

Differential proteins expression between gastric cancer and normal cell lines[☆]

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Abstract

Objective. In search of novel mechanisms leading to the development of gastric cancer, we analyzed differences of protein expression pattern between gastric cancer cell lines of MGC80-3, BGC-823, SGC-7901, AGS and normal gastric epithelial cell line GES-1. **Methods.** Cells were cultured *in vitro* and analyzed by proteomics approach. **Results.** Twenty-two different expression proteins were detected and identified. Nine of them were up-regulated, 10 of them were down-expressed in gastric cancer cells, and 3 of them only expressed in normal gastric epithelial cell. **Conclusion.** The results suggest that expressed proteins are different between gastric cancer cells and normal cells. These identified proteins may be important in gastric cancer formation and represent potential targets for tumor markers. [Life Science Journal. 2008; 5(4): 28 – 32] (ISSN: 1097 – 8135).

Keywords: gastric carcinoma; two-dimensional electrophoresis; proteomics

1 Introduction

Carcinogenesis has long been recognized as a multi-step process, which involves not only genetic changes conferring growth advantage but also factors that disrupt regulation of growth and differentiation^[1,2]. It is possible that some of these factors could be identified and their functions evaluated during tumorigenesis. Emerging evidence indicates that most tumor-associated biomarkers are cellular proteins whose aberrant regulation of function could be linked to malignancy^[3]. The proteome-based approaches are currently available for the identification of biomarkers in cancer, and some of these identified proteins may have potential values in cancer diagnosis. Some of these protein changes may be associated with tumorigenesis, and there is a substantial interest in the application of proteomics for the discovery of biomarkers in cancer detection^[4]. The aim of the present study was

to compare differential protein expressed between gastric cancer (GC) cell and normal gastric epithelial cell by proteomics approach. It is expected that a more detailed analysis of these changes will give new hints about the mechanisms that lead to the development of gastric cancer and may eventually help to propose novel targets for an effective diagnosis and therapy.

2 Materials and Methods

2.1 Cell culture and protein preparation

The gastric normal cell lines GES-1 and four human gastric cancer cell lines were used in this study. These cancer cell lines were well-differentiated gastric epithelial cell line AGS, moderately differentiated cell line SGC-7901, poorly differentiated cell line MGC80-3 and BGC-823. All the cell lines were kindly provided by Chinese Center for Disease Control and Prevention. They were cultivated in parallel, removed from the flask by incubating them with trypsin-EDTA, and then harvested for further study.

The cell pellets were dissolved in 20% trichloroacetic

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acid (TCA) in acetone containing 0.2% DTT and kept at -20°C over night. The suspensions were centrifuged at 2000 g for 10 minutes. The precipitation were washed in acetone containing 0.2% DTT and kept in -20°C for 30 minutes. The pellets were lyophilized under vacuum after spun again and resuspended in fresh lyses buffer (8 M urea, 4% CHAPS, 1 mM phenylmethylsulfonyl fluoride (PMSF), 20 g/L IPG buffer; pH 3 – 10). 1% DTT was added at 4°C for 1 hour, and then centrifuged (12000 g, 30 minutes, 4°C). The supernatant was the extracted proteins. The concentration was determined by the Bradford method with bovine serum albumin as standard. 0.1 M HCl was added to protein solution before quantification, and aliquots of protein samples were kept in -80°C until use.

2.2 2-DE, silver staining and image analysis

Isoelectric focusing (IEF) was performed on Ettan IP-Gphor Isoelectric Focusing System. Proteins of 100 μg for analytical gels and 300 μg for preparative gels were utilized for IEF and subsequent second dimensional separation. The protein spots were visualized in gel by silver staining and Coomassie brilliant blue staining. Stained two-dimensional electrophoresis (2-DE) gels were captured by transmission scan (LabScan). Target gels were analyzed with ImageMaster 5.0 2D platinum analysis software including spot detection, background subtraction, and matching. Total intensity in valid spots was used as the normalization method in the analysis. Spots differing by 3-fold or more with an intraspot covariance less than 20% were considered as proteins with differential expression.

2.3 MALDI-TOF and MALDI MS/MS analysis for protein identification

2.3.1 In-gel digestion. Gel particles excised from gels were washed with 25 mM NH_4HCO_3 , 20 minutes, and destained with 30% (v/v) Acetonitrile (ACN) twice for 30 minutes each; acetonitrile in 100 mM NH_4HCO_3 , until all traces of Coomassie Brilliant Blue were removed. Proteins were in-gel reduced with 10 mM DTT (60 minutes, 56°C) and S-alkylated with 55 mM iodoacetamide (30 minutes, in the dark), both in 100 mM NH_4HCO_3 . Gel particles were washed with 25 mM NH_4HCO_3 , and then dried under vacuum, rehydrated with the digestion solution (40 $\mu\text{g}/\text{ml}$ of trypsin in 25 mM NH_4HCO_3).

2.3.2 MALDI-TOF and MALDI-MS/MS. All mass spectra were obtained on a 4700 Proteomics analyzer with TOF/TOF optics in the positive ion reflector mode with a mass accuracy of about 50 ppm. The MALDI tandem

mass spectrometer used a 200 Hz frequency-tripled Nd : YAG laser operating at a wavelength of 355 nm. MS spectra were obtained in the mass range between 800 and 4000 Da with ca. 1000 laser shots. MS/MS spectra were acquired with 2000 laser shots using air as the collision gas. The singly charged peaks were analyzed using an interpretation method present in instrument software, where the five most intense peaks were selected and MS/MS spectra were generated automatically, excluding those from the matrix, due to trypsin autolysis peaks. Spectra were processed and analyzed by the Global Protein Server Workstation, which uses internal Mascot v2.0 software for searching the peptide mass fingerprints and MS/MS data. Searches were performed against the NCBI non-redundant protein database (updated 1 August 2007). Total ion scores are calculated from weighted ion scores for individual peptides that are matched to a given protein. Protein scores lower than 65 indicate that the proteins were not identified by random matches of peptide mass data.

2.4 Statistical analysis

The spots were visualized using PDQuest 2-DE analysis software as described in the manufacturer's manual (Amersham Pharmacia Biotechnology, Uppsala, Sweden). A statistical evaluation of the results is performed by *t*-test, the variation of the spot intensity within the sample map and between the two maps is analyzed.

3 Results and Discussion

3.1 Overview analysis of the protein expression profiles of cells

2-DE was performed three times for each cell line to ensure reproducibility. MGC80-3 cell was used to evaluate the reproducibility of 2-DE gel. 2-DE maps of MGC80-3 cell were established on three individual gels which ran simultaneously and were analyzed. Average 884 protein spots were detected with matching above 95% and in pI and Mr orient the warp of these spots were not obvious.

Approximately 800 protein spots were detected on each silver-stained gel by ImageMaster (810 ± 8 spots in GES-1, 650 ± 20 spots in AGS, 747 ± 31 spots in SGC-7901, 884 ± 13 spots in MGC80-3, 675 ± 14 spots in BGC-823), as shown in Figure 1. The different expressing protein spots were defined that those showed changes in expression of more than five-fold. In Figure 2, the different protein spots were described: Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), only appeared in GES-1 cells; dihydrolipoamide dehydrogenase, expressed increasing in cancer cells; glyoxalase, reducing expression in cancer

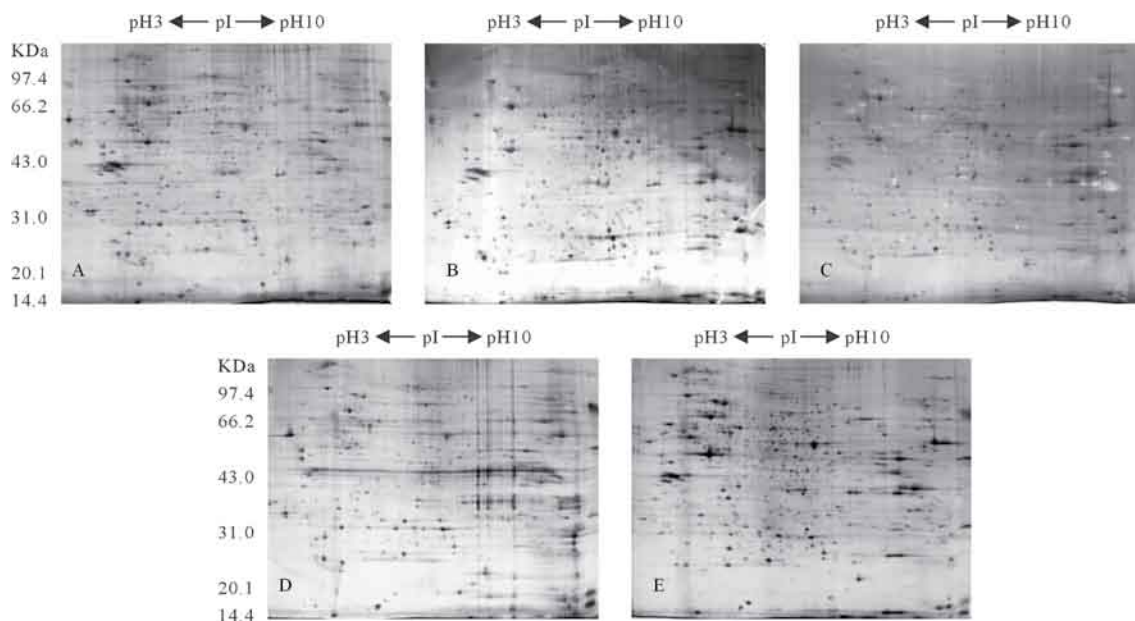


Figure 1. 2-DE maps of human gastric cell lines. A: GES-1; B: AGS; C: 7901; D: 80-3; E: 823. Proteins were separated on pH 3 – 10 linear IPG strip in the first dimension and 12.5% SDS-PAGE in the second dimension, gel were silver stained.

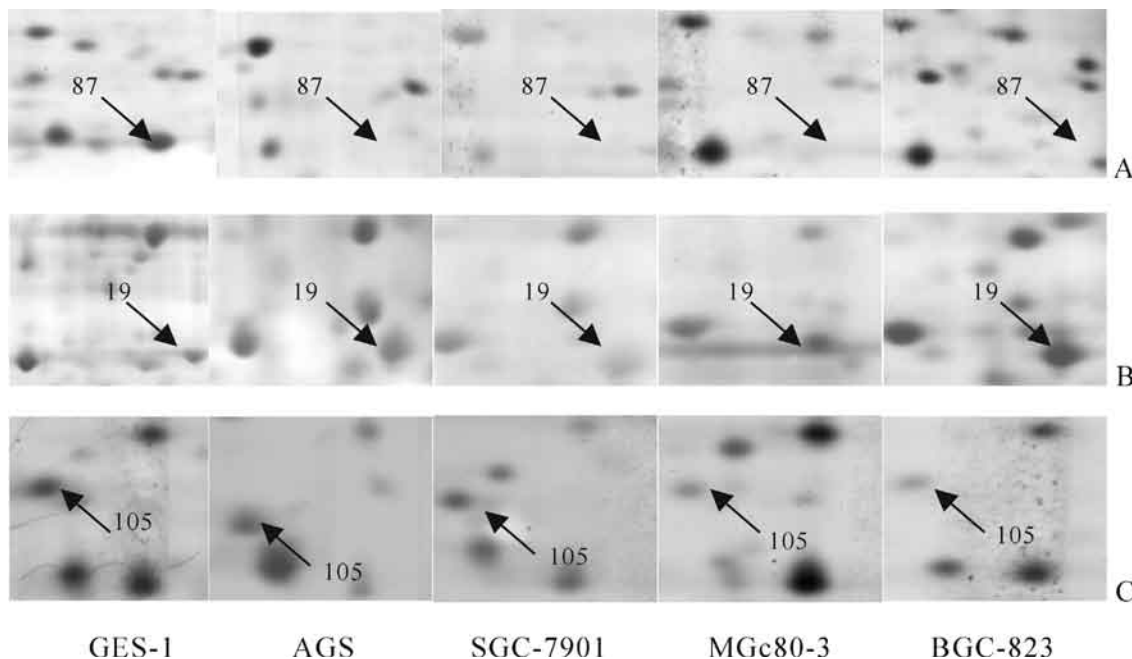


Figure 2. Cropped images of up- and down-regulated protein spots in normal cells and gastric cancer cells. A: Spot #87, UCH-L1, only appeared in GES-1 cells; B: Spot #19, dihydrolipoamide dehydrogenase, up-expressed in cancer cells; C: Spot #105, glyoxalase, down-expressed in cancer cells.

cells.

Although the cell lines are derived from different patients, the distinct growth patterns make it a meaningful model for investigation of cancer forming. Comparative study on the protein expression profile may disclose the

differential functional proteins and provide useful information. In this study, the different expressed proteins between distinct differentiation type gastric cancer cells and normal gastric cells were investigated via a proteomic approach. Twenty-two protein spots were identified. Of

Table 1. Differential expressed proteins in gastric cancer cells and normal gastric cells

Spot	Protein name	Accession	Protein	Total ion	Pep.	Mr/pI		Expressed cell lines				
No.		No.(gi/)	Score	Score	Count	Theoretic	Observed	823	80-3	7901	AGS	GES
Upregulated in GC cells												
15	Heat shock protein 60 (HSP60)	77702086	159	79	12	61/5.7	55/5.1	+	+	+	+	+
19	Dihydrolipoamide dehydrogenase	62088986	121	23	13	56/8.7	60/8.2	+	+	+	+	+
28	Migration-inducing gene 10 protein	41350401	356	238	15	45/8.3	51/9.0	+	+	+	+	+
46	Actin capping protein (CapZ)	55665442	202	66	15	31/5.4	33/5.0	+	+	+	+	+
47	Electron transfer flavoprotein	2781202	118	30	11	33/7.0	41/7.5	+	+	+	+	+
115	DEAD/H box polypeptide 3	62087546	168	17	22	75/7.7	81/7.0	+	+	+	+	+
18	Chain D,Deoxy Rhb 1.1	9256889	206	84	11	16/7.1	22/7.8	+	+	+	+	-
37	hnRNP A2/B1	4504447	361	153	20	36/8.7	38/8.5	+	+	+	+	-
129	HNRPA1 protein	75517570	445	328	13	29/9.2	32/9.5	+	+	+	+	-
Downregulated in GC cells												
7	KHSRP protein	54648253	169	58	16	73/8.0	80/9.0	+	+	+	+	+
24	ATP synthase	32189394	328	247	12	57/5.3	58/5.5	+	+	+	+	+
38	PDZ and LIM domain 1 (elfin)	13994151	159	65	11	36/6.6	29/6.1	+	+	-	+	+
104	rho GDP dissociation inhibitor	36038	388	334	7	23/5.0	31/6.2	+	+	+	+	+
105	Glyoxalase	15030212	259	144	10	21/5.1	19/5.5	+	+	+	+	+
63	hnRNPK	55958544	158	61	13	48/5.5	55/6.4	+	+	+	+	+
42	Nuclear chloride channel	4588526	263	144	12	28/5.0	27/5.2	+	-	+	+	+
60	TIF eIF-4c-human	1082871	160	106	7	16/5.0	19/5.5	-	+	+	+	+
72	creatine kinase	180570	300	193	13	48/5.3	43/5.9	-	-	+	+	+
73	Human Pirin	40889062	70	25	7	32/6.4	36/7.1	+	-	+	+	+
Expressed in normal cell only												
81	SNW domain containing 1	48146983	65	22	10	61/9.5	66/8.9	-	-	-	-	+
86	Cathepsin D	5822091	88	23	8	26/5.3	27/5.8	-	-	-	-	+
87	UCH-L1	4185720	209	99	11	23/5.3	28/6.3	-	-	-	-	-

the 22 proteins, 9 proteins were increased, 10 were down-regulated proteins in gastric cancer cells, and 3 proteins were only expressed in normal gastric epithelial cell. The proteins and parameters were shown in Table 1.

3.2 Proteins generally accepted to associate with gastric cancer

Among these identified proteins, some had been reported connecting with cancer, especially gastric cancer. Heat shock 60 KD (HSP60, spot #15) protein was ascended in gastric cancer carcinogenesis. The results agreed with

mitochondrial alteration in Kim's study^[3]. The same condition happened that HSP60 may be associated with the adhesion of *H. pylori* to human gastric cells^[5,6]. HSP60 in gastric cancer may be connected to apoptosis. The protein's functions in gastric cancer need to be researched deeply.

The ATP synthase was down regulated in cancer cells in this study. Zhao found that it was downregulated in the poorly differentiated MGC80-3 cells^[7]. In addition, the relative expression of beta-F1-ATPase was significantly reduced in gastric adenocarcinoma^[8]. So ATP synthase

may be associated with metabolism of gastric cancer.

3.3 Proteins found to be associated with gastric cancer in this study

The proteins in second group was never found to have association with gastric cancer before. The Rho GDP dissociation inhibitors (GDIs, spot #104) were pivotal regulators of Rho GTPase function, which was decreased^[9]. GDIs were downregulated in gastric cancer on the basis of this result. GDIs had been found as the overexpressed protein in dyspeptic patients with *H. pylori* infection and pancreatic carcinoma patients' serum^[10]. So GDIs may play a role in gastric carcinogenesis.

Heterogeneous nuclear ribonucleoproteins A2 and B1 (hnRNP A2/B1, spot #37) were two of the abundant nuclear RNA-binding proteins involved in alternative splicing. The diversity of A/B group hnRNP proteins may have important effects on the post-transcriptional regulation of cell-specific gene expression^[11]. Overexpression of hnRNP A2/B1 was shown in moderately and poorly differentiated gastric cancer cells, and it promoted epithelial cell proliferation in response to epidermal growth factor (EGF)^[12]. Consequently, hnRNP A2/B1 may be involved in gastric cancer cell proliferation.

KH-type splicing regulatory protein (KHSRP, spot #7) expression decreased in gastric cancer cells. Hall's study indicated that interaction of KHSRP and other proteins change the differentiated state of the cell^[13], and would activate splicing through intronic splicing enhancer sequences^[14]. KHSRP, as a multifunctional RNA-binding protein, was critically involved in the post-transcriptional regulation of human iNOS expression^[15]. Accordingly, KHSRP may activate splicing and regulate cellular differentiated state in gastric cancer.

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1, spot #87) was associated with Parkinson's disease^[16]. Up-regulated expression of UCH-L1 gene were observed in cigarette smokers and invasive breast cancer^[17]. The results of this research were UCH-L1 presented not in gastric cancer cells but in normal gastric cell as well as SNW domain containing 1 (spot #81) and Cathepsin D (spot #86). A subset of these loss expression proteins may be relevant to gastric cancer carcinogenesis.

4 Conclusion

In the present study, we have applied a proteomic approach to identify 22 gastric cancer-associated proteins, different expressed proteins in distinct differentiation type gastric tumor cells and normal gastric cells, representing

the major alterations in cell physiology during gastric cancer development. The functions of these proteins are associated with cancer cells proliferation, differentiation, invasion, metastasis, cellular information transduction and apoptosis. These identified proteins may serve as biomarkers, and this observation forms the basis for further biological, pathogenetic and possibly clinically relevant studies.

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