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Differential protein expression between EBV-positive and negative epithelial cells

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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-Gel Electrophoresis (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 accrues 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 Hyclone 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 ± 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 MALD-I-TOF-MS/MS. The PMF are obtained and identified by FlexAnalysis 3.0 software from NCBI nr 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 line. The different expressed Raichu404X and Hsp70 protein were validated by Western blot. The results are consistent with results of MALD-I-TOF/MS/MS 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 different expressed protein spots were chosen and identi-
<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Accession No.</th>
<th>Protein name</th>
<th>Level</th>
<th>Function</th>
<th>Fold</th>
<th>p-value</th>
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<tr>
<td>574</td>
<td>gi</td>
<td>62896815</td>
<td>Heat shock 70 kDa protein 8 isoform 2 variant</td>
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<td>598</td>
<td>gi</td>
<td>42543698</td>
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<td>83754516</td>
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<td>gi</td>
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<td>62324</td>
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<td>14595132</td>
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<td>RAS signal pathway</td>
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<td>gi</td>
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<td>2.16</td>
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<td>4584423</td>
<td>AKAP450 protein</td>
<td>Down</td>
<td>Immunity/induce Tcell to combine</td>
<td>2.17</td>
</tr>
</tbody>
</table>

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

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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

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