Reproductive Sciences
20(11) 1356-1364

DOI: 10.1177/1933719113485291
rs.sagepub.com
SAGE

Proteomic Identification of Neoadjuvant Chemotherapy-Related Proteins in Bulky Stage IB-IIA Squamous Cervical Cancer

Shuangwei Zou, MD[1], Qi Shen, MD[1], Ying Hua, MD[1], Wenxiao Jiang, MD[1], Wenwen Zhang, MS[1], and Xueqiong Zhu, MD, PhD[1]

Abstract

Objective: The aim of this study was to investigate the effect of neoadjuvant chemotherapy (NAC) on the human squamous cervical cancer using proteomics profiling and to obtain related proteins to NAC exposure and response. **Methods:** Paired samples of early-stage bulky squamous cervical cancer before and after NAC treatment from patients who responded to NAC were obtained and submitted to 2-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MS). The expression and localization of the interesting proteins in additional paired samples were confirmed by Western blot analysis and immunohistochemistry. **Results:** The comparison of the proteins present before and after NAC revealed that 116 protein spots were significantly changed. In all, 31 proteins were analyzed by MS, and 15 proteins were upregulated in the cancer tissue after NAC relative to the level before NAC, whereas 16 proteins were downregulated after NAC. The significantly higher expression of peroxiredoxin 1 and significantly lower expression of galectin 1 after NAC treatment were confirmed by Western blot. **Conclusions:** Proteomics can be used to identify the NAC-related proteins in squamous cervical cancer. The change in proteins may be associated with NAC exposure and response, but insight into their relevance requires further study.

Keywords

squamous cervical cancer, neoadjuvant chemotherapy, proteomics, peroxiredoxin 1, galectin 1

Introduction

Uterine cervical cancer is the second most common cancer in women. According to the staging system of the International Federation of Gynecology and Obstetrics (FIGO), the 5-year survival rate for patients with stage IB1 cervical cancer exceeds 90%. In contrast, patients with bulky (>4 cm in diameter) stage IB-IIA disease have poor local control and/or distant failures and a low survival rate than those with a tumor of 4 cm or smaller, regardless of the treatment regimen.[1]

Neoadjuvant chemotherapy (NAC) prior to surgery or radiotherapy has been applied as a new therapeutic strategy for early-stage bulky or locally advanced cervical cancer. The NAC can decrease the surgical stage of the cancer and increase the rates of radical hysterectomy and long-term tumor-free survival.[2,3] Its main advantages are the potential elimination of micrometastases, shrinkage of the primary tumor to achieve radical operability, and surgical downstaging of patients.[4] However, NAC is not effective in all patients, and using NAC may delay the radical surgery or the use of radiation therapy past the optimal time frame in patients who do not respond to NAC. Therefore, there is an urgent need to develop markers that can identify patients who would benefit from NAC.

Currently, there is a growing body of evidence revealing the great potential of proteomics technology in the analysis of the response and resistance of tumor cells to cytotoxic drugs.[5,6] Therefore, this study plans to explore the effect of NAC in the human squamous cervical cancer using proteomics profiling. Additionally, the expression and localization of the interesting proteins in additional paired samples before and after NAC were confirmed by means of Western blot and immunohistochemistry. The information obtained should be important for us to have a better understanding of the chemotherapeutic effects of NAC and possibly help to discover the mechanism of NAC treatment and response in patients with squamous cervical cancer.

[1] Department of Obstetrics and Gynecology, The Second Affiliated Hospital of Wenzhou Medical College, Wenzhou, China

Corresponding Author:
Xueqiong Zhu, Department of Obstetrics and Gynecology, The Second Affiliated Hospital of Wenzhou Medical College, No. 109 Xueyuan Xi Road, Wenzhou, Zhejiang 325027, China.
Email: zjwzzxq@163.com

Materials and Methods

Patients and Tissue Specimens

Women with previously untreated bulky stage IB-IIA cervical cancers (primary tumor >4 cm in diameter) who had histologically confirmed invasive cervical cancer and received NAC and radical hysterectomy between January 2007 and July 2009 were selected for this study. The lesions in nonpregnant patients who had normal bone marrow, liver, and kidney function were measured by physical examination, transvaginal ultrasound, and magnetic resonance imaging (MRI). The histological classification was based on the criteria of the World Health Organization (WHO), and the clinical staging was based on the criteria of the FIGO. A total of 16 patients, 9 with FIGO stage IB and 7 with stage IIA, were included in this analysis. The mean age of the patients at diagnosis was 41.6 years (range, 33-57 years). We obtained approval from the local research ethics committees, and all the patients provided written informed consent for voluntary participation before any procedures were performed. The trial protocol met the standards of the Declaration of Helsinki.

Neoadjuvant Chemotherapy and Therapeutic Effect Evaluation

All the patients received 1 or 2 courses of NAC (uterine artery injection of cisplatin 60 mg/m^2 + 5-fluorouracil 750 mg/m^2 + mitomycin C 8 mg/m^2). The assessment of how the tumors respond to the treatment has been traditionally performed by measuring the tumor's 2 dimensions (the longest diameter and its perpendicular diameter) using transvaginal ultrasound or by MRI. The chemotherapeutic response was scored 2 weeks after the last course, according to the WHO criteria, as follows: complete resolution (CR) of the tumor; partial response (PR), >50% decrease in the tumor volume; stable disease (SD), <50% decrease or a <25% increase in the tumor volume; and progressive disease (PD), >25% increase in the tumor volume. The NAC responders include the patients with a CR or a PR, whereas the non-NAC responders were the patients with SD or PD. All the patients in the present study were NAC responders and underwent radical hysterectomy and bilateral pelvic lymphadenectomy 2 to 3 weeks after completion of the NAC regimen.

Specimen Collection

Cancer tissues before and after NAC were obtained by cervical biopsy and during surgery. All the samples were carefully examined, and the cytological and histological results were confirmed by a senior pathologist, who confirmed that the cancer specimens contained more than 80% of cancer cells. Six paired cases were selected for the 2-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS). The other 10 pairs of tissues were selected to confirm the expression and localization of the interesting proteins using Western blot analysis and immunohistochemistry.

Instrumentation and Chemicals

Urea, tris(hydroxymethyl) aminomethane (Tris), and EDTA were obtained from Gibco BRL (Grand Island, New York). The 18-cm nonlinear immobilized pH gradients (IPGs) strips of pH 3 to 10, IPG buffer, mineral oil, and silver-staining PlusOne kits were obtained from Amersham Pharmacia (Piscataway, New Jersey). Acrylamide, sodium dodecylsulfate (SDS), bromophenol blue, tetramethylethylenediamine, and N,N′-methylene bisacrylamide were purchased from Sigma (St Louis, Missouri). Dithiothreitol (DTT), α-cyano-4-hydroxycinnamic acid (CCA), trifluoroacetic acid, iodoacetamide (IAM), and tolylsulfonyl phenylalanyl chloromethyl ketone-trypsin were acquired from Promega (Madison, Wisconsin). The protease inhibitor (phenylmethanesulfonylfluoride [PMSF]) was from Shenzhen Cy-tech Biotech Co Ltd (China). 3-[(3-Cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPS) was from Calbiochem (La Jolla, California). Mouse anti-human peroxiredoxin 1 antibody was from Abcam (Cambridge, Massachusetts). Mouse anti-human galectin 1 and β-actin monoclonal antibodies were from Santa Cruz, California. Enhanced chemiluminescence (ECL) reagents were from Beyotime (China). Polyvinylidene fluoride (PVDF) membranes were from Bio-Rad (Hercules, California). All the solutions were prepared with Milli-Q water (MilliPore, Billerica, Massachusetts).

Sample Preparation for Protein Extraction

The tissues, each weighing approximately 50 mg, were cut with scissors and ground into powder in liquid nitrogen. Then, buffer (8 mol/L urea, 4% CHAPS, 10 mmol/L DTT, 0.5% ampholyte [pH 3-10 nonlinear], 1 mmol/L PMSF, 2 mmol/L EDTA, 40 mmol/L Tris-HCL, and pH 8.5) was added at 1 mL/100 mg to the tissue. The homogenate was then sonicated in an ice bath for 5 minutes. After incubation in an ice bath for 20 minutes, the lysates were centrifuged for 30 minutes (40 000 rpm, 4°C), and the protein content of the supernatant was measured using a modified Bradford method. The remaining supernatant was frozen at −80°C.

Two-Dimensional Electrophoresis

The method for the extraction of the proteins from cancer tissues for the 2-dimensional electrophoresis analysis was based on our previous study.[7]

In brief, the isoelectric focusing procedure used 18 cm strips at 20°C. The strips were rehydrated at 0 V for 4 hours and at 50 V for 8 hours. Then, isoelectric focusing was performed in gradient mode using an IPGphor (Amersham, Piscataway, New Jersey). The samples were first focused for 3 hours at 3 separate voltages, 500, 1000, and 8000 V, and then kept at 8000 V until a total of 50 kV for 1 hour was reached. The strips with the focused proteins were subsequently treated with DTT and IAM in equilibrated buffer containing 6 mol/L urea, 20% glycerol, 2% SDS, and 1.5 mol/L Tris–HCl, pH 8.8. The equilibrated strips were placed in 12% polyacrylamide gels, and SDS-polyacrylamide gel electrophoresis (PAGE) was carried out at

2.5 W/gel for 30 minutes and 15 W/gel using an Ettan DALT II (Amersham, Piscataway, New Jersey) until the bromophenol blue dye reached the bottom of the gel. The 2-dimensional electrophoresis spots were visualized by silver staining. The stained gels were scanned using an Amersham ImageScanner (Amersham Biosciences, Piscataway, New Jersey), and the tagged image file format images were imported into Amersham ImageMaster Platinum for analysis.

Protein Identification by MALDI-TOF-MS

Spots that differed between the samples before and after chemotherapy were excised from the gel with a razor blade and placed in Eppendorf tubes. The gel particles were reduced with DTT and alkylated with IAM and then washed, dried, and incubated in 25 mmol/L NH_4HCO_3, 50% acetonitrile (ACN), and 100% ACN, respectively. The tryptic digests were cocrystallized with a matrix of CCA and spotted into the target wells. The peptide mass fingerprinting signals were obtained in the reflectron mode, with the *m*/*z* range from 600 to 4000. The MS/MS data were exported in a suitable format and used in a database (NCBInr) search with the MASCOT program (Matrix Science, London, United Kingdom). A mass accuracy tolerance of 100 ppm was used.

Western Blot

The tissue samples were lysed as described above for the 2-dimensional electrophoresis, and aliquots of the protein extracts (50 μg) were subjected to 12% SDS-PAGE at 100 V until the bromophenol blue dye reached the bottom of the gels. The proteins were then electrotransferred onto PVDF membranes at 300 mA for 1 hour. The membranes were blocked for 2 hours with 10% skim milk in Tris-buffered saline (TBS) at room temperature and incubated with 1:300 mouse anti-human peroxiredoxin 1 monoclonal antibody, 1:500 mouse anti-human galectin 1 monoclonal antibody, or 1:1000 mouse anti-human β-actin monoclonal antibody at 4°C overnight. The membranes were rinsed and incubated with a horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1:5000). The peroxidase activity was visualized using ECL reagents according to the manufacturer's instructions. The blots were reprobed using anti-β-actin antibody as control for equal protein loading. The films were scanned using a GS-800 scanner (Bio-Rad, Hercules, California), and Quantity One software (Bio-Rad, Hercules, California) was used to analyze the band pixel values and the net pixel values of peroxiredoxin 1 and galectin 1. The relative expression levels of the proteins were evaluated using the net pixel value/β-actin net pixel value.

Immunohistochemistry

Paraffin-embedded sections (4 μm) were cut and mounted on 2% silanized slices, dried, dewaxed, rehydrated, and washed with phosphate-buffered saline. The slides were placed in 80 mL plastic jars containing citrate buffer and repeatedly heated in a commercial microwave oven (25 minutes at 750 W). The tissue slices were incubated for 15 minutes in 0.3% hydrogen peroxide in methanol to suppress the endogenous peroxidase activity and then incubated with nonimmune serum for 15 minutes to prevent nonspecific binding. After incubation overnight at 4°C with either 1:100 mouse anti-human peroxiredoxin 1 antibody or 1:200 mouse anti-human galectin 1 monoclonal antibody, the samples were incubated with a biotinylated goat anti-mouse antibody as the secondary antibody for 30 minutes at room temperature. The samples were exposed to streptavidin peroxidase as a label for 20 minutes and to diaminobenzidine, a chromogen, for 10 minutes. The sections were counterstained with Mayer hematoxylin to enhance the nuclear detection, dehydrated, and mounted in distrene dibutylphthalate xylene. Appropriate positive and negative control slides were stained in parallel.

Evaluation of Immunoreactivity

Peroxiredoxin 1 and galectin 1 immunoreactivity were observed in the cytoplasm. Positive cells were recognized as cells that showed yellowish brown. All counting was done by 2 observers (XWZ and XQZ), without any knowledge of the diagnosis and results of each other's observations. All immunopositive cells were counted in at least 10 high-power fields (×40 objective, ×10 eyepiece) chosen at random. The number of positive cell was given as a percentage for each case. Extent of immunostaining (based on the percentage of positive cells) was scored as 0 (0-5%), 1 point (6%-24%), 2 points (25%-49%), 3 points (50%-74%), and 4 points (75%-100%). Staining intensity was graded as 0 (negative), 1 point (weak), 2 points (moderate), and 3 points (strong). The immunohistochemical staining for peroxiredoxin 1 or galectin 1 was assessed according to the immunoreactive score (IRS) value by multiplying the individual scores of extent by intensity.

Statistical Analyses

Student *t* test was used to identify the proteins whose expression levels changed significantly as determined by 2-dimensional electrophoresis and immunohistochemistry. The Wilcoxon test was applied to the comparison of the expression of the peroxiredoxin 1 and galectin 1 proteins detected by Western blot between the samples before and after NAC, because the data exhibited a non-normal distribution. A *P* value less than .05 was accepted as significant. SPSS 13.0 was used for the statistical analysis.

Results

Two-Dimensional Electrophoresis Maps of Cervical Cancer Tissues Before and After NAC

All the experiments were performed in triplicate, and well-resolved and reproducible 2-dimensional electrophoresis patterns were obtained (Figure 1).

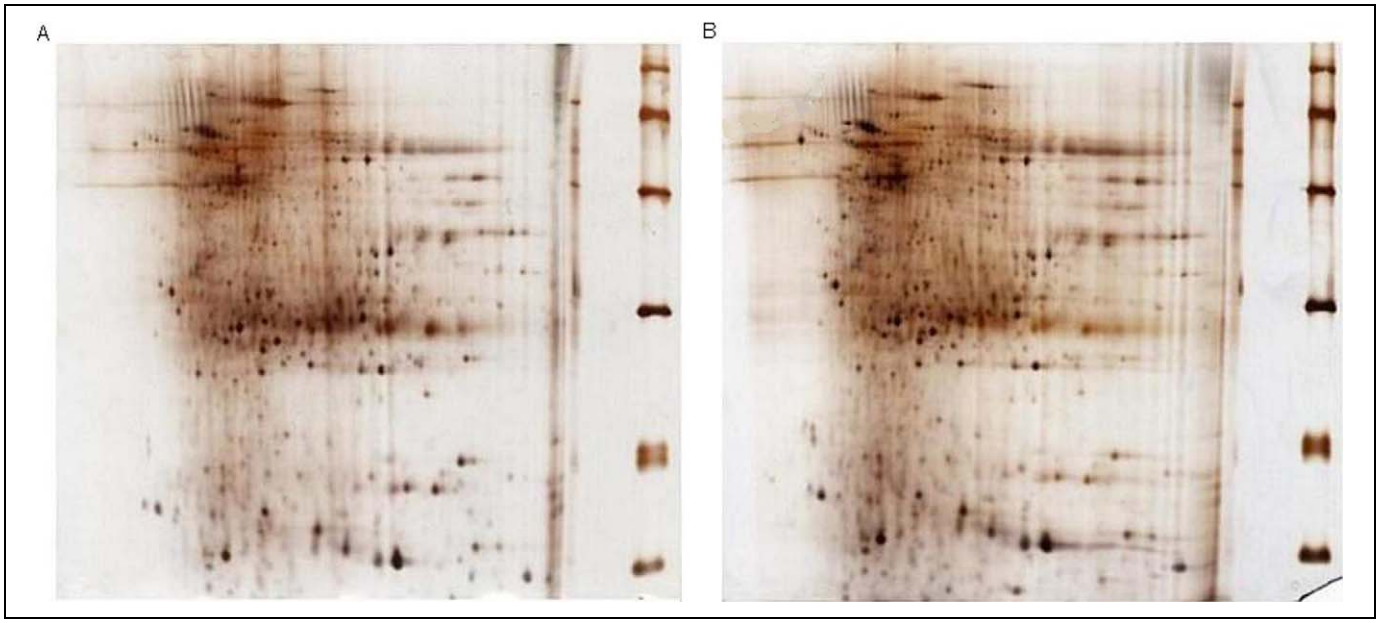

Figure 1. Two-dimensional electrophoresis maps of squamous cervical cancer tissue samples collected before and after neoadjuvant chemotherapy (NAC). A, Before chemotherapy and (B) after chemotherapy.

Using ImageMaster software, the comparison of the 2-dimensional electrophoresis patterns between before and after NAC revealed 116 proteins exhibiting a consistent, more than 3-fold difference in the level of expression; 54 protein spots increased in intensity, and 62 protein spots decreased in intensity in the tissue after NAC relative to the intensities before chemotherapy.

Identification of Differentially Expressed Proteins Before and After NAC in Cervical Cancer by MS

A total of 31 differential proteins were successfully identified by MALDI-TOF/TOF MS, including 15 proteins that were upregulated and 16 proteins that were downregulated with NAC relative to the levels present before the chemotherapy. The functions of the differentially expressed proteins were predominantly related to cell proliferation and metabolism, tumor progression, chemoresistance, transcription, transport, cellular structural component, and DNA repair; some of the proteins have unknown functions. The accession numbers in Swiss-Prot, the theoretical molecular mass and isoelectric point (p*I*), the sequence coverage, and functions are presented in Tables 1 and 2.

As shown in Table 1, there is a substantial number of upregulated proteins that are implicated in the mechanisms of chemoresistance (annexin A2, MutS homolog 2 protein, peroxiredoxin 1, and human pyruvate kinase M2) and tumorigenesis (transthyretin, Tbx3, vasodilator-stimulated phosphoprotein, and α-1 antitrypsin). Several proteins involved in the regulation of protein transport (RAB7B and KIF21A variant) or cellular structure (centrosomal protein 290 kDa and mutant desmin) were also found.

Table 2 shows a list of selected proteins that were downregulated after NAC in the tumors, including proteins that participate in the process of drug action in vivo either directly or indirectly. We also found that some proteins were associated with chemoresistance (galectin 1, tumor protein p53 mutant, heat shock protein 27, MRP 4, and peroxiredoxin 6) and the regulation of protein transport (albumin, PRO2044, FLOT1, and transferrin). Several proteins involved in cell proliferation (cyclin-dependent kinase 2, and putative double homeobox protein 2) were identified.

The Change in Peroxiredoxin 1 and Galectin 1 Expression Before and After NAC Confirmed by Western Blot

Western blot was used to confirm the expression levels of peroxiredoxin 1 and galectin 1 in 10 pairs of cervical cancer specimens collected before and after NAC (representative of paired specimens were shown in Figure 2). Equal protein loading was verified by parallel β-actin immunoblotting, and signal quantification was performed using densitometry scanning.

Relative to the expression before chemotherapy, the expression of peroxiredoxin 1 was significantly higher ($P < .05$), whereas the expression of galectin 1 was significantly lower after NAC ($P < .05$; pooled data were shown in Table 3).

Expression and Localization of Peroxiredoxin 1 and Galectin 1 in Cervical Cancer Before and After NAC by Immunohistochemistry

In all the specimens, peroxiredoxin 1 expression was detected in the cytoplasm of cervical cancer cells, whereas galectin 1

Table 1. Proteins That Were Upregulated in Cervical Cancer After NAC Relative to the Level Before Chemotherapy.

Accession	Description	Mass	Score	p*I*	Sequence Coverage, %	Function
gi|55959887	Peroxiredoxin 1	19135	90	6.41	37	Cell proliferation, chemoresistance[8]
gi|55669575	Transthyretin	12836	79	5.33	74	Transporter, tumorigenesis[9]
gi|3041821	Tbx3	52080	71	8.1	17	Transcription factor, tumorigenesis[10]
gi|119577769	Vasodilator-stimulated phosphoprotein	39848	54	9.05	15	Cell growth, tumorigenesis[11]
gi|119617819	Centrosomal protein 290 kDa	250593	56	5.76	5	Cellular structural component
gi|119611451	Chromosome 1 open reading frame 125	85818	66	5.35	14	Uncharacterized protein
gi|5822002	Profilin 1	15085	92	8.48	74	Tumor suppressor
gi|38490537	RAB7B, member RAS oncogene family	22709	67	6.32	41	Transporter
gi|147712797	MutS homolog 2 protein	4508	66	7.77	87	DNA repair, chemoresistance[12]
gi|119597993	Annexin A2	32600	46	5.93	21	Cell proliferation, chemoresistance[13]
gi|10439911	Unnamed protein product	60448	67	4.78	20	
gi|83582518	KIF21A variant	181739	69	6.07	10	Transporter
gi|28637	α-1 antitrypsin	22871	95	6.11	42	Tumorigenesis
gi|23194177	Mutant desmin	53446	67	5.21	22	Cytoskeleton
gi|73535278	Human pyruvate kinase M2	62570	50	7.01	18	Cell metabolism, chemoresistance[14]

Abbreviations: NAC, neoadjuvant chemotherapy; p*I*, isoelectric point.

Table 2. Proteins That Were Downregulated in Cervical Cancer After NAC Relative to the Level Before Chemotherapy.

Accession	Description	Mass	Score	p*I*	Sequence Coverage, %	Function
gi|42542978	Galectin 1	14 917	111	5.34	51	Cell proliferation, chemoresistance[15]
gi|119587704	Transgelin	23 748	133	8.54	55	Tumor suppressor
gi|94442889	Tumor protein p53 mutant	38 539	48	6.14	17	Cell cycle, chemoresistance[16]
gi|149242354	Cdk2	33 035	73	8.3	43	Cell growth
gi|662841	Heat shock protein 27	22 427	74	7.83	37	Cell apoptosis, chemoresistance[17]
gi|21928235	Seven transmembrane helix receptor	1 06 780	75	9.58	13	Signal transduction
gi|74762113	Putative double homeobox protein 2	9312	73	10.73	77	Cell growth
gi|10439439	Unnamed protein	13 908	65	6.02	51	
gi|8928546	MRP 4	1 50 358	66	8.48	6	Chemoresistance
gi|119626065	Albumin	61 122	95	6.96	26	Transport
gi|4758638	Peroxiredoxin 6	25 133	81	6	38	Chemosensitivity[18]
gi|6650826	PRO2044	30 084	211	6.97	54	Transport
gi|51574061	PCLO protein	39 007	76	9.65	30	Neurotransmitter
gi|48146009	FLOT1	47 536	70	7.08	17	Transport
gi|119613361	SCC-112	1 52 662	50	8.23	6	Cell proliferation, tumorigenesis
gi|110590599	Transferrin	76 988	307	6.85	46	Transport

Abbreviations: NAC, neoadjuvant chemotherapy; p*I*, isoelectric point.

expression was detected in the cytoplasm of stromal cells, not in the cancer cells.

The expression of peroxiredoxin 1 was statistically higher after NAC in comparison with that before NAC, while the expression of galectin 1 was significantly lower after NAC ($P < .05$; pooled data were shown in Table 4, representative of paired specimens was shown in Figure 3).

Discussion

The NAC is defined as the use of chemotherapy to treat the bulky stage IB-IIA and locally advanced cervical cancer (stages IIB-IV) before surgery or radiotherapy.[19] Screening and validating differential proteins between before and after NAC treatment who responded to NAC would find the related proteins to NAC exposure and response and improve our understanding of the mechanisms of chemotherapeutic effects of NAC and provide the thread to treat cervical cancer effectively. In the past, research at the protein level was hampered by the complexity of the proteome. Rapid development of the proteomic technologies integrated with advanced bioinformatics tools has changed the situation in recent years and allows the comparison of thousands of proteins simultaneously.

In the present study, using 2-dimensional electrophoresis analysis, 116 protein spots were significantly changed when comparison of the proteins between before and after NAC. In all 31 proteins were identified by MALDI-TOF-MS, and 15 proteins were upregulated after NAC, whereas 16 proteins were downregulated, which belong to various families with different functions. Rather than comparing the histological variants from

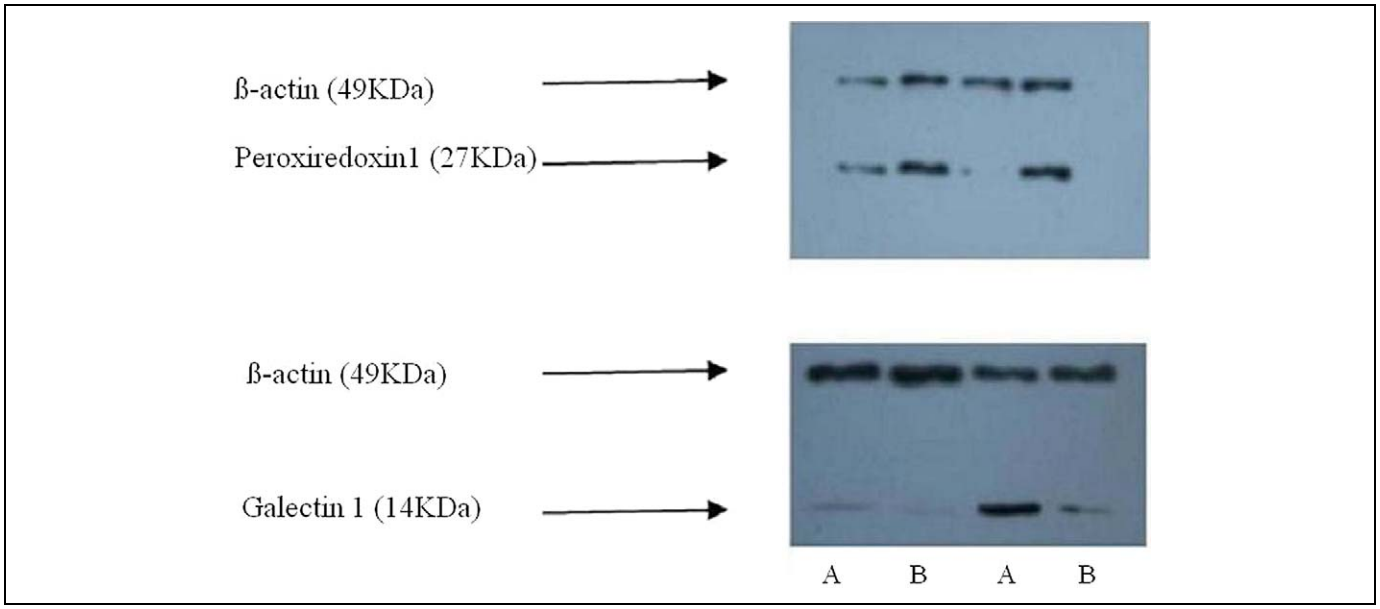

Figure 2. Western blot analysis of peroxiredoxin 1 and galectin 1 protein expression before and after neoadjuvant chemotherapy (NAC) in representative of cervical cancer. A, Before chemotherapy and (B) after chemotherapy.

Table 3. Peroxiredoxin 1 and Galectin 1 Expression in Cervical Cancer Before and After NAC (Mean ± SD).

Groups	Number of Cases	Peroxiredoxin 1	Galectin 1
Before NAC	10	0.632 ± 0.294[a]	0.865 ± 0.399[a]
After NAC	10	1.103 ± 0.216	0.116 ± 0.079

Abbreviations: NAC, neoadjuvant chemotherapy; SD, standard deviation.
[a]$P < .05$.

Table 4. Peroxiredoxin 1 and Galectin 1 Immunoreactivity in Cervical Cancer Before and After NAC (Mean ± SD).

Groups	Number of Cases	Peroxiredoxin 1	Galectin 1
Before NAC	16	3.13 ± 0.96[a]	6.06 ± 1.71[a]
After NAC	16	5.81 ± 1.23	2.88 ± 1.52

Abbreviations: NAC, neoadjuvant chemotherapy; SD, standard deviation.
[a]$P < .05$.

randomly selected tissue samples of different patients, we chose paired cancer samples from the same patient before and after NAC to study the proteomic changes taking place at the most basic level, thus avoiding any potential confounders due to individual genetic variations. It was demonstrated that the proteomic approach was effective for screening differentially expressed proteins in cervical cancer tissues before and after chemotherapy.

In the second part of the study, we performed Western blot and immunohistochemical analysis to confirm the prevalence of some of the proteins selected through 2-dimensional electrophoresis. Further studies are needed to verify the rest of the differentially expressed proteins. The main outcome of the present survey is the discovery of the dramatically increased expression of peroxiredoxin 1 and reduced expression of galectin 1 after NAC in all 16 squamous cervical cancer tissues. The consistency and magnitude of peroxiredoxin 1 and galectin 1 change after NAC raise the possibility that these proteins are associated with NAC exposure and play a role in NAC response.

Peroxiredoxins comprise a novel group of proteins with efficient antioxidant capacity. There are 6 isoforms in humans, and peroxiredoxin 1 is an important member of this family of 25-kDa peroxidases that can reduce H_2O_2 using an electron from thioredoxin or other substances. The expression of peroxiredoxin 1 has been found to be elevated in several human cancer cells and tissues, such as breast, esophageal, and lung cancers, and has been implicated in diverse cellular processes, including cell survival, proliferation, apoptosis, chemotherapy, and radiotherapy resistance.[8,20] Elevated peroxiredoxin 1 expression was implicated in the chemotherapy resistance of breast cancer and in the radiotherapy resistance of lung cancer cells. Accordingly, the downregulation of peroxiredoxin 1 was shown to enhance the radiosensitivity of lung cancer and intestinal cancer cells.[20,21] Cisplatin-induced oxidative damage evokes peroxiredoxin 1 expression thus increasing cellular protection by preventing oxidative damage. In a proteomic investigation into neuroblastoma cell lines, Urbani et al[8] observed the overexpression of peroxiredoxin 1 in chemoresistant cells, possibly indicating that peroxiredoxin 1 is a chemoresistance protein. Ma et al[22] observed that peroxiredoxin 1-deficient mice showed an increased sensitivity to cisplatin-induced cell apoptosis. Butzke et al[23] observed the knockdown of peroxiredoxin 1-sensitized HeLa cells to *Aplysia punctata* ink toxin-induced cell death. To our knowledge, this is the first report of the relationship between peroxiredoxin 1 and NAC treatment in squamous cervical cancer. We found that an

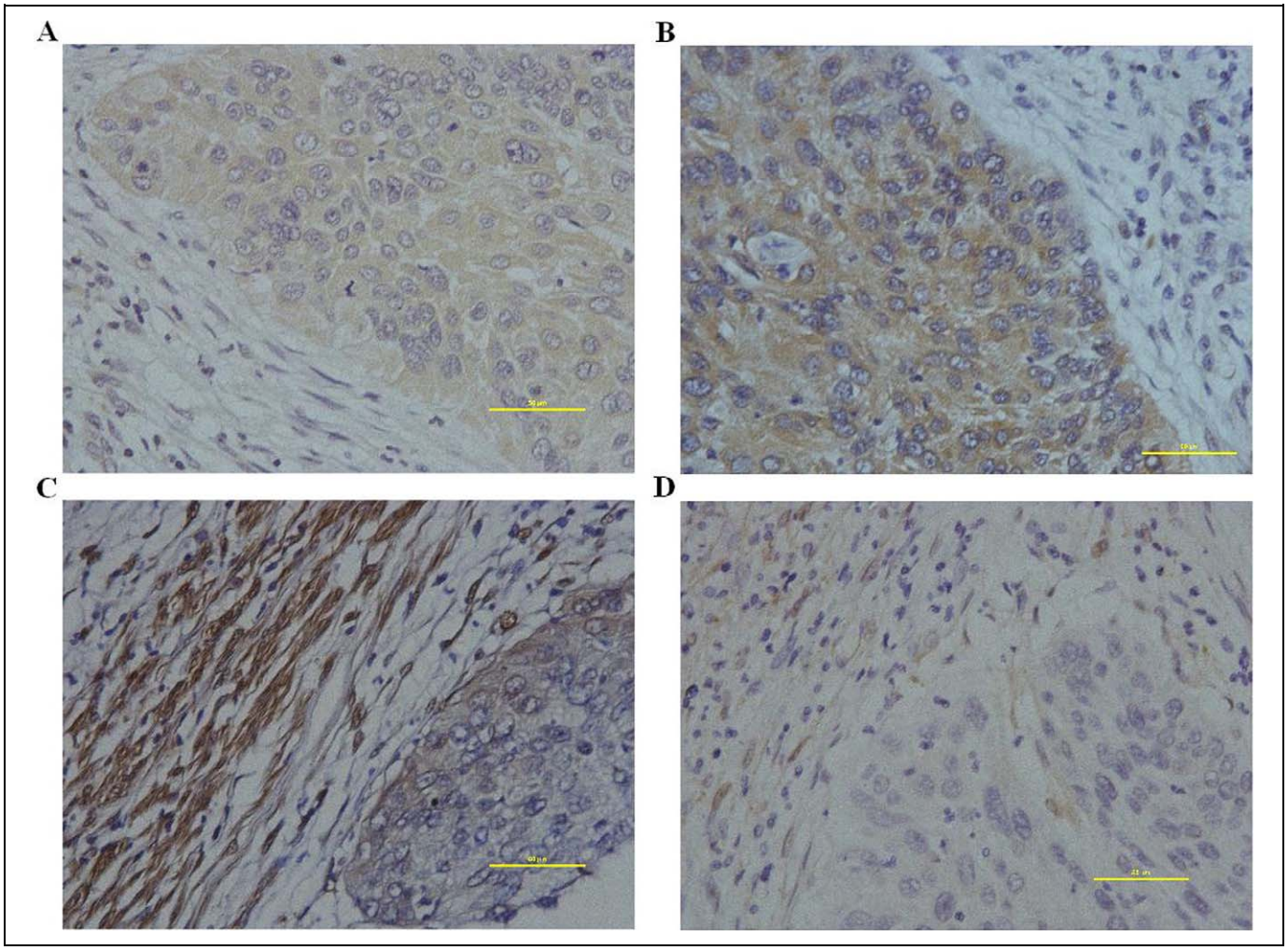

Figure 3. Peroxiredoxin 1 and galectin 1 protein expression in representative of cervical cancer before and after neoadjuvant chemotherapy (NAC). SP ×400. A, Peroxiredoxin 1 before chemotherapy, (B) peroxiredoxin 1 after chemotherapy, (C) galectin 1 before chemotherapy, and (D) galectin 1 after chemotherapy.

upregulation of peroxiredoxin 1 after NAC treatment in squamous cervical cancer, which suggests peroxiredoxin 1 is associated with NAC exposure and may play a role in NAC response.

Galectin 1 is an important member of the β-galactoside-binding galectin protein family. Its expression has been reported in thyroid, colon, glioma, and ovarian cancer tissues, and this protein is involved in the transformation, apoptosis, and migration of cancer cells.[24] Kohrenhagen et al[25] observed that the expression of galectin 1 is associated with the progression of squamous cervical cancer. Galectin 1 is predominantly expressed in the cytoplasm of benign tissue and in the stromal cells of malignant tumors. Its expression in the stromal cells of malignant tumors is closely correlated with the aggressiveness of tumors and the acquisition of the metastatic phenotype. Recently, Kim et al[26] revealed that high galectin 1 expression in peritumoral stroma was significantly correlated with the depth of invasion in stage IB-IIA cervical cancer and lymph node metastasis of cervical cancer, and that galectin 1 may be functionally involved in cell proliferation and invasion. In the present study, galectin 1 expression was also detected only in the stromal cells in cervical cancer by immunohistochemistry either before or after NAC.

Galectin 1 mediates the radioresistance of cervical cancer cells through H-Ras-dependent pathways involved in DNA damage repair.[27] Targeting galectin 1 may be considered a radiosensitizing therapy for cancer. Puchades et al[28] found that galectin 1 was significantly downregulated when cells were treated with wild-type TP53 and the cytotoxic chemotherapeutic SN38 relative to the level in untreated glioblastoma cell lines. Decreasing galectin 1 expression in human Hs683 glioma cells increases their sensitivity to the antitumor effects of various chemotherapeutic drugs, in particular temozolomide, both in vitro and in vivo.[15] In experimental melanomas, galectin 1 knockdown increases the sensitivity to temozolomide in a B16F10 mouse metastatic melanoma model.[29] In contrast, Kuramitsu et al[30] showed that galectin 1 was downregulated in gemcitabine-resistant cells relative to the level in gemcitabine-sensitive cells in human pancreatic cancer cell lines. In the present study, we found that galectin 1 was downregulated in stroma cells in cervical cancer after NAC, a change that was also observed in colorectal cancer after drug treatment,[31]

suggesting that the antiproliferative activity of the drug against cervical cancer might be related to its direct or indirect interaction with galectin 1.

In summary, our novel application of proteomic technique has identified a few known proteins associated with NAC exposure in patients with squamous cervical cancer. Our data point to the possible importance of 2 of these proteins, peroxiredoxin 1 and galectin 1, which may play roles in NAC response. Further studies are necessary to investigate the relationship between their expressions and NAC exposure, to evaluate their expression in NAC-sensitive cervical cancer and in NAC-resistant cervical cancer, and to understand their role in predicting cisplatin-based NAC treatment response. Moreover, detailed investigation of the functional role of the molecular targets identified in this study would improve our understanding of the chemotherapeutic effects of NAC and, in the long run, may lead to a more effective chemotherapeutic treatment of this most common cancer.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by a grant from the National Natural Science Foundation of China (No. 30700195) and sponsored by Zhejiang Provincial Program for the Cultivation of High-level Innovative Health Talents..

References

1. Lee JY, Kim YH, Kim MJ, et al. Treatment of stage IB2, IIA bulky cervical cancer: a single-institution experience of neoadjuvant chemotherapy followed by radical hysterectomy and primary radical hysterectomy. *Arch Gynecol Obstet.* 2011;284(2):477-482.
2. Chen H, Liang C, Zhang L, Huang S, Wu X. Clinical efficacy of modified preoperative neoadjuvant chemotherapy in the treatment of locally advanced (stage IB2 to IIB) cervical cancer: randomized study. *Gynecol Oncol*. 2008;110(3):308-315.
3. Kobayashi K, Furukawa A, Takahashi M, Murata K. Neoadjuvant intra-arterial chemotherapy for locally advanced uterine cervical cancer: clinical efficacy and factors influencing response. *Cardiovasc Intervent Radiol*. 2003;26(3):234-241.
4. Cho YH, Kim DY, Kim JH. Comparative study of neoadjuvant chemotherapy before radical hysterectomy and radical surgery alone in stage IB2-IIA bulky cervical cancer. *J Gynecol Oncol*. 2009;20(1):22-27.
5. Gong F, Peng X, Zeng Z, Yu M, Zhao Y, Tong A. Proteomic analysis of cisplatin resistance in human ovarian cancer using 2-DE method. *Mol Cell Biochem*. 2011;348(1-2):141-147.
6. Wong CS, Wong VW, Chan CM, et al. Identification of 5-fluorouracil response proteins in colorectal carcinoma cell line SW480 by two-dimensional electrophoresis and MALDI-TOF mass spectrometry. *Oncol Rep*. 2008;20(1):89-98.
7. Zhu X, Lv J, Yu L, et al. Proteomic identification of differentially-expressed proteins in squamous cervical cancer. *Gynecol Oncol*. 2009;112(1):248-256.
8. Urbani A, Poland J, Bernardini S, et al. A proteomic investigation into etoposide chemo-resistance of neuroblastoma cell lines. *Proteomics*. 2005;5(3):796-804.
9. Moore LE, Fung ET, McGuire M, et al. Evaluation of apolipoprotein A1 and posttranslationally modified forms of transthyretin as biomarkers for ovarian cancer detection in an independent study population. *Cancer Epidemiol Biomarkers Prev*. 2006;15(9): 1641-1646.
10. Lomnytska M, Dubrovska A, Hellman U, Volodko N, Souchelnytskyi S. Increased expression of cSHMT, Tbx3 and utrophin in plasma of ovarian and breast cancer patients. *Int J Cancer*. 2006;118(2):412-421.
11. Han G, Fan B, Zhang Y, et al. Positive regulation of migration and invasion by vasodilator-stimulated phosphoprotein via Rac1 pathway in human breast cancer cells. *Oncol Rep*. 2008;20(4): 929-939.
12. Lan L, Hayashi T, Rabeya RM, et al. Functional and physical interactions between ERCC1 and MSH2 complexes for resistance to cis-diamminedichloroplatinum(II) in mammalian cells. *DNA Repair (Amst)*. 2004;3(2):135-143.
13. Zhang F, Zhang L, Zhang B, et al. Anxa2 plays a critical role in enhanced invasiveness of the multidrug resistant human breast cancer cells. *J Proteome Res*. 2009;8(11):5041-5047.
14. Shin YK, Yoo BC, Hong YS, et al. Up regulation of glycolytic enzymes in proteins secreted from human colon cancer cells with 5-fluorouracil resistance. *Electrophoresis*. 2009;30(12): 2182-2192.
15. Le Mercier M, Lefranc F, Mijatovic T, et al. Evidence of galectin-1 involvement in glioma chemoresistance. *Toxicol Appl Pharmacol*. 2008;229(2):172-183.
16. Hiro J, Inoue Y, Toiyama Y, Miki C, Kusunoki M. Mechanism of resistance to chemoradiation in p53 mutant human colon cancer. *Int J Oncol*. 2008;32(6):1305-1310.
17. Zhang Y, Shen X. Heat shock protein 27 protects L929 cells from cisplatin-induced apoptosis by enhancing Akt activation and abating suppression of thioredoxin reductase activity. *Clin Cancer Res*. 2007;13(10):2855-2864.
18. Pak JH, Choi WH, Lee HM, et al. Peroxiredoxin 6 overexpression attenuates cisplatin-induced apoptosis in human ovarian cancer cells. *Cancer Invest*. 2011;29(1):21-28.
19. Behtash N, Nazari Z, Ayatollahi H, Modarres M, Ghaemmaghami F, Mousavi A. Neoadjuvant chemotherapy and radical surgery compared to radical surgery alone in bulky stage IB-IIA cervical cancer. *Eur J Surg Oncol*. 2006;32(10):1226-1230.
20. Kim JH, Bogner PN, Baek SH, et al. Up-regulation of peroxiredoxin 1 in lung cancer and its implication as a prognostic and therapeutic target. *Clin Cancer Res*. 2008;14(8):2326-2333.
21. Zhang B, Wang Y, Liu K, et al. Adenovirus-mediated transfer of siRNA against peroxiredoxin I enhances the radiosensitivity of human intestinal cancer. *Biochem Pharmacol*. 2008;75(3):660-667.
22. Ma D, Warabi E, Yanagawa T, et al. Peroxiredoxin I plays a protective role against cisplatin cytotoxicity through mitogen activated kinase signals. *Oral Oncol*. 2009;45(12):1037-1043.

23. Butzke D, Machuy N, Thiede B, Hurwitz R, Goedert S, Rudel T. Hydrogen peroxide produced by Aplysia ink toxin kills tumor cells independent of apoptosis via peroxiredoxin I sensitive pathways. *Cell Death Differ*. 2004;11(6):608-617.
24. Jung EJ, Moon HG, Cho BI, et al. Galectin-1 expression in cancer-associated stromal cells correlates tumor invasiveness and tumor progression in breast cancer. *Int J Cancer*. 2007;120(11):2331-2338.
25. Kohrenhagen N, Volker HU, Kapp M, Dietl J, Kammerer U. Increased expression of galectin-1 during the progression of cervical neoplasia. *Int J Gynecol Cancer*. 2006;16(6):2018-2022.
26. Kim HJ, Do IG, Jeon HK, et al. Galectin 1 expression is associated with tumor invasion and metastasis in stage IB to IIA cervical cancer. *Hum Pathol*. 2013;44(1):62-68.
27. Huang EY, Chen YF, Chen YM, et al. A novel radioresistant mechanism of galectin-1 mediated by H-Ras-dependent pathways in cervical cancer cells. *Cell Death Dis*. 2012;3:e251.
28. Puchades M, Nilsson CL, Emmett MR, et al. Proteomic investigation of glioblastoma cell lines treated with wild-type p53 and cytotoxic chemotherapy demonstrates an association between galectin-1 and p53 expression. *J Proteome Res*. 2007;6(2):869-875.
29. Mathieu V, Le Mercier M, De Neve N, et al. Galectin-1 knockdown increases sensitivity to temozolomide in a B16F10 mouse metastatic melanoma model. *J Invest Dermatol*. 2007;127(10): 2399-2410.
30. Kuramitsu Y, Taba K, Ryozawa S, et al. Identification of up- and down-regulated proteins in gemcitabine-resistant pancreatic cancer cells using two-dimensional gel electrophoresis and mass spectrometry. *Anticancer Res*. 2010;30(9):3367-3372.
31. Wang YJ, Zhang GY, Xiao ZQ, Wang HM, Chen ZC. Preliminary proteomic analysis of indomethacin's effect on tumor transplanted with colorectal cancer cell in nude mice. *J Biochem Mol Biol*. 2006;39(2):171-177.