The screening and analysis of protein signatures and signaling associated with chemoresistance based on Protein Pathway Array technology in gastric cancer

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Abstract. The present study was aimed to identify proteins associated with signaling pathways involved in chemoresistance, and establish a predictive model for chemoresistance in gastric cancer patients after radical surgery. A total of 140 clinically-staged III gastric cancer samples from patients after D2 radical gastrectomy were enrolled in the present study. Protein Pathway Array (PPA) and 286 antibodies were used to assess the protein expression in tumor tissues of patients. The Significance Analysis of Microarray (SAM) software and clustering and discriminant analysis were used to identify differentially expressed proteins between chemosensitive and chemoresistant subsets, and a predictive model for chemoresistance was established using the independent predictive factors. The Ingenuity Pathway Analysis (IPA) software was also used to investigate the relationship between proteins and the signaling transduction network. A total of 23 proteins were differentially expressed between 67 chemosensitive and 73 chemoresitant tumor tissues. Six proteins including PLK1 and DACH1 were independent risk factors for chemoresistance. A predictive model for chemoresistance by these proteins was established, and the accuracy, the sensitivity, and the specificity of this modal was 89.3, 90.3 and 88.2%, respectively. In addition, the present study revealed that differentially expressed proteins were closely related to cellular activity, DNA methylation and DNA damage and repair, and also involved in the ERK/MAPK, Wnt/β-catenin, PI3K/AKT, apoptosis and p53 signaling pathways. In conclusion, the predictive model established by PPA may be an effective detection system for predicting the chemosensitivity of gastric cancer patients after D2 gastrectomy.

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Introduction

Worldwide, gastric cancer is the fourth most common malignant tumor, accounting for ~8% of new cancer cases (1). It is the third leading cause of cancer-related mortality, and the gastric cancer incidence in China accounts for nearly half of all worldwide cases (2).

Currently, surgical resection is possibly the only modality for a cure. However, since 70-90% of gastric cancer patients are first diagnosed at an advanced stage, surgical excision alone could not achieve a cure. Adjuvant chemotherapy has been adopted as the standard treatment for advanced cancer for disease-free survival and overall survival (3-6). The MAGIC trial revealed that the 5-year survival rate of patients receiving adjuvant chemotherapy was significantly higher than those undergoing resection only (36 vs. 23%) (7).

The chemotherapeutic drugs 5-fluorouracil (5-FU), cisplatin and paclitaxel are widely used anticancer agents. However, chemotherapeutic drug resistance is a major cause of treatment failure in patients with cancer. Previous studies suggested a variety of resistance mechanisms in cancer cells underlying the resistance to chemotherapeutic drugs. The methylation of MLH1 promoter (8), the overexpression of Bcl-2 (9), Bcl-xL (9,10) and Mcl-1 (11), and the increased activity of deoxyuridine triphosphatase (DUT) have been found to be associated with drug resistance to 5-FU. AKT (12), c-ABL (13) and P53 signaling (14), as well as the proteins are related to drug resistance to cisplatin. To identify the drug sensitivity of gastric cancer patients in a chemotherapy regimen, and to choose a personalized treatment plan for them are currently essential for physicians.

The Protein Pathway Array (PPA) technology is a proteomic method that can globally characterize proteins and identify changes in protein expression (15). In the present study, the PPA method was used to identify differentially expressed proteins which contributed to chemotherapy resistance and were involved in signaling pathways in patients with gastric cancer. In addition, the independent risk factors among proteins were screened by multivariable logistic regression analysis. Moreover, the prediction model for chemotherapy resistance was built based on the risk factors and risk coefficient. The present study may help clinicians to develop an individualized course of treatment for patients with gastric cancer.

Materials and methods

Patients and samples. From February 2008 to July 2010, a total of 140 patients with clinical stage III gastric cancer (7th AJCC) undergoing D2 radical gastrectomy and postoperative chemotherapy at the First Hospital of Jilin University were enrolled in the present study. All patients were given a written informed consent document before treatment. All patients were followed up for at least 2 years: in the first year, the patients were followed-up for disease status, physical examination, serum tumor marker and abdominal ultrasonography every 3 months, and a routine CT scan of the abdomen every 6 months; in the second year, disease status, physical examination, serum tumor marker and abdominal ultrasonography every 6 months, and a routine CT scan of the abdomen each year. Disease-free survival in patients was the period after curative treatment when no disease could be detected.

All tumor samples were resected from freshly biopsied tumor specimens: ~3 x 3 x 5 mm^3 of tumor tissues were obtained to avoid necrotic tumor tissues and the surrounding tissues, and the tumor tissues were frozen in liquid nitrogen for 24 h, and stored at -80˚C. The other specimens were fixed in neutral-buffered formalin, embedded in paraffin, and confirmed histologically by two pathologists for tumor location, tumor size, depth of invasion, degree of lymph node metastasis and histological type.

Total protein extraction. The 3 x 3 x 5 mm^3 frozen tissues were ground and homogenized. Then, the protein concentration was quantified by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA) and the solution was adjusted to ~1 $\mu g/\mu l$.

SDS-PAGE for protein separation. SDS-PAGE 10% gels were prepared. The protein solution was heat denatured at 95˚C for 5 min. Approximately 300 μl of protein solution with a commercial protein ladder [8 μl; BenchMark™; Invitrogen, Carlsbad, CA, USA)] were subjected to SDS-PAGE using 100 V for 30 min by the stacking gel and 130 V by the separating gel. The proteins on the gel were transferred to a nitrocellulose membrane for 2 h at 100 V. The membranes were stained with Ponceau S to confirm successful transfer.

Antibodies used in PPA analysis. A total of 286 antibodies were used to assess the protein expression involved in multiple pathways including cell proliferation and apoptosis, cell invasion and metastasis, cell cycle, cell metabolism, cell resistance and angiogenesis.

Phosphorylation-specific antibodies were obtained from Cell Signaling Technology, except for p-protein kinase Cα (Ser657) which was obtained from Upstate Biotech (Lake Placid, NY, USA), and p-Met (Tyr1234), p-c-Jun kinase (G-7) and p-focal adhesion kinase (Tyr397) which were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The non-phosphorylation-specific antibodies including Stat1, HER2/ErbB2, β-catenin, p44/42 mitogen-activated protein kinase [MAPK; extracellular signal-regulated kinase (Erk)1/2], Akt, Notch4, eIF4B, NF-κB p50, cAMP responsive element binding, estrogen receptor α, Bcl-xL, RIP, aurora A/AIK, matrix metalloproteinase-9 and Snail were purchased from Cell Signaling Technology. X-linked inhibitor of apoptosis and glutamine synthetase were obtained from BD Biosciences (San Jose, CA, USA). Transforming growth factor (TGF)-β was purchased from R&D Systems (Minneapolis, MN, USA). Hsp90 was obtained from ENZO Life Sciences (Farmingdale, NY, USA). Hypoxia-inducible factor-2α was obtained from Novus Biologicals (Littleton, CO, USA). Cytokeratin 18 was purchased from Dako Corporation (Carpinteria, CA, USA) and FAH from ProteinTech Group (Chicago, IL, USA). Keratin 10 was obtained from Covance Research Products (Berkeley, CA). G protein of vesicular stomatitis virus was purchased from Abcam Corporation (Cambridge, MA, USA). All other antibodies were obtained from Santa Cruz Biotechnology.

PPA analysis. The nitrocellulose membranes were placed in blocking solution [3% bovine serum albumin (BSA)] for 1 h, and then placed in a 20-well slot blotting manifold apparatus (Mini-PROTEAN II Multiscreen Apparatus Ca#170-4017; Bio-Rad Laboratories, Inc., Hercules, CA, USA). Approximately 600 μl containing 1-2 types of primary antibodies were added to lanes 1-19, and lane 20 was added with protein ladders. The membranes were incubated for 12 h at 4˚C.

Subsequently, the primary antibodies were retrieved and the membranes were washed by 600 μl Tris-buffered saline (TBS) for 3 times and 600 μl Tris-buffered saline with Tween-20 (TBST) for 2 times. Binding was detected using horseradish peroxidase-conjugated secondary antibodies (anti-rat/lamb/rabbit; Bio-Rad Laboratories, Inc.) for 1 h at room temperature and washed by TBST.

Immun-Star™ HRP peroxide buffer and the Immun-Star™ HRP luminol enhancer (Cat#94547; Bio-Rad Laboratories, Inc.) in a 1:1 volume ratio was used for enhanced chemiluminescent assay. The luminescent images were captured using a ChemiDoc XRS system (Bio-Rad Laboratories, Inc.) and the images were quantified and calculated by Quantity One 4.5.0 software based on the global median subtraction to decrease the variation between the batches of experiments.

Finally, the antibodies were eluted from the nitrocellulose membranes using Restore™ Western Blot stripping buffer (Cat# 21059; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The thoroughly cleaned membranes were blocked again using 3% BSA solution for 1 h and re-analyzed as aforementioned.

Statistical analysis. The Student's t-test and the χ^2 test were used to evaluate the relationship between the clinicopathological

Table I. Clinicopathological characteristics of 140 gastric cancer cases based on chemosensitivity.

	Number (%)	Chemosensitive (n=67)	Chemoresitant (n=73)	P-value
Age (years)				1
≤60	67 (47.9)	32 (47.8)	35(47.9)	
>60	73 (52.1)	35 (52.2)	38 (52.1)	
Sex				0.494
Male	117 (83.6)	58 (86.6)	59 (80.8)	
Female	23 (16.4)	9 (13.4)	14 (19.2)	
Family history of cancer				0.644
Positive	21 (15.0)	9 (13.4)	12 (16.4)	
Negative	119 (85.0)	58 (86.6)	61 (83.6)	
Curative surgery for gastric cancer				0.085
Subtotal gastrectomy	103 (73.6)	54 (80.6)	49 (67.1)	
Total gastrectomy	37 (26.4)	13 (19.4)	24 (32.9)	
Histological feature				0.159
Histological grade				
Moderate/ high	48 (34.3)	27 (40.3)	21 (28.8)	
Low	92 (65.7)	40 (59.7)	52 (71.2)	
Vascular invasion				0.444
Positive	103 (73.6)	47 (70.1)	56 (76.7)	
Negative	37 (26.4)	20 (29.9)	17 (23.3)	
Tumor size (cm)				0.041
≤5	79 (56.4)	44 (65.7)	35 (47.9)	
>5	61 (43.6)	23 (34.3)	38 (52.1)	
Tumor location				0.728
Proximal (upper panel)	53 (37.9)	24 (35.8)	29 (39.7)	
Distal (lower panel)	87 (62.1)	43 (64.2)	44 (60.3)	
Depth of invasion[a]				0.525
T1	0 (0.0)	0 (0.0)	0 (0.0)	
T2	6 (4.3)	1 (1.5)	5 (6.8)	
T3	13 (9.3)	7 (10.4)	6 (8.2)	
T4	121 (86.4)	59 (88.1)	62 (84.9)	
Degree of lymph node metastasis[a]				0
N0	0 (0.0)	0 (0.0)	0 (0.0)	
N1	33 (23.6)	24 (35.8)	9 (12.3)	
N2	33 (23.6)	19 (28.4)	14 (19.2)	
N3	74 (52.8)	24 (35.8)	50 (68.5)	
Outcome				0
The last follow-up				
Death	71 (50.7)	4 (6.0)	67 (91.8)	
Survival	69 (49.3)	63 (94.0)	6 (8.2)	

[a]According to the 7th edition of the AJCC Cancer Staging Manual (AJCC-7). The p-values for T and N stages were analyzed by Spearman test, the other p-values were obtained by χ^2 test. A p-value <0.05 was considered statistically significant.

factors and drug resistance of patients. A Spearman test was performed to determine the association between the different T and N stages, and chemotherapy resistance. The Significance Analysis of Microarray (SAM; http://www-stat.stanford. edu/~tibs/SAM/) software was used to screen differentially expressed proteins, and the further clustering and discriminant analysis for proteins were performed using k-fold cross-validation and hierachical clustering analysis (htt://linus.nci.nih.gov/BRB-ArrayTools.html) of BRB Array Tools v.3.3.0. SPSS 17.0 (SPSS v17.0 software; SPSS, Inc.,

Table II. Differentially expressed proteins between chemosensitive and chemoresistant gastric cancer.

Protein	Gene ID	Average		Fold change	P-value	q-value (%)
		Chemosensitive	Chemoresistant			
Up						
cdk2	CDK2	12305.4	14555.1	1.18	0.04	3.8
cdk4	CDK4	964.1	2109.4	2.19	0	0
WT1	WT1	1749.3	3271.4	1.87	0	0
NFκBp50	NFKB1	2213.3	2865.3	1.29	0.04	3.8
H-Ras	HRAS	873.3	1268	1.45	0	1.8
Bcl-xL	BCL2L1	7748.7	10261.4	1.32	0.01	0
ERCC1	ERCC1	4749.1	6226.5	1.31	0.03	3.8
HMG-1	HMGB1	1667.7	3973.9	2.38	0	0
FKHR	FOXO1	5075.6	7949.6	1.57	0	0
HDAC1	HDAC1	6297.3	8257.4	1.31	0	0
NMT1	NMT1	8433.7	10922	1.3	0	0
PLK1	PLK1	3190	6405.5	2.01	0	0
P-cadherin	CDH3	781.9	1051.3	1.34	0	3.8
β3 tubulin	TUBB3	946.9	1775	1.87	0	1.8
V-ATPase H	ATP6V1H	2562.4	3252.5	1.27	0.01	0
tsg 101	TSG101	632.5	971.4	1.54	0	0
Calpastatin	BIRC3	764.3	1114.5	1.46	0	1.8
Calpain 2	CAPN2	12604.6	14698.4	1.17	0	0
Down						
P-JNK	JNK	2945.6	1247.1	0.42	0	0
DACH1	DACH1	3070.7	1599.8	0.52	0	0
E-cadherin	CDH1	1448.8	577.3	0.4	0	0
Cytokeratin 18	KRT18	30968.5	19842.4	0.64	0	0
ADH	ADH1	38848.6	23953.8	0.62	0	0

Up and down represent overexpressed and dowregulated proteins in chemoresistant tissues.

Chicago, IL, USA) was used to identify the relationship between the PPA and the clinical data. P<0.05 indicated a statistically significant difference. The Ingenuity Pathway Analysis (IPA), version 9.0 (Ingenuity Systems, Inc., Redwood City, CA, USA) was used for pathway analysis.

Results

General and pathological characteristics of subjects. The clinical data and pathological characteristics of patients are shown in Table I. According to the evaluation criteria of post-operative disease-free survival, 73 patients had a disease-free interval <12 months and were designated as the chemotherapy-resistant group; while 67 patients with a disease-free survival of >36 months, were designated as the chemotherapy-sensitive group.

The preoperative average age was 60.5 years old, and the global sex distribution was 5.1:1. No significant difference was found in age, sex distribution family history of cancer, the ratio of radical subtotal gastrectomy, histopathological grade, vascular invasion, tumor location and depth of invasion between chemoresistant and chemosensitive patients (p=0.726, p=0.494, p=0.644, p=0.085, p=0.159, p=0.444, p=0.728 and p=0.525, respectively). The tumor size of chemoresistant patients was bigger than that of chemosensitive patients (p=0.041), and the

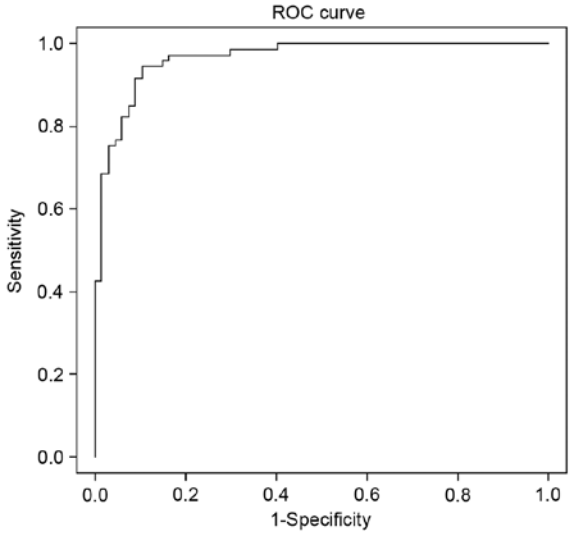


Figure 1. Receiver operating characteristic (ROC) curve based on a compound covariate model classifier. The area under ROC curve is 96.5%.

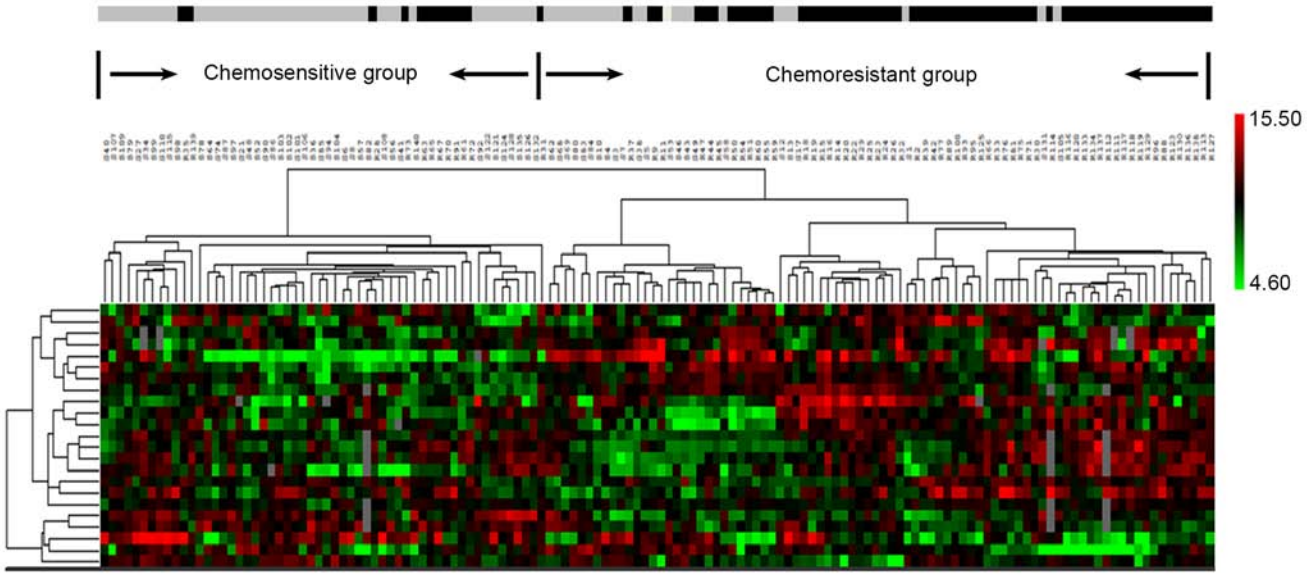


Figure 2. Hierarchical clustering analysis of differentially expressed proteins in chemosensitive and chemoresistant gastric cancer. The right vertical color scale indicates protein expression: red indicates increased expression, green indicates decreased expression, black indicates no difference in expression, and gray indicates no expression. The top horizontal black-gray bars represent the different groups: the gray represents the chemosensitive group, and the black represents the chemoresistant group. Each column of numbers and bars corresponds to the coding and grouping of the specimens. Each row in the heat map represents a protein.

number of malignant nodes of chemoresistant patients was more than that of chemosensitive patients ($p<0.001$). There was a significant difference in mortality, which was 6.0% in the chemosensitive group and 91.8% in the chemoresistant group ($p<0.001$).

Differentially expressed proteins in signaling pathways between chemosensitive and chemoresistant patients. After comparing the differential expression of proteins between 73 chemoresistant tumors and chemosensitive tumors, total 23 differentially expressed proteins (18 overexpressed and 5 downexpressed) were identified (t-test $p<0.05$; SAM $q<0.05$; Table II). SVMs for protein classification revealed 18 proteins, including PLK1, FKHR, HDAC1, calpain 2, WT1, cdk4, β3 tubulin, HMG-1, NMT1, Bcl-xL, V-ATPase H, tsg 101, calpastatin, P-cadherin, ADH, P-JNK, DACH1 and E-cadherin ($p<0.01$) yielded an accuracy of 93.6%, a sensitivity of 94.8% and a specificity of 93.0%. While 11 proteins were identified by KNN method, including PLK1, FKHR, HDAC1, WT1, cdk4, β3 tubulin, HMG-1, ADH, P-JNK, DACH1 and E-cadherin ($p<0.01$) with an accuracy of 89.3%, a sensitivity of 90.3%, and a specificity of 88.2%. The Bayesian ROC curve estimation revealed an area under the curve (AUC) = 96.5% (Fig. 1). The hierarchical clustering analysis of 23 proteins performed by BRB is shown in Fig. 2.

Logistic regression model for chemotherapy response. Univariate logistic regression analysis revealed that 15 proteins, including PLK1, FKHR, HDAC1, WT1, CDK4, HMG-1, NMT1, Bcl-xL, H-Ras, ERCC1, ADH, P-JNK, DACH1, cytokeratin 18 and E-cadherin, as well as 2 clinicopathologic factors of AJCC-N and AJCC-TNM stages, had significant association with chemotherapy response.

The multivariate logistic regression analysis was used to determine the independent predictors associated with chemotherapeutic sensitivity in human gastric cancer. The results in Table III revealed that PLK1, DACH1, E-cadherin, FKHR, ADH and ERCC1 could be the independent predictors. The Cox and Snell R^2 and Nagelkerke R^2 in the logistic model were 0.571 and 0.762, respectively.

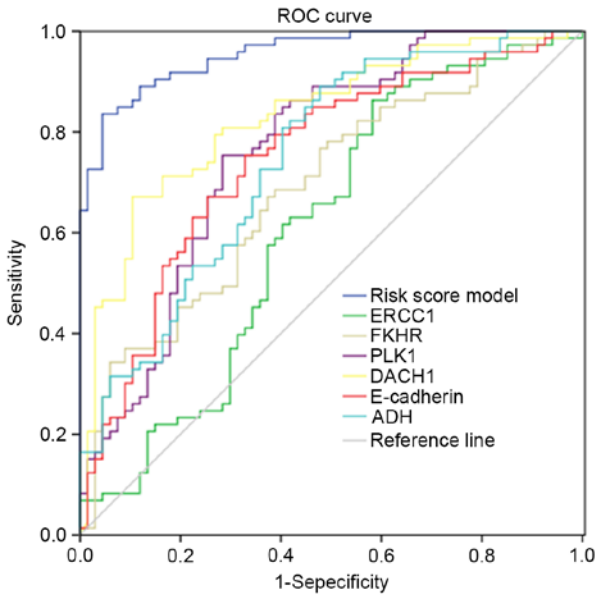


Figure 3. Receiver operating characteristic (ROC) curves of different predictor models for chemoresistivity.

The ROC curve analysis of 6 independent factors revealed the overall predictive value with a AUC of 96%, which was higher than the area of a single predictor (61-83%). This indicated the good prediction efficiency of the predictive risk score model (Fig. 3).

Moreover, the Risk Assessment System (RAS) of chemotherapy resistance in gastric cancer was built: Risk score = $e^z/(1+e^z)$ x 100;

Table III. Independent predictors of chemoresistance based on the multivariate logistic regression analysis.

			EXP (B) 95% CI		
Variables	B	Sig.	Exp (B)	Lower	Upper
PLK	1.80	0.00	6.03	2.79	13.03
DACH1	-0.48	0.04	0.62	0.39	0.98
E-cadherin	-0.77	0.00	0.46	0.30	0.71
FKHR	0.94	0.01	2.57	1.32	4.99
ADH	-2.05	0.00	0.13	0.05	0.36
ERCC1	0.65	0.02	1.92	1.10	3.35
Constant	1.95	0.84	7.05		

B, regression coefficients; OR, odds ratio; CI, confidence interval.

$Z = B0 + B1 \times V1 + B2 \times V2 + \ldots\ldots + Bn \times Vn$. Where e denotes the natural logarithm; Z is the results of logistic regression; B0 represents the logistic regression coefficient for the constant; 'V1…Vn' is the independent variable for multivariate regression; 'B1…Bn' is the regression coefficient corresponding to 'V1…Vn'. Fig. 4A revealed that most of the patients had a risk score of 0-20 and 80-100, and the risk of chmoresistance increased according to the increased risk score (Fig. 4B). Therefore, all of the patients were subjected to 3 groups: the high-risk group with a risk score of >80, and a high risk of chemotherapeutic resistance; the medium-risk group with a risk score between 20-80, and a moderate risk of chemotherapeutic resistance; the low-risk group with a risk score of <20, and a low risk of chemotherapeutic resistance (Fig. 4C).

Finally, the RAS was validated in each subject. The results revealed that a total of 87.9% samples were correctly predicted, among which, 89.6% were correct in the chemosensitive group, and in the chemoresistant group, 86.3% of cases were predicted correctly (data not shown).

IPA system and the signaling pathways associated with chemotherapeutic sensitivity in gastric cancer. To identify the roles of differentially expressed proteins in the signal transduction pathway contributing to chemotherapy resistance in gastric cancer, the IPA system (version 9.0) revealed that 23 proteins were mainly participated in 5 types of signaling pathways (Fig. 5A), such as cancer, cell cycle (12 proteins) and cell death and survival, cellular growth and proliferation (10 proteins). Two significant signaling networks with the most differentially expressed proteins are shown in Fig. 5B and C and each node in the network indicated a classic signaling pathway. In addition, the top 30 signaling pathways ($p<0.01$) with the highest correlation are shown in Fig. 5D, such as ErbB signaling, p53 signaling, 14-3-3-mediated signaling, and the PTEN signaling.

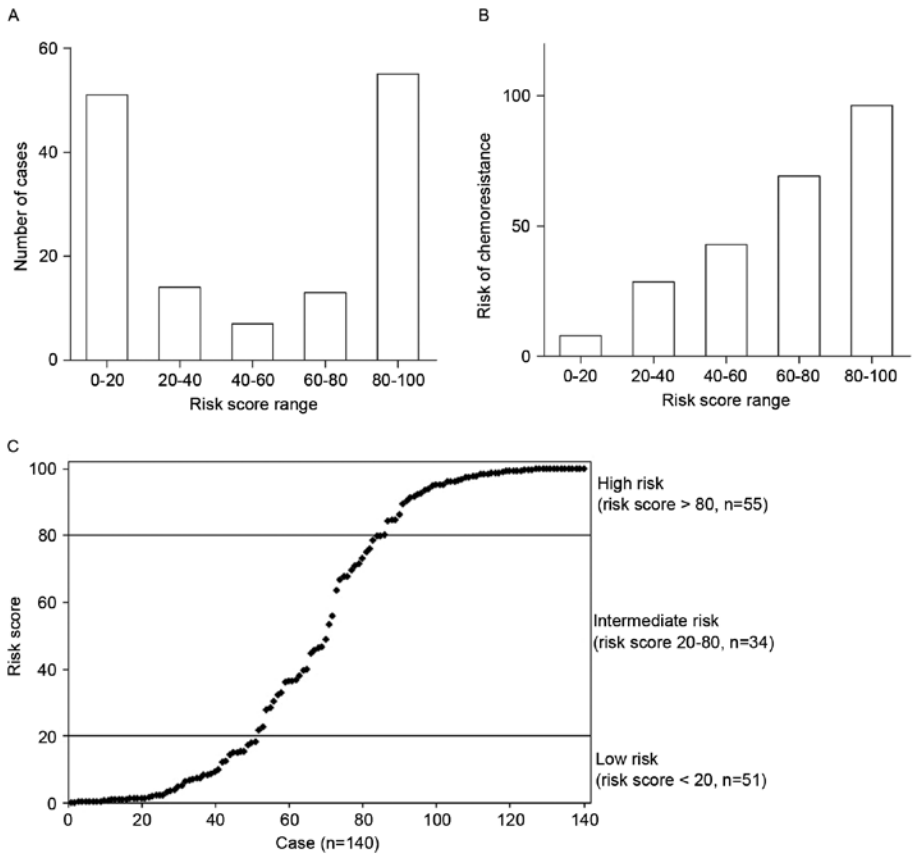
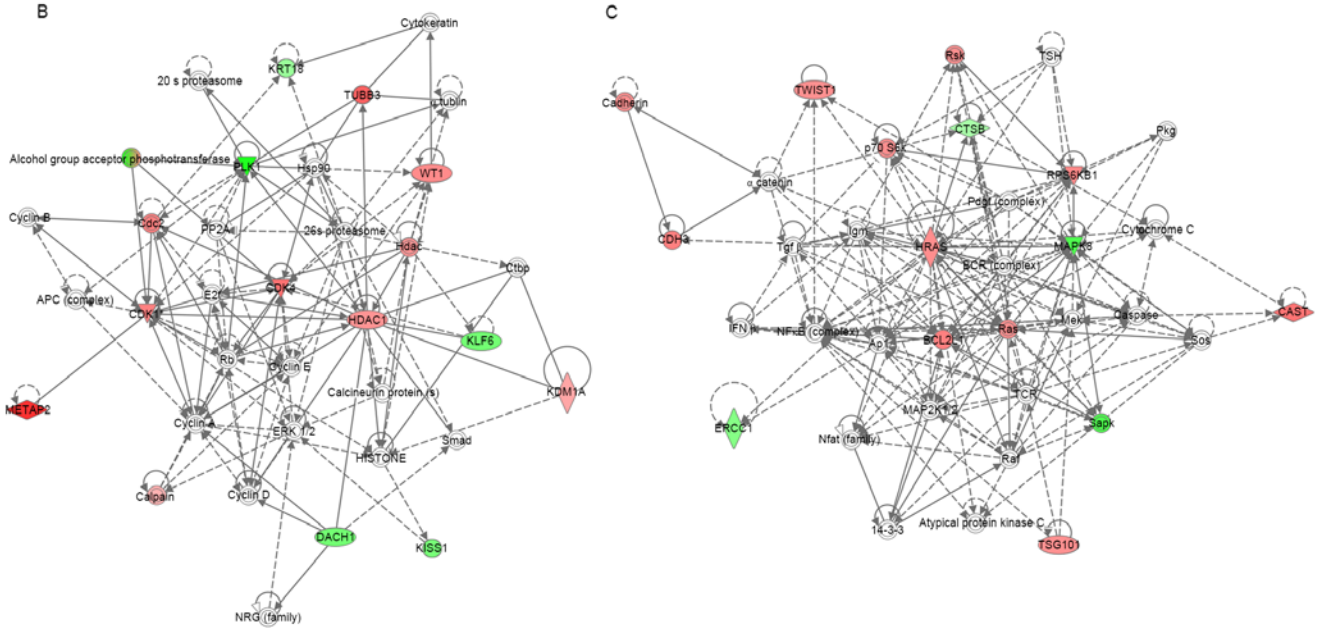


Figure 4. Characteristics of chemoresistant and the chemoresistance risk scores of 140 gastric cancer patients. (A) Most of the patients were distributed in the extremes of the risk score ranges (0-20 and 80-100), whereas a few patients were located in the middle of the range. (B) The risk of chemoresistance was correlated with score levels. (C) Gastric cancer patients (140) were ranked according to their chemoresistance risk scores, and the two lines divided 51 cases into a low-risk group (risk scores ≤20), 34 cases into an intermediate-risk group (risk scores >20 and <80) and 55 cases into a high-risk group (risk scores ≥80).

A

	Top functions	Score
1	Cancer, cell cycle	27
2	Cell death and survival, cellular growth and proliferation	20
3	Cell morphology, cancer	11
4	Carbohydrate metabolism, cellular movement, small molecule biochemistry	5
5	Cell-to-cell signaling and interaction, embryonic development	3



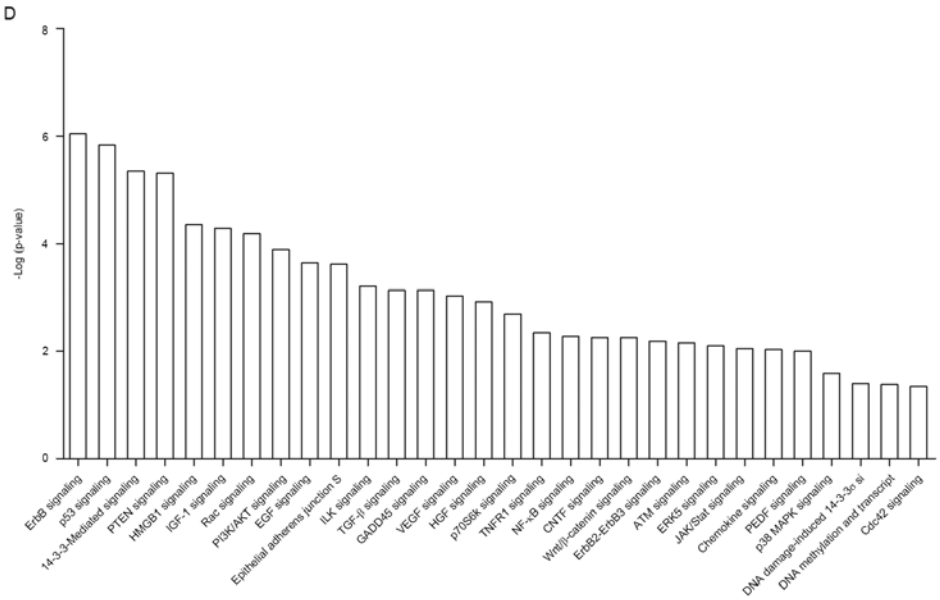


Figure 5. (A) Top 5 functions, (B and C) 2 most relevant signaling networks, (D) and top 30 pathways based on the differentially expressed proteins between chemoresistant and chemosensitive groups. (B and C) Green and red represents the low and high expression of proteins in the chemoresistance group, respectively; the degree of color shade represents the degree of protein increase or decrease in expression level; the solid and the dotted line represents the direct and indirect interaction between two proteins, respectively. (D) The -log p-value indicates the significance of the signaling pathways on the basis of the number of differentially expressed proteins.

Discussion

More individualized treatment regimens to decrease side-effects induced by adjuvant chemotherapy may be essential and the molecular mechanisms underlying resistance to chemotherapeutic drugs was widely investigated (16). In the present study, the PPA method was used to explore the underlying mechanisms of chemoresistance in gastric cancer. The results revealed that 23 proteins obtained between 73 chemotherapy-resistant patients and 67 chemotherapy-sensitive patients were mainly associated with cell adhesion, proliferation, migration, cell cycle, cell signaling and interaction. In addition, 11 proteins distinguished 2 groups of patients with an accuracy of 89.3%,

a sensitivity of 90.3% and a specificity of 88.2%. These results may help surgeons to classify the gastric cancer tissues into chemotherapy sensitive and chemotherapy resistant.

The differentially expressed proteins identified in the present study were consistent with various studies. For example, calpain, ERCC1 and β3 tubulin were observed to be highly expressed in gastric cancer cells resistant to chemotherapeutic agents (17-19), while E-cadherin and DACH1 were lowly expressed (20,21). This may indicate the prediction features of proteins in chemotherapy drug-resistance. Since single predictors usually do not provide accurate predictions at the individual-level, the multivariable risk prediction model which included multiple predictors, facilitates clinical decision-making (22). The similar prediction models were built in various types of cancer, such as lung cancer (23,24). The PPA analysis built a predictive model for chemoresistance based on the protein expression profiling. Moreover, the efficacy and the good prediction strength was explained. This model may help to predict drug resistance to chemotherapeutic agents in gastric cancer patients with high specificity and sensitivity.

Previous studies have demonstrated the correlation between chemotherapy resistance in gastric cancer tissues and a large number of aberrantly expressed proteins in signal transduction pathways, such as protein phosphorylation (25), methylation (8) and aberrant expression (9). IPA is commonly used to combine differentially expressed genes with associated-networks, functions and canonical pathways (26). The results revealed that the differentially expressed proteins were mainly associated with cell actions, cancers and cell signaling and interaction. Moreover, the ErbB, p53 and 14-3-3-mediated signaling, and the PTEN signaling pathway were enriched by 23 differentially expressed proteins. This is consistent with previous studies that revealed that chemoresistance in cancer is mediated via various signaling pathways (27-30).

Our results revealed that multiple signaling pathways and proteins were involved in chemotherapy and chemotherapy resistance in gastric cancer. The phosphorylation, methylation and aberrant expression of proteins are closely related to the chemoresistance of gastric cancer. The predictive risk model established by PPA technique, the model classifier and the logistic regression for chemoresistance are feasible, and could have important clinical implications in predicting the chemoresistance in radically resected gastric cancer patients.

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References

1. Fox JG and Wang TC: Inflammation, atrophy, and gastric cancer. J Clin Invest 117: 60-69, 2007.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
3. Nakajima T, Kinoshita T, Nashimoto A, Sairenji M, Yamaguchi T, Sakamoto J, Fujiya T, Inada T, Sasako M and Ohashi Y; National Surgical Adjuvant Study of Gastric Cancer Group: Randomized controlled trial of adjuvant uracil-tegafur versus surgery alone for serosa-negative, locally advanced gastric cancer. Br J Surg 94: 1468-1476, 2007.
4. Sasako M, Sakuramoto S, Katai H, Kinoshita T, Furukawa H, Yamaguchi T, Nashimoto A, Fujii M, Nakajima T and Ohashi Y: Five-year outcomes of a randomized phase III trial comparing adjuvant chemotherapy with S-1 versus surgery alone in stage II or III gastric cancer. J Clin Oncol 29: 4387-4393, 2011.
5. Bang YJ, Kim YW, Yang HK, Chung HC, Park YK, Lee KH, Lee KW, Kim YH, Noh SI, Cho JY, *et al*; CLASSIC trial investigators: Adjuvant capecitabine and oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): A phase 3 open-label, randomised controlled trial. Lancet 379: 315-321, 2012.
6. Di Costanzo F, Gasperoni S, Manzione L, Bisagni G, Labianca R, Bravi S, Cortesi E, Carlini P, Bracci R, Tomao S, *et al*; Italian Oncology Group for Cancer Research: Adjuvant chemotherapy in completely resected gastric cancer: A randomized phase III trial conducted by GOIRC. J Natl Cancer Inst 100: 388-398, 2008.
7. Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, *et al*; MAGIC Trial Participants: Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 355: 11-20, 2006.
8. Arnold CN, Goel A and Boland CR: Role of hMLH1 promoter hypermethylation in drug resistance to 5-fluorouracil in colorectal cancer cell lines. Int J Cancer 106: 66-73, 2003.
9. Violette S, Poulain L, Dussaulx E, Pepin D, Faussat AM, Chambaz J, Lacorte JM, Staedel C and Lesuffleur T: Resistance of colon cancer cells to long-term 5-fluorouracil exposure is correlated to the relative level of Bcl-2 and Bcl-X_L in addition to *Bax* and *p53* status. Int J Cancer 98: 498-504, 2002.
10. Liu R, Page C, Beidler DR, Wicha MS and Núñez G: Overexpression of Bcl-x_L promotes chemotherapy resistance of mammary tumors in a syngeneic mouse model. Am J Pathol 155: 1861-1867, 1999.
11. Shi X, Liu S, Kleeff J, Friess H and Büchler MW: Acquired resistance of pancreatic cancer cells towards 5-Fluorouracil and gemcitabine is associated with altered expression of apoptosis-regulating genes. Oncology 62: 354-362, 2002.
12. Guinea Viniegra J, Hernández Losa J, Sánchez-Arévalo VJ, Parada Cobo C, Fernández Soria VM, Ramón y Cajal S and Sánchez-Prieto R: Modulation of PI3K/Akt pathway by E1a mediates sensitivity to cisplatin. Oncogene 21: 7131-7136, 2002.
13. Machuy N, Rajalingam K and Rudel T: Requirement of caspase-mediated cleavage of c-Abl during stress-induced apoptosis. Cell Death Differ 11: 290-300, 2004.
14. Vekris A, Meynard D, Haaz MC, Bayssas M, Bonnet J and Robert J: Molecular determinants of the cytotoxicity of platinum compounds: The contribution of in silico research. Cancer Res 64: 356-362, 2004.
15. Shu C, Liu Z, Cui L, Wei C, Wang S, Tang JJ, Cui M, Lian G, Li W, Liu X, *et al*: Protein profiling of preeclampsia placental tissues. PLoS One 9: e112890-e112890, 2014.
16. Wildiers H and Brain E: Different adjuvant chemotherapy regimens in older breast cancer patients? Ann Oncol 26: 613-615, 2015.
17. Nabeya Y, Suzuki T, Furuya A, Koide N, Ohkoshi M, Takiguchi M, Ochiai T, Matsubara H and Hiwasa T: Calpain regulates thymidylate synthase-5-fluoro-dUMP complex levels associated with response to 5-fluorouracil in gastric cancer cells. Cancer Sci 102: 1509-1515, 2011.
18. Yin M, Yan J, Martinez-Balibrea E, Graziano F, Lenz HJ, Kim HJ, Robert J, Im SA, Wang WS, Etienne-Grimaldi MC, *et al*: *ERCC1* and *ERCC2* polymorphisms predict clinical outcomes of oxaliplatin-based chemotherapies in gastric and colorectal cancer: A systemic review and meta-analysis. Clin Cancer Res 17: 1632-1640, 2011.
19. Sève P and Dumontet C: Is class III beta-tubulin a predictive factor in patients receiving tubulin-binding agents? Lancet Oncol 9: 168-175, 2008.
20. Carneiro P, Figueiredo J, Bordeira-Carriço R, Fernandes MS, Carvalho J, Oliveira C and Seruca R: Therapeutic targets associated to E-cadherin dysfunction in gastric cancer. Expert Opin Ther Targets 17: 1187-1201, 2013.
21. Yamada Y, Arao T, Gotoda T, Taniguchi H, Oda I, Shirao K, Shimada Y, Hamaguchi T, Kato K, Hamano T, *et al*: Identification of prognostic biomarkers in gastric cancer using endoscopic biopsy samples. Cancer Sci 99: 2193-2199, 2008.
22. Ahmed I, Debray TP, Moons KG and Riley RD: Developing and validating risk prediction models in an individual participant data meta-analysis. BMC Med Res Methodol 14: 3, 2014.

23. Spitz MR, Hong WK, Amos CI, Wu X, Schabath MB, Dong Q, Shete S and Etzel CJ: A risk model for prediction of lung cancer. J Natl Cancer Inst 99: 715-726, 2007.
24. Marcus MW, Raji OY, Duffy SW, Young RP, Hopkins RJ and Field JK: Incorporating epistasis interaction of genetic susceptibility single nucleotide polymorphisms in a lung cancer risk prediction model. Int J Oncol 49: 361-370, 2016.
25. Oki E, Baba H, Tokunaga E, Nakamura T, Ueda N, Futatsugi M, Mashino K, Yamamoto M, Ikebe M, Kakeji Y, *et al*: Akt phosphorylation associates with LOH of PTEN and leads to chemoresistance for gastric cancer. Int J Cancer 117: 376-380, 2005.
26. Krämer A, Green J, Pollard J Jr and Tugendreich S: Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics 30: 523-530, 2014.
27. Tan M and Yu D: Molecular mechanisms of erbB2-mediated breast cancer chemoresistance. Adv Exp Med Biol 608: 119-129, 2007.
28. Rohwer N, Dame C, Haugstetter A, Wiedenmann B, Detjen K, Schmitt CA and Cramer T: Hypoxia-inducible factor 1α determines gastric cancer chemosensitivity via modulation of p53 and NF-kappaB. PLoS One 5: e12038-e12038, 2010.
29. Zhao J, Meyerkord CL, Du Y, Khuri FR and Fu H: 14-3-3 proteins as potential therapeutic targets. Semin Cell Dev Biol 22: 705-712, 2011.
30. Li J, Zhang Y, Zhao J, Kong F and Chen Y: Overexpression of miR-22 reverses paclitaxel-induced chemoresistance through activation of PTEN signaling in *p53*-mutated colon cancer cells. Mol Cell Biochem 357: 31-38, 2011.