

Differential protein expression between EBV-positive and negative epithelial cells

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Received 27 February 2013; revised 29 March 2013; accepted 7 April 2013

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ABSTRACT

Epstein Barr virus infection is believed to play a role in the development of nasopharyngeal carcinoma. In order to investigate the function of EBV in epithelial cell, proteomic methods were used to find and identify the differential proteins and expected to elucidate the mechanism of EBV. Altered protein expressions were found between 293 cell (HEK293) and EBV infected cell (293-EBV). In this study, we separated differential expressed proteins using 2D-DIGE method while matrix-assisted laser desorption/ionization tandem time of flight mass spectrometry (MALDI-TOF-MS) method was used to identify proteins. The results showed that 14 proteins were up regulated and 3 proteins were down regulated in 293-EBV cells. Bioinformatic analysis showed that these proteins are involved in cell proliferation, metastasis, apoptosis, metabolism, and signal transduction. Western blotting analysis was further carried out to verify the MS results. Thus, EBV may exert its functions by mediating differential expression of these proteins.

Keywords: EBV; Differential In-Gelelectrophoresis (DIGE); Mass Spectrometry

1. INTRODUCTION

Epstein Barr virus infection has been believed to play a key role in the development of many tumors such as

nasopharyngeal carcinoma (NPC) which prevalently occurs in southern China and Southeast Asia. In our previous study, the proliferation rate of epithelial cell was faster after transfection with EBV genome. The growth of xenografts was also enhanced after the transfected cells were injected into nude mice *in vivo*. The mechanism of EBV is still unclear, thus in this work powerful proteomic technologies were used to elucidate the potential roles of EBV. As it is known, protein is the ultimate life performer. How does EBV regulate the protein profile of epithelial cell? Our study uses the differential in-gel electrophoresis DIGE and MALDI-TOF-MS (Matrix-assisted laser desorption/ionization time of flight mass spectrometry) to select and identify differential expressed protein compared 293 cells with 293-EBV cells. The results are analyzed to illuminate the mechanism of EBV in promoting cell proliferation and differentiation in protein level, which supply a new target and clue for cure and prognostic of EBV associated cancer.

2. Materials and methods

2.1. Cell Types and Protein Preparation

293 and The EBV-infected cell, 293-EBV, were preserved and propagated in our laboratory. They were cultured in DMEM medium (GIBCO) supplemented with 10% fetal bovine serum, 5% CO₂ atmosphere at 37°C. Cells were collected during the exponential growth phase. DMEM (Dulbecco's Modified Eagle's medium) purchased from Hgclone company; 2D-Clean up protein purification kit and 2D-Quant Kit purchased from the Amersham Biosciences company. The cells were extracted with DIGE lysis buffer. After lysed for 40 min on ice, the supernatant was transferred into a new microcentrifuge tube. The concentration of protein was determined with

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Bio-Rad protein assay reagent.

2.2. Two-Dimensional Fluorescence Difference Gel Electrophoresis (2D-DIGE)

For two-dimensional fluorescence difference gel electrophoresis (2D-DIGE), the IPG-strip was re-hydrated with sample at 30 V for 12 hours, and then IEF was conducted at 500 V for 1 hour, 1000 V for 1 hour, 10,000 v for 8 h, 500 v for 10 h. After IEF, the strips were equilibrated in equilibration buffer. Afterward, the IPG strips were electrophoresed on 12.5% acrylamide gels in the second dimension with electrophoresis parameter 3 w per gel for 30 min then 16 w per gel. The gels were scanned with a Typhoon 9400 fluorescence scanner (GE Healthcare). DeCyder 2D soft was used to match and select the different expressed proteins (folds > 1.8). Then, preparation of gel to cut different expressed proteins and the different expressed proteins identified by MALDI-TOF-MS/MS.

2.3. Western Blotting

Western blotting was carried out as described previously. Antibody to HSP-70, β -actin was purchased from Sigma-Aldrich (St Louis, MO).

2.4. Statistical Analysis

SPSS11.5 software was adopted to do student's t-test analysis. All data showed by $X \pm S$. $p < 0.05$ means that the difference was statistically significant.

3. Results

3.1. Protein Expression Profile in 293 and 293-EBV Cells

We obtained the images of proteins from 293 cells, 293-EBV cells and from the internal standard using different emission filters on the Typhoon 9400 fluorescence scanner. The obtained images were analyzed by DeCyder 5.0 software. We compared the protein expression in each group, and the results of the matched spots from the different gels were analyzed by Student's t test. We found 17 protein spots which showed consistent expression differences (fold > 1.8) between the two groups (**Figure 1**, **Table 1**). The differentially expressed proteins were selected and analyzed by the MALDI-TOF-MS/MS. The PMF are obtained and identified by FlexAnalysis 3.0 software from NCBI data. Take the number of 1940 protein spot (Raichu404X) as an example showed in **Figures 2-4**. Raichu404X was up-regulated significantly in 293-EBV cell. To validate the results of MALDI-TOF/MS/MS, two different expressed proteins are detected by western blot between 293 cell line and 293-EBV cell

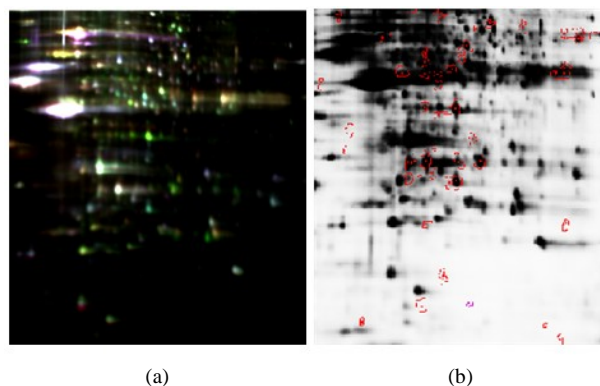


Figure 1. Overlapping image of three different emission filters. Red circles represent the differentially expressed proteins detected (a) separated proteins dye overlay with Cy2, Cy3, Cy5 (b) elected and labeled significant difference proteins.

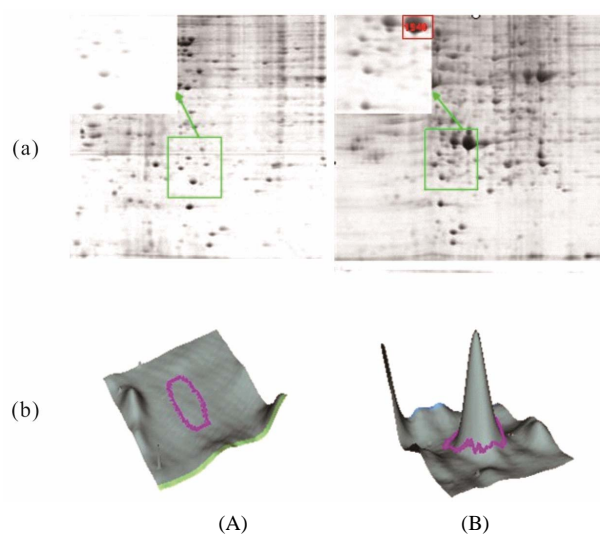


Figure 2. (A) Raichu404X (the spot number 1940) protein expression level in the 293 cell line (control). (B) Raichu404X protein expression level in the 293-EBV cell line. As a graphic showed, the spot 1940 field within the magnified boxes (top-left) was up-regulated in 293-EBV cell line; the same result was showed in b (three-dimension graphics).

line. The different expressed Raichu404X and Hsp70 protein were validated by Western blot. The results are consistent with results of MALDI-TOF/MS/MSAs which it is known. Raichu404X and Hsp70 proteins are closely associated with carcinogenesis. They are involved in several biological functions including signal transduction, Cell migration, DNA synthesis, cell proliferation, cell apoptosis and invasion of many cancers.

4. Discussion

According to DIGE analysis, 14 up-regulated proteins and 3 down-regulated proteins in the 293-EBV, the differentially expressed protein spots were chosen and identi-

Table 1. The identified differentially expressed proteins between 293 and 293-EBV cells.

Spot No.	Accession No.	Protein name	Level	Function	Fold	p-value
574	gi 62896815	Heat shock 70 kDa protein 8 isoform 2 variant	Up	Cellcycle, proliferation	3.78	0.0018
598	gi 42543698	Chain A, the crystal structure of the human Hsp70 Atpase domain	Up	Cellcycle, proliferation	3.546	0.0024
701	gi 83754516	Chain B, crystal structure of the Chip-Ubc13-Uev1a complex	Up	Ubiquitylation	2.83	0.0028
760	gi 4506753	RuvB-like 1	Up	Proliferation	1.86	0.036
853	gi 119597640	Protein disulfide isomerase family A, member 3, isoform CRA_a	Up	Metabolism	1.85	0.021
920	gi 5453603	Chaperonin containing TCP1, subunit 2	Down	Cellcyclecytoskeletal proteins	1.89	0.00083
1248	gi 34234	Laminin-binding protein	Up	Invasion and metastasis	1.96	0.05
1862	gi 5668560	Serine/threonine phosphatase 1 gamma	Up	Cellcycle, proliferation	2.16	0.041
1878	gi 62324	Epstein-Barr nuclear antigen [Human herpesvirus]	Up	Generegulation, extrachromosomal replication	6.51	0.00021
1926	gi 70995211	Peroxisomal enoyl-coenzyme A hydratase-like protein	Up	Metabolism the fatty acid beta-oxidation pathway	2.95	0.022
1940	gi 14595132	Raichu404X [Homo sapiens]	Up	RAS signal pathway	22.22	8.40E-05
1979	gi 6755214	Proteasome activator subunit 3	Up	Cell cycle, proliferation	2.14	0.0013
2071	gi 6912586	6 phosphogluconolactonase	Up	Metabolism	2.46	0.017
2081	gi 5803013	Endoplasmic reticulum protein 29 isoform 1 precursor [Homo sapiens]	Up	Stress	8.13	0.00085
2479	gi 4758504	Hydroxysteroid (17-beta) dehydrogenase 10 isoform 1 [Homo sapiens]	Up	Metabolism steroid hormones and fats	1.86	0.045
3392	gi 3891774	Crystal structure of a human low molecular weight phosphotyrosyl phosphatase	Down	Metabolism	2.16	0.0056
3600	gi 4584423	AKAP450 protein	Down	Immunity/induce Tcell to combine	2.17	0.014

fied as heat shock 70, Chip-Ubc13-Uev1a, RuvB-like 1, protein disulfide isomerase, TCP1, laminin-binding protein, serine/threonine phosphatase, Epstein-Barr nuclear antigen, peroxisomal enoyl-coenzyme A hydratase-like protein, Raichu404X, proteasome activator subunit 3, 6-phosphogluconolactonase, endoplasmic reticulum protein 29 isoform 1 precursor, hydroxysteroid (17-beta) dehydrogenase 10 isoform 1, Human Low Molecular Weight Phosphotyrosyl Phosphatase and AKAP450 protein. The identified proteins involved in numerous crucial intracellular functions such as cell cycle and apoptosis, regulation of signal transduction pathways, cell proliferation and so on. Since it has been suggested that Raichu404X

contains Ras interaction domain and that Cdc42Hs is a member in Ras/MAPK pathways Raichu404X is differently expressed in stem cells [1]. The function of Raichu404X needs further investigated. Hsp70 as central components of the cellular chaperone network, are frequently recruited by viruses and is important factors in developmental and pathological processes such as oncogenesis, neurodegenerative and autoimmune diseases, viral infections and aging [2-5]. Hsp70 interacts with key regulators of many signal transduction pathways controlling cell homeostasis, proliferation, differentiation and cell death and Hsp70 are ubiquitous molecular chaperones that function in a myriad of biological processes, modulating

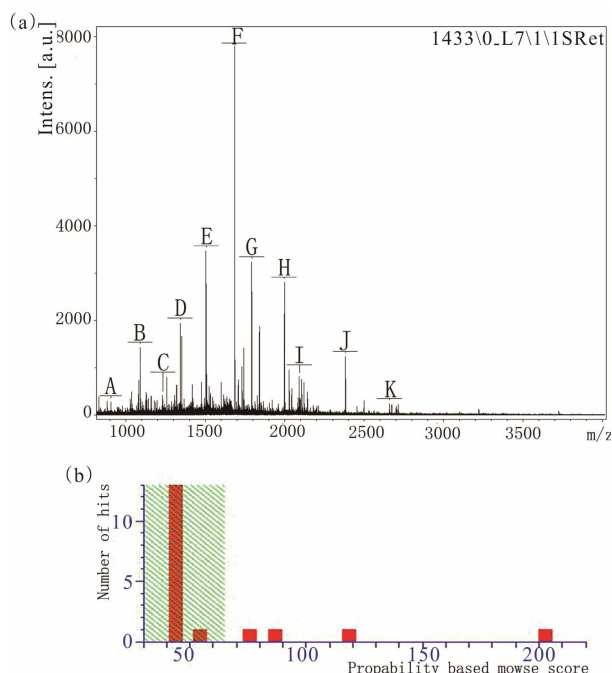


Figure 3. (a) Database searches of the masses of the trypsinized peptides identified the protein based on the 11 spikes from A to K. (b) Mass spectrometric characterization of Raichu404X.

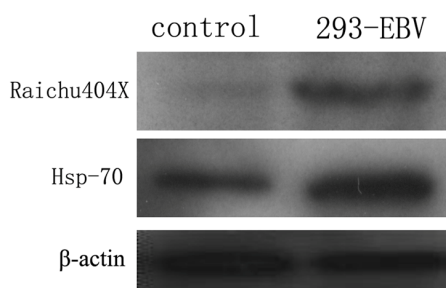


Figure 4. Westernblot results show the Raichu404X and Hsp70 proteins are up-regulated in 293-EBV cell line. (A) Control (293) (B) 293-EBV.

polypeptide folding, degradation and translocation across membranes, and protein–protein interactions [6-10]. Hsp70 and other members of the Hsp family have been shown to inhibit apoptosis at several different stages [7,9]. This work will provide us significant clue to clarify the happen of EBV associated diseases and new molecular will be used for prognosis and drug targets of clinic.

5. ACKNOWLEDGEMENTS

This work was supported by Projects of Hunan Provincial Natural Science Foundation of China (11JJ2043), the National Natural Science Foundation of China (81171931), the Research and Development Pro-

ject of Hunan Development and Reform Commission (2009-13910), Graduate Degree Thesis Innovation Foundation of Central South University (2010ybfz102), Doctor Innovation Research Project of Hunan Province (CX2010B040).

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