Differential Display of the Basic Protein in 5-fluorouracil Resistance of Human Colon Cancer Cell Line Using the Radical-free and Highly Reducing Method of Two-dimensional Polyacrylamide Gel Electrophoresis

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ABSTRACT

5-fluorouracil (5-FU) is one of the most potent and widely used for treatment of advanced colorectal cancer. However, it is common for such patient to develop resistance to 5-FU, and this drug resistance becomes a critical problem for chemotherapy. The mechanisms underlying this resistance are largely unknown. To screen for the proteins possibly responsible for 5-FU resistance, cells resistant to 5-FU were derived human colon cancer cell lines and two-dimensional gel electrophoresis-based comparative proteomics was done. The study wishes the first comparative proteomic analysis of basic proteins between the HT-29 human colon cancer cell line and HT-29/5-FU (its 5-FU resistant sub-line) using the radical-free and highly reducing method of two-dimensional polyacrylamide gel electrophoresis, which has a superior ability in the separation of basic proteins and the quantification of post-translational modification. A densitometric analysis was performed to quantify the modulated proteins, and protein spots showing significant changes were identified by matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry. Four basic proteins significantly modulated between HT-29 and HT-29/5-FU were identified. The identified proteins were mitochondrial transcription factor A (TFAM), ribosomal protein L5, L9, and L10. Our findings could lead to understand and manipulate resistance in 5-FU therapy to colon cancer.
INTRODUCTION

5-fluorouracil (5-FU) is one of the most widely used chemotherapeutic treatments for advanced colorectal cancer patients and is still considered a mainstay of therapy [1]. However, 5-FU produces major responses in only about 10% of such patients [1]. Many attempts to enhance its therapeutic effectiveness [2,3] have resulted in only minor improvements in patients survival [4,5]. A common critical issue for patients responding to 5-FU is the development of resistance to the drug, which has become a major obstacle in chemotherapy [6,7].

Numerous efforts have been made to identify mechanisms underlying 5-FU resistance [8-10]. Some mechanisms seem to involve thymidylate synthase (TS), which is crucial in de novo synthesis of thymidylate [11]. DPD (dihydropyrimidine dehydrogenase) is important in the catabolism of not only the naturally occurring pyrimidines uracil and thymine, but also the widely used antimetabolite cancer chemotherapy agent 5-fluorouracil (5-FU) [12]. Understanding 5-FU resistance mechanisms at a molecular level seem essential to design strategies to overcome this resistance.

To identify the predictive biomarkers for 5-FU resistance, several comprehensive studies have investigated 5-FU resistance by DNA microarray analysis technique [13-15]. However, the genome is only a carrier of genetic information, whereas the protein is the executor of living activities. Moreover, changes at the mRNA level do not always correlate with alterations in the protein level, and genomics does not analyze the regulation of protein-protein interactions and post-translational modifications that contribute to the protein changes. In this respect, a proteome-based approach that can identify quantitative and qualitative protein changes associated with 5-FU resistance is an important and useful for the research approach. However, few studies have used proteomic-based approaches, or specifically, comprehensive proteomics using two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) to screen proteins from human colon cancer cell lines with induced 5-FU resistance [16,17].

In comparative proteomics for identifying novel biomarkers of drug resistance, 2-D PAGE have been performed mainly using an immobilized pH gradient (IPG) method as the mainstream procedure. Actually, the IPG method can separate proteins within a limited range of isoelectric points, but it cannot separate basic proteins with isoelectric points greater pH 10. On the other hand, with the radical-free and highly reducing (RFFHR) 2-D PAGE, the migration rate of protein is determined by the net charge of the protein at pH 8.6 in the first dimension electrophoresis, and the net charge at pH 3.8 and molecular sieving effect in the second dimension electrophoresis. Therefore separation of proteins in the RFFHR 2-D PAGE is not limited by the isoelectric points of protein. Basic proteins, even if the isoelectric points are very high, can be sufficiently separated by the RFFHR 2-D PAGE as well as neutral and acidic proteins [18,19]. Furthermore, changes of proteins' net charge by posttranslational modification are detected as shifts of protein spots by RFFHR 2-D PAGE. This high potential for separation of basic proteins in RFFHR 2-D PAGE has been utilized through much of the analysis of the ribosomal proteins (RPs) [19].

5-FU is transformed into two active metabolites, fluorouridine triphosphate (FUTP) and fluordeoxuridine monophosphate (FdUMP), and induces tumor cells to undergo apoptosis in response to DNA and RNA damage. FUTP is incorporated into RNA instead of uridine triphosphate, and interferes with the maturation and finally the function of RNA; FdUMP forms a covalent ternary complex with thymidylate synthase (TS) and 5, 10-methylene tetrahydrofolate, thereby inhibiting deoxythymidine monophosphate synthesis from deoxyuridine monophosphate by TS and finally, DNA synthesis [20]. Because the basic proteins can bind to DNA/RNA with a negative protein charge, it may be thought that identification of basic proteins related to DNA and RNA damage by 5-FU, using the RFFHR 2-D PAGE, could provide further evidence for elucidation of the 5-FU resistant mechanism in colon cancer cells.

Therefore, this study was the first comparative proteomic analysis between HT-29 human colon cancer cell line and its 5-FU resistant line, HT-29/5-FU, using an improved RFFHR 2-D PAGE. An analysis of the enzyme activity associated with 5-FU metabolism has been indicated that orotate phosphoribosyl transferase (OPRT), a key enzyme of 5-FU anabolism, was lower in HT-29/5-FU than HT-29 [21]. In the cells with lower OPRT, a decreased metabolism of FUTP from 5-FU induces the attenuation of RNA damage [20]. Four basic proteins which were modulated between HT-29 and HT-29/5-FU were identified in the current study. The identification of the novel biological markers related to 5-FU resistance may therefore greatly help to achieve not only an improvement in both chemotherapy regimens and the patient prognosis, but also it may also help in the development of novel idea for pharmaceutical products.

MATERIAL and METHODS

Human colon cancer cell lines and establish
ment of 5-fluorouracil-resistant cell line. Human colon cancer cell line, HT-29 and its 5-FU/ resistant cell was obtained from Taiho Pharmaceutical, Japan. HT-29/5-FU was selected by stepwise increase in 5-FU concentrations from 0.2 μM to 100 μM [21]. Cells were cultured in RPMI 1640 supplemented with 10% FBS at 37°C with 5% CO2 in a humidified atmosphere. The cells (106 for each cell culture in 150cm2 flasks) harvested in 300 μL of solubilization buffer (9 mol/L urea, 2% NP40, 2% β-mercaptoethanol, 10mmol/L phenylmethylsulfonyl fluoride) using a cell scraper an stored at -80°C until use.

3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazoi-
ium bromide assay. A colorimetric assay using the tetrazolium salt, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), was used to assess suppression of cell proliferation by 5-FU (Taiho Pharmaceutical, Tokyo, Japan). In brief, the cells (5 x 104 cells/well) were seeded into 96-well microplates. After 24 hours, the cells were cultured with 200μL ZnSO4 supplemented in growth medium and treated with varying doses of 5-FU. At 4 days after exposure to drugs, 0.1mg of MTT was added to each well and incubated at 37°C for further 2 hours. Plates were centrifuged at 450 x g for 5 minutes at room temperature and medium removed. DMSO (0.15ml) was added to each well to solubilize the crystals and the plates were read at 540nm using a microplate reader (Bio-
Rad Laboratories, Inc. CA, USA). All experiments were done six times and the mean and SD of the IC50 (μM) values were calculated. The nonparametric Mann-Whitney’s U test was used to evaluate the differences between groups by Microsoft Office Excel software. Statistical significance was set at a P value of <0.05.

Protein preparation

For protein preparation, 6 sheets of 150 cm2 flask (80% confluent) of HT-29 and HT-29/5-FU were used, respectively. Sample proteins were prepared by the acetic acid method [22]. Briefly, a cocktail of protease inhibitors in a lysis buffer (50 mM Tris-HCl pH 7.8, 2% CHAPS) was added to the cells. After vortexing, the samples were later disrupted in 20 cycles of 15 minutes sonication and 15 minutes off at 4°C. One-tenth volume of 1 M MgCl2 and two volumes of acetic acid were added, and the mixture was stirred for 1 hour at 4°C. After centrifugation at 15,000 x g for 15 minutes, the supernatant was dialyzed against 2% acetic acid three times. The proteins were lyophilized and stored at -80°C until use.

Electrophoresis

Extracted proteins (approximately 1.5mg/ml) were analyzed by the RFHR method of 2-D gel electrophore-
sis as previous described with some modifications [18,19]. Apparatus for RFHR 2-D electrophoresis was obtained from Nihon Eido (Tokyo, Japan). Briefly, protein samples were dissolved in a lysis buffer containing 8 M urea and 0.2 M mercaptoethanol and incubated at 37°C for 60 min. Pyronine-G (Merck Ltd, Germany) and recrystallized acridine orange (Wako Purified Reagent, Kyoto, Japan) were added to the sample solution as a migration marker for sample charging electrophoreses. After prerun was at 100 V for 60 minutes, sample charging electrophoresis was carried out on an 8% polyacrylamide gel containing 10 M urea, 40 mM KOH, and 0.37% acetic acid at 100 V for 30 min on NA1460 apparatus (Nihon Eido, Tokyo, Japan). Pyronine-G and acridine orange in the sample solution were concentrated rapidly and migrated into gels, and sample proteins just followed them. When Pyronine-G and acridine orange had run inside the gels at least about 10 mm, the run was finished. Sample gel pieces containing proteins were obtained by cutting the gels with a razor at a position immediately below the band of Pyronine-G and within the length of 10 mm. Subsequently, the first dimensional separation was performed on 8% polyacrylamide gel containing 8 M urea, 400 mM Tris, 500 mM boric acid, and 21.5 mM EDTA-2Na on NA1460 apparatus (Nihon Eido, Tokyo, Japan). After prerun was performed at 100 V for 6 hours with air cooling, the sample gel pieces were inserted into the spaces that were cut out with a ground spatula to the same length as the sample gel pieces. 1-D electrophoresis was performed at 170 V for 20 hours with air cooling. The second dimensional separation was then carried out on an 18% polyacrylamide gel containing 8 M urea 50 mM KOH and 5% acetic acid (16 x 16 x 0.2 cm). After prerun was performed at 100 V for 15 hours, the 1-D and sample gels were put on the 2-D gels. The 2-D electrophoresis was performed at 100 V for 22 hours with air cooling.

Image analysis

Following separation by electrophoresis, gels were stained with Coomassie Brilliant Blue (CBB) R-250 (Nacalai Tesque, Kyoto, Japan). For differential analysis, gels were scanned with GS-800 Calibrated Densi-
tometer, the gel images were analyzed using PDQuest™ version 7.1. (Bio-Rad Laboratories, Inc. CA,USA.) And the gel images were analyzed Quantity One™ version 4.5.0. software (Bio-Rad Laboratories, Inc. CA, USA.). For each gel, spot detection, area calculation and background subtraction was done, and unmodulated spot of each gel (identified as Elongation factor 1- alpha 1, accession No. EFA1 in Swiss-Prot) were used as internal control. The detection of change in protein
maps between the two classes (HT-29 and HT-29/5-FU) was confirmed by visual inspection.

**In-gel enzyme digestion and MS**

Following separation by electrophoresis, protein identification was done. Briefly, gel pieces were alternatively washed with 25 mmol/L ammonium bicarbonate/30% acetonitrile (1:1, v/v). After the liquid was discarded, 10 mmol/L DTT-100 mmol/L NH₄HCO₃ was added with shaking for 15 minutes. Then the liquid were exchanged for 50 mmol/L iodoacetamide-100 mmol/L NH₄HCO₃ and shaked for 30 minutes. After the pieces were shaken with 10% acetonitrile/methanol (1:1, v/v), 50 mmol/L NH₄HCO₃ was added. After the liquid was discarded, the pieces were dehydrated with 10% acetonitrile finally. These pieces were completely dehydrated in a Speedvac device at room temperature, then covered with 24 µL of L-(tosylamide-2-phenyl)ethyl chloromethyl ketone-modified trypsin (0.02 mg/ml; Promega, Madison, WI) in 100 mmol/L NH₄HCO₃ buffer and left at 37°C overnight. After enzymatic digestion, the resultant peptides were extracted in 30 µL of 0.1% (v/v) trifluoroacetic acid/acetoneitrile (1:1, v/v) and then in 40 µL of 0.1% (v/v) trifluoroacetic acid/acetoneitrile (1:2, v/v). Extraction was conducted in an ultrasonic bath for 10 minutes each time. Each extracts were brought to dryness in a Speedvac for 90 minutes and then resuspended with 10 µL of 0.1% (v/v) trifluoroacetic acid/acetoneitrile (95:5, v/v) trifluoroacetic acid/acetoneitrile with 15 minutes ultrasonication. The extracted peptides were desalted in ZipTip C18 micricolumns (Millipore, Bedford, MA, USA).

**Protein identification by mass spectrometry**

For identification, using the peptide mass fingerprinting (PMF) method, 0.7µL aliquots were deposited on target plate (Bruker Daltonik GmbH, Bremen, Germany) followed by deposition of 0.7µL of matrix (10mg/ml α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 0.1% trifluoroacetic acid).

The identification of each protein was carried out by PMF analysis using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometer and by tandem mass spectrometry (MS/MS) analysis using Ultraflex (Bruker Daltonik, GmbH, Germany.). Proteins were identified by matching the PMF results with Swiss-Prot protein database using the Mascot Search engine.

**Protein identification**

The peptide mass fingerprint was used for protein identification from the tryptic fragment size using the Mascot Search engine based on the entire NCBI and Swiss-Prot protein databases, using the assumption that peptides are monoisotopic, oxidized at methionine residues and carbamidomethylated at the cystine residues. Up to one missed trypsin cleavage was allowed and a tolerant of 100 ppm was the window of error allowed for matching the peptide mass values. Probability-based MOWSE scores were estimated by comparison of the search results against estimated random match population and were reported as \(-10\log_{10}(p)\), where \(p\) is the absolute probability. All protein identifications were in the expected size range based on the position in the gel.

**RESULTS**

**Cytotoxic effects by 5-FU treatment in human colon cancer cell lines**

The cytotoxic effects by 5-FU were determined in HT-29 and HT-29/5-FU human colon cancer cell lines. Following in vitro treatment of the cells with 5-FU at several different drug concentration for 4 days, cell proliferation was evaluated using MTT assay. The degree of resistance to 5-FU was estimated at the ratio of the IC₅₀, the concentration inhibiting growth by 50%, of resistant line and to that of its parent line. The IC₅₀ values of 5-FU were 0.66±0.31 µM and 6.11 ±1.19µM against HT-29 cells and HT-29/5-FU cells, respectively. The cytotoxic effects of 5-FU against 5-FU resistant line was ×9.23-fold lower than that against the parental cells (P<0.3×10⁻⁶) (Table 1). The degree of resistance to 5-FU of resistant line seems to be quite

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>IC₅₀(µM) ± 5-FU</th>
<th>Relative resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT-29</td>
<td>0.66±0.31</td>
<td>9.23</td>
</tr>
<tr>
<td>HT-29/5-FU</td>
<td>6.11±1.19</td>
<td></td>
</tr>
</tbody>
</table>

† IC₅₀ is defined as the drug concentration that produced a 50 % reduction in 540-nm absorbance compared with untreated controls in MTT assays. † Fold changes in 5-FU IC50 in resistant cells compared with parent cells.
stable because the IC₅₀ was consistent event after sequential passage without 5-FU (data not shown).

**Separation of protein by RFHR 2-DE**

To analyze the underlying mechanisms and identify downstream mediators that are unique to the effects of 5-FU resistance in colon cancer cells, we performed a comprehensive proteome analysis to identify target-specific proteins. In each cell line, soluble basic proteins were separated by RFHR 2-D electrophoresis with high reproducibility. For each of the cell lines, 3 lysates (from 3 independent cultures) were analyzed in duplicate (i.e., six experiments for each cell line). Analytical CBB staining was performed for detection of differentially expressed basic proteins for each cell line. Typical separations of soluble basic proteins isolated from HT-29 and HT-29/5-FU are shown in Fig. 1. In these figures, all proteins that have been identified were marked with arrows. The arrows and circles indicate regulated proteins in HT-29/5-FU than HT-29.

![RFHR 2-D PAGE maps of HT-29 and HT-29/5-FU](image1)

**Fig. 1** RFHR 2-D PAGE maps of HT-29 and HT-29/5-FU. Proteins were separated and visualized by CBB staining. Protein spots marked on the maps were considered differentially expressed and identified by Ultiflex MALDI TOF/TOF MS. Elongation factor 1-α 1 was used as an internal control (IC) for normalization purposes. Each cell line from 3 independent cultures was analyzed in duplicate. The arrows and circles indicate regulated proteins in HT-29/5-FU than HT-29.

![Magnification of the spots containing the modulated expression](image2)

**Fig. 2** Magnification of the spots containing the modulated expression. The corresponding identities are listed in Table 1.
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Table 2 MALDI-TOF/TOF MS identification of the modulated expression of basic protein in HT-29/5-FU aldeitified/confirmed by MS/MS analysis (TOF/TOF), thus indicated fragments are indicated their scores. bThese values show the combined Mascot score, representing the total score of PMF and MS/MS(TOF/TOF) score. cThe means calculated from each 3 lysates of HT-29 and HT-29/5-FU.

<table>
<thead>
<tr>
<th>Spot no.</th>
<th>Identification</th>
<th>Gene name</th>
<th>Mass (Da)</th>
<th>pI</th>
<th>% Sequence coverage</th>
<th>Mascot score</th>
<th>Function</th>
<th>Change factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ribosomal protein L5</td>
<td>RL5</td>
<td>34,569</td>
<td>9.73</td>
<td>49</td>
<td>235</td>
<td>p53 stability</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>Ribosomal protein L9</td>
<td>RL9</td>
<td>21,964</td>
<td>9.96</td>
<td>59</td>
<td>180</td>
<td>Inactivator of ribosomes linking with rRNA A</td>
<td>0.49</td>
</tr>
<tr>
<td>3</td>
<td>Transcription factor A, mt precursor</td>
<td>TFAM</td>
<td>29,306</td>
<td>9.74</td>
<td>74</td>
<td>172</td>
<td>Maintenance and replication of mtDNA</td>
<td>4.00</td>
</tr>
<tr>
<td>4</td>
<td>Ribosomal protein L10</td>
<td>RL10</td>
<td>28,044</td>
<td>10.11</td>
<td>44</td>
<td>145</td>
<td>Tumor growth suppressor</td>
<td>0.14</td>
</tr>
</tbody>
</table>

aIdentified/confirmed by MS/MS analysis (TOF/TOF), thus indicated fragments are indicated their scores.
bThese values show the combined Mascot score, representing the total score of PMF and MS/MS(TOF/TOF) score.
cThe means calculated from each 3 lysates of HT-29 and HT-29/5-FU.

Transcription factor A (TFAM)

Sequence coverage=74%

Intensity coverage=66%

Fig. 3 An example of PMF pattern of a band excised from RFHR 2-D electrophoresis gel as mitochondrial transcription factor A (TFAM). Peptides was extracted from excised gel after in-gel enzyme digestion and was analyzed using Ultraflex MALDI-TOF/TOF mass spectrometry. Acquired spectra were processed and searched against a Mascot Search engine based on the entire NCBI and SWISS-PROT databases.
Identification of differentially expressed basic proteins between HT-29 and HT-29/5-FU

The peptide was extracted from excised gel after in-gel enzyme digestion and then analyzed using Ultraflex MALDI-TOF/TOF MS. The acquired spectra were processed and searched against a Mascot Search engine based on the entire NCBI and Swiss-Prot protein databases with high confident based on high scores and sequence coverage. Four basic proteins significantly modulated between HT-29 and HT-29/5-FU were identified. An example of the PMF pattern of TFAM identified by MALDI TOF/TOF is demonstrated in Fig. 3. Table 2 presents these four identifications with NCBI accession numbers, theoretical molecular weights and pI, sequence coverage rates (all above 30%), as well as the Mascot score, representing the total score of PMF and MS/MS (TOF/TOF) score.

DISCUSSION

Recently, the systemic therapy for colorectal cancer has advanced from essentially from a single, partially effective agent, 5-fluorouracil, to a combination of cytotoxics and antibodies offering increased survival. In addition to damage of DNA through agents, and inhibition of DNA replication, a promising approach involves modifying the control of gene expression, including epigenetic control.

The RHFR 2-DE in this study was developed as an improvement of the Kalschmidt and Wittmann method with the aim of separating and quantifying proteins that are small, basic and rich in disulphide bridges [23]. Extremely small basic proteins are difficult to separate because their mobility is exceedingly high. In RHFR method, the proteins can be mainly separated by their charge in acid-urea gels in the first dimension, whereas in the second dimension separation is based mainly on mobility rate. Precursors using radical scavengers were carried out to estimate residual free radicals from gels and thereby decrease loss of proteins caused by such radicals. The sample charging electrophoresis, before the first dimensional electrophoresis, also was designed to limit protein loss and, in addition, to concentrate a dilute sample solution as a sharp band. The second dimensional electrophoresis was carried out at a more acidic pH 3.6 instead of pH 4.5 to obtain better separation of extremely small basic proteins with avoiding formation of art factual disulphide bridges during migration. With these modifications, quantitative yield and reproducibility became much better. In addition, even protein alterations such as post-translational forms can be clearly separated and quantified [18,19]. The sample which we used in this experiment was the mixed equal amount of fractionated one. The reason of that is some of the proteins have not migrated in the sample gel during sample charging electrophoresis because of aggregation, and it was recognized that it depended on non-specific protein-protein interactions among each fraction. To avoid this interaction, we improved sample preparation (see materials and methods), and could get the reproducible results.

Four spots were identified by MALDI-TOF/TOF MS which were modulated between HT-29 and HT-29/5-FU. The four identified proteins were mitochondrial transcription factor A (TFAM), ribosomal protein L5, L9 and L10. One of these basic proteins, TFAM showed up-regulated expression in HT-29/5-FU in comparison to HT-29. The other proteins showed down-regulated expression in HT-29/5-FU in comparison to HT-29.

Recent studies have shown ribosomal proteins L5, L9 and L10 may play an important role in mediating p53 activation and several physiological functions [24-34]. However, in the current studies, TFAM is only one of up-regulated expression in HT-29/5-FU. Accordingly we focus on just TFAM, besides ribosomal proteins, in the following discussion.

TFAM is a member of a high-mobility group (HMG) of proteins named on the basis of their electrophoretic mobility in polyacrylamide gels, and was first cloned as a transcription factor of the mitochondrial DNA. This group is composed of nonhistone chromatin proteins and transcription factors that can bind DNA either nonspecifically or in a sequence-dependant manner [35]. TFAM is encoded in the nucleus and is synthesized on cytoplasmic ribosomes as a precursor, which is converted, upon mitochondrial importation, to a mature form. The mature form contains two HMG boxes, HMG-like domain 1 (HMG1) and HMG-like domain 2 (HMG2), joined by a basic 36 amino acid linker and followed by a basic 27 amino acid tail [36]. Most of the TFAM proteins have been shown to stimulate transcription of mitochondrial DNA by binding to the D-loop region, and it is thought that TFAM not only contributes to the mitochondrial DNA stability by packaging of the mitochondrial DNA, but also maintains the mitochondrial DNA [37]. Recently, it was shown that TFAM preferentially recognizes cisplatin-damaged DNA and oxidized DNA. In addition, increased apoptosis has been observed in cells lacking mitochondrial DNA gene expression, thus suggesting that TFAM is involved in apoptosis because mitochondria act as a pivotal decision center in many types of apoptotic response [38, 39]. Yoshida et al. reported that TFAM expression was up-regulated in mitochondria interacted by p53 after human epidermoid cancer cells (KB cells) were treated with cisplatin. Up-regulated expression of TFAM was recognized in mitochondria.
when HCT116 colon cancer cell was treated with 5-FU [40]. Nuclear HMG-box protein, HMG1, has been shown to be enhanced binding of cisplatin-damaged DNA in association with p53 [41], and cisplatin-damaged DNA binding of TFAM is activated by p53. Therefore, since in TFAM transfected HeLa cell, the susceptibility of three chemotherapeutic drugs, etoposide, camptothecin and cisplatin, are decreased in comparison to the parental cell line, up-regulated expression of TFAM may contribute to the prevention of chemotherapeutic drug induced apoptosis [42].

CONCLUSIONS

We demonstrated the high reproducibility of basic protein maps of colon cancer cell lines by RPFR 2-DE. The 5-FU resistant and its parent cell lines clearly differed regarding in their protein expression profile. Four basic proteins which were modulated between HT-29 and HT-29/5-FU were identified. Among them, several proteins were associated with the biological basis of 5-FU pharmacologic DNA damage in tumor cells, and may play potentially be associated with the biological basis of 5-FU resistance. These proteomic approaches may not only be a powerful tool that can lead to the identification of novel potential markers involved in 5-FU resistance of colon cancer cells, but also be useful in completing the understanding of these complex mechanisms. Moreover, these observations will facilitate the development of novel therapeutic strategies against colon cancer.

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