axis and its corresponding A_{595} values on the Y-axis. Protein concentrations in samples could be determined from this curve.

Two-dimensional electrophoresis (2-DE)

An IPG gel strip was dipped in 350 μL rehydration sampling buffer with a total protein content of 300μg. The following electrophoresis program was set: active rehydration at 50V for 16 h; first-dimension: IEF was run at 10 000V and 60 000 V after removing salt and increasing pressure followed by equilibration for 15 min in equilibrium buffer I and II, respectively.

An equilibrated gel strip was loaded onto a 12% SDS-PAGE gel for the second-dimension: SDS-PAGE began with low voltage at 70V for 30 min and it was gradually increased to 180V. Running was stopped when the dye front had just reached the anode buffer strip. Silver staining was performed on the 2-DE gels after electrophoresis. 2-DE was performed 3 times on both normal and HSV-1 infected L-02 cell lysates.

Image collection and identification of protein spots

Images were collected by scanning proteomic assays of normal and HSV-1 infected L-02 cell lysates, respectively. Different protein spots were analyzed by PDQuest professional software analysis followed by MALDI-TOF-MS identification. The bioinformatic retrieval of the obtained PMF data was processed in the Matrixscience database (http://www. Matrixscience.com).

RESULTS

Protein standard curve and sample detection

The Bradford Standard Curve was made by using a standard protein with known concentrations and their A_{595} values. The amount of protein sampling was calculated based upon the concentrations determined according to the A_{595} values, and the primary protein amount added to the gel strips was adjusted accordingly.

As for the IPG gel strips with lengths of 17cm, pH3-pH10, 100 ~ 300 μg protein in 350 μL sampling volume was required for silver staining.

Variation of protein expression

Samples were collected from HSV-1 infected and control L-02 cells and loaded onto IEF and SDS-PAGE gels respectively. Protein expression assays of HSV-1 infected and control L-02 cells at 24 h post-infection were obtained by silver staining (Fig. 1). The scanned images were processed by PDQuest professional software and 14 different protein spots were noted. The MALDI-TOF-MS assay indicated that among the HSV-1 infected L-02 cell at 24 h post-infection, the expression of 10 proteins were highly enhanced, 2 proteins were strikingly reduced, and 1 additional protein was identified as comparable to the controls (Table 1).

Variation of acidic ribosomal phosphoprotein expression

2-DE immunoblotting of protein extracted at 24 h

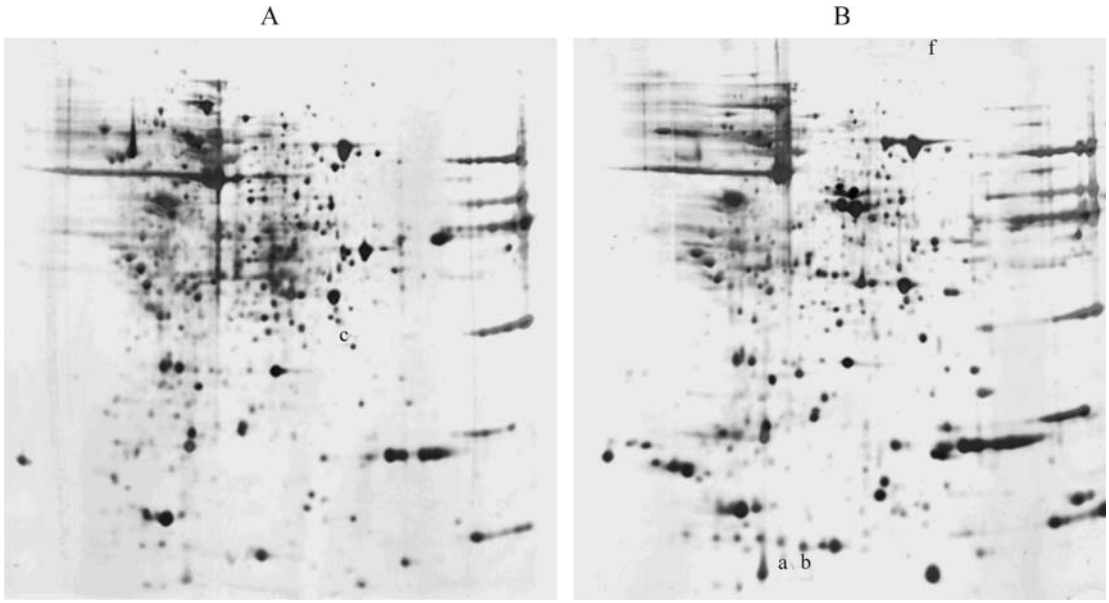

Fig.1. Two-dimensional gel electrophoresis analysis on L-02 cells infected by HSV-1. A: Uninfected L-02 cells. B: HSV-1 infected L-02 cells.

Table 1. Differential expression proteins in L-02 cells induced by HSV Ⅰ infection

Differential protein	Protein spots	Species	Expression level (compared with uninfected cells)
Acidic ribosomal phosphoprotein P1	2	Homo sapiens	increased
UL26.5	2	Human herpesvirus 1	increased
Heterogeneous nuclear ribonucleoprotein H2	1	Homo sapiens	decreased
KH-type splicing regulatory protein	1	Homo sapiens	increased
Unnamed Protein（GI: 16552881）	1	Homo sapiens	increased
Triosephosphate isomerase 1	1	Homo sapiens	decreased
Thioredoxin Peroxidase B	1	Homo sapiens	increased
Pericentrin B	1	Homo sapiens	increased
DBI	1	Homo sapiens	increased
Transmembrane protein 4	1	Homo sapiens	increased
Ubiquitin	1	Homo sapiens	increased
MRCL3 protein	1	Homo sapiens	increased

post-infection revealed the expression levels of 2 proteins in HSV-1 infected L-02 cells were higher than those in control cells (Fig. 2A, 2B). The following MALDI identification and MASCOT retrieval demonstrated that these 2 proteins were acidic ribosomal phosphoproteins (P1) (Fig. 2C and 2D) and their position differences resulted from different modifications.

Variation of hnRNP H2 protein expression

By 2-DE immunoblotting of protein extracted from HSV-1 infected L-02 cells at 24 h post-infection, one protein was inhibited by HSV-1 infection (Fig. 3A and 3B). The following MALDI identification demonstrated that this protein was heterogeneous nuclear ribonucleoprotein H2 (hnRNP H2) (Fig. 3C).

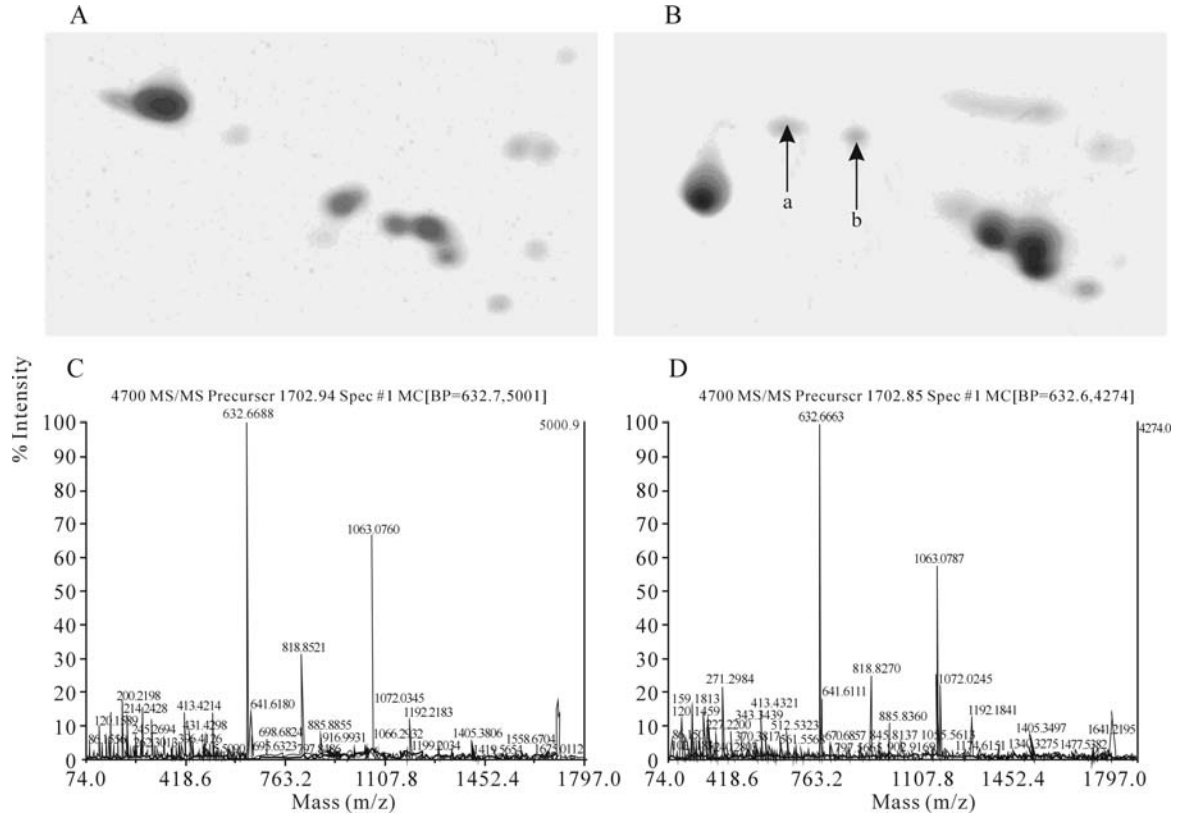

Fig.2. Expression of RPLP1in HSV-1-infected L-02 cells.A: Uninfected L-02 cells. B: HSV-1 infected L-02 cells. C: MALDI-TOF-MS analysis on differential spot A. D: MALDI-TOF-MS analysis on differential spot B.

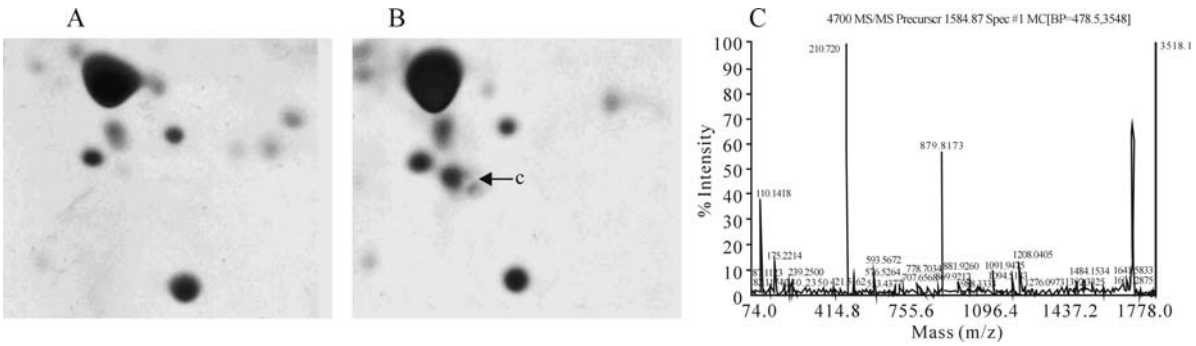

Fig. 3. Expression of hnRNP Hin HSV-1-infected L-02 cells. A: HSV-1 infected L-02 cells. B: Uninfected L-02 cells; C: MALDI-TOF-MS analysis on differential spot c.

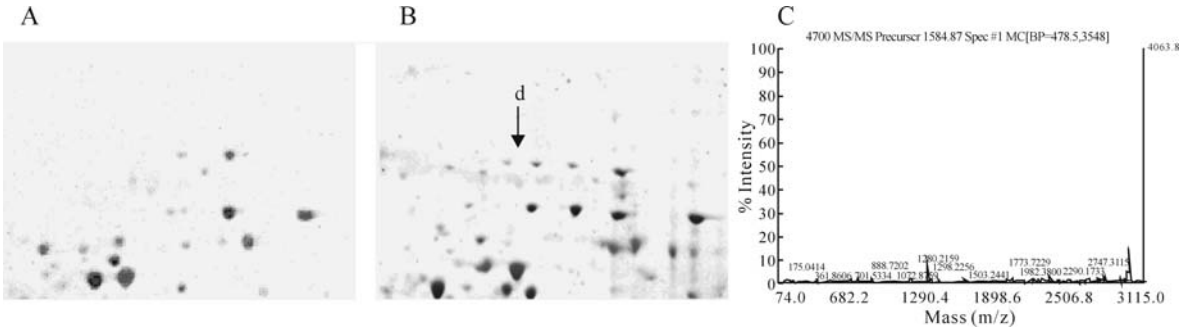

Fig. 4. Expression of KHSRP in HSV-1-infected L-02 cells. A: Uninfected L-02 cells. B: HSV-1 infected L-02 cells. C: MALDI-TOF-MS analysis on differential spot d.

Variation of KHSRP protein expression

A KH-type splicing regulatory protein (KHSRP) identified by the MALDI assay was highly expressed in HSV-1 infected L-02 cells at different times (Fig. 4).

DISCUSSION

Modulation of host cellular protein synthesis is an essential event in most viral infections. This is particularly important for the herpes simplex virus family, including HSV, since accurate control of host cellular protein synthesis is required by the systematic viral pathways involved in the complicated proliferation and regulatory mechanisms of herpes simplex viruses (3). Currently, many studies have provided evidence for such events. For example, it has been reported that all of the transcripts synthesized by 3 cellular transcription enzymes are reduced dramatically to only 50% of the levels found in control cells within 4 h after HSV-1 infection (18). RNA polymerase II is phosphorylated by the immediate early protein ICP 22 and protein kinase UL13 in some cell strains(4); whereas another immediate early protein, ICP27, can directly bind to the Aly/REF protein, which is associated with the splicing complex that colocalizes with sites of HSV-1 transcription(12). Although it is not clear how these events influence host cell pathways, HSV-1 infection has been shown to mediate host cellular protein synthesis via a certain comprehensive pathway. Therefore, the details reported in this study help elucidate mechanisms of this synthesis system.

2-DE immunoblotting of HSV-1 infected L-02 cell lysates revealed expression of tens of different proteins. Several distinct proteins that were associated with cellular protein synthesis are discussed in this paper. Among these, RPLP1 constitutes one of the elements of the lateral stalk in the 60S ribosomal subunit (9) and it was usually phosphorylated after synthesis (11). This protein and P-proteins contain P2 and P0 which are able to bind to 28S mRNA and are actively involved in GTP hydrolysis during protein translation, resulting in direct control of the mRNA translation functions of ribosomes. The expression of RPLP1 is strikingly enhanced when L-02 cells were infected with HSV-1. The up-regulated expression level of the RPLP1 implies that HSV-1-mediated control of cellular protein synthesis contributes to regulation of ribosomes and related functions regardless of the lack of observation of P2 and P0 elements. Furthermore, it is interesting that the expression level of another protein, hnRNP H2, which is responsible for cellular pre-mRNA splicing processing, is down-regulated, whereas the expression level of RPLP1 is

up-regulated. hnRNP H2 is a member of the hnRNP H protein family which contain an RNA recognition motif (RRM) and an A1-binding site (12) that can specifically recognize the sequence GGGA in an intron or exon of pre-mRNA (2) to form pre-mRNA complexes with RNA polymerase II for selective splicing (14). It is of great importance that hnRNP H2 confers a positive effect on cellular gene expression(1). Nevertheless, expression hnRNP H2 in HSV-1 infected L-02 cells is diminished dramatically, sug gesting that expression levels of a number of proteins may vary in HSV-1 infected cells as a consequence of diminished hnRNP H2 expression and inhibition of splicing after pre-mRNA transcription in some cells. Of course, not only are abnormal mRNA synthesis, splicing and translation present during infection, but the variation of mRNA stability may be related to an essential factor that modulates protein synthesis. The expression level of the RNA decay activating factor KHSRP is enhanced during HSV-1 infection. KHSRP promotes rapid mRNA decay by recruiting degradation machinery, including the exosome and poly (A) ribonuclease, to ARE-containing mRNAs (7, 8). It is reported that decay of mRNAs is dramatically accelerated in HSV-1 infected cells. This cannot be fully understood by the current studies. However, our observation that the expression level of KHSRP is enhanced by HSV-1 infection reveals that its cellular mRNA levels might be diminished by over-active RNA decay pathways, resulting in facilitation of better establishment of viral infection. In light of these observations, we may conclude that HSV-1 might regulate cellular protein synthesis by several pathways, such as by changing the preliminary function of cellular proteins. This may result in an increase of the complexity of this synthesis system. Although the detailed functions of RPLP1, hnRNP H2 and KHSRP during HSV-1 infection need further investigation, these data provide new insights into the targeting of regulatory protein synthesis mechanisms in HSV-1 infected cells.

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