Published OnlineFirst March 29, 2011; DOI: 10.1158/1078-0432.CCR-10-2180

Clinical Cancer Research

Gene Expression Signature–Based Prognostic Risk Score in Gastric Cancer

Jae Yong Cho, Jae Yun Lim, Jae Ho Cheong, et al.

Clin Cancer Res 2011;17:1850-1857. Published OnlineFirst March 29, 2011.

Updated version Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-10-2180

Supplementary Material Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2011/03/31/1078-0432.CCR-10-2180.DC1.html

Cited Articles This article cites by 30 articles, 6 of which you can access for free at: http://clincancerres.aacrjournals.org/content/17/7/1850.full.html#ref-list-1

Citing articles This article has been cited by 5 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/17/7/1850.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.

Downloaded from clincancerres.aacrjournals.org on November 4, 2013. © 2011 American Association for Cancer Research.

Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Gene Expression Signature–Based Prognostic Risk Score in Gastric Cancer

Jae Yong Cho[1,4], Jae Yun Lim[1,4], Jae Ho Cheong[1,5], Yun-Yong Park[1], Se-Lyun Yoon[7], Soo Mi Kim[1,9], Sang-Bae Kim[1], Hoguen Kim[6], Soon Won Hong[6], Young Nyun Park[6], Sung Hoon Noh[5], Eun Sung Park[1], In-Sun Chu[8], Waun Ki Hong[2], Jaffer A. Ajani[3], and Ju-Seog Lee[1]

Abstract

Purpose: Despite continual efforts to develop a prognostic model of gastric cancer by using clinical and pathologic parameters, a clinical test that can discriminate patients with good outcomes from those with poor outcomes after gastric cancer surgery has not been established. We aim to develop practical biomarker-based risk score that can predict relapse of gastric cancer after surgical treatment.

Experimental Design: Microarray technologies were used to generate and analyze gene expression profiling data from 65 gastric cancer patients to identify biomarker genes associated with relapse. The association of expression patterns of identified genes with relapse and overall survival was validated in independent gastric cancer patients.

Results: We uncovered two subgroups of gastric cancer that were strongly associated with the prognosis. For the easy translation of our findings into practice, we developed a scoring system based on the expression of six genes that predicted the likelihood of relapse after curative resection. In multivariate analysis, the risk score was an independent predictor of relapse in a cohort of 96 patients. We were able to validate the robustness of the six-gene signature in an additional independent cohort.

Conclusions: The risk score derived from the six-gene set successfully prognosticated the relapse of gastric cancer patients after gastrectomy. *Clin Cancer Res; 17(7); 1850–7. ©2011 AACR.*

Introduction

Gastric cancer is the second leading cause of cancer-related death in the world (1). Surgery remains the gold standard in the treatment of gastric cancer (2, 3). In the United States, however, only a small fraction of patients with gastric cancer who undergo curative resection have early-stage disease, and the prognosis for patients with more advanced stage (II or III) remains poor because of the high rate of relapse after gastrectomy (4, 5). Preoperative staging techniques, including laparoscopy and noninvasive imaging systems (i.e., endoscopic ultrasonography and positron emission tomography), have a relatively low sensitivity for discriminating patients with favorable clinical biology versus poor clinical biology (6). Moreover, the outcomes of patients considered to have a similar clinical or pathologic stage remain unpredictable, especially when patients are treated similarly. For example, not all patients with stage III tumor survive 5 years even after successful curative resection (7, 8), and the outcomes remain uneven whether or not preoperative or postoperative therapy is administered. This inherent clinical heterogeneity is most likely due to the diverse molecular profile of gastric cancer. Thus, identifying the diversity in the molecular profile of gastric cancer that governs the clinical behavior of tumors could lead to new and more effective clinical strategies. Microarray technologies have been successfully used to predict clinical outcomes and survival, as well as classify different types of cancer (9–13). Recent studies in gastric cancer have identified genes that differ according to histologic factors and age, as well as those for gastric cancer prognosis prediction (14–16). However, these studies have failed to create molecular prognostic tests that could be practical for gastric cancer patients.

In the present study, we characterized tumor transcriptome at the systems level to identify potential markers that could be used to divide patients into distinct subclasses that have not been recognized by current staging system.

Authors' Affiliations: Departments of [1]Systems Biology, [2]Thoracic/Head and Neck Medical Oncology, and [3]Gastrointestinal Medical Oncology, Division of Cancer Medicine, The University of Texas M. D. Anderson Cancer Center, Houston,Texas; Departments of [4]Medical Oncology, [5]Surgery, and [6]Pathology, Yonsei University College of Medicine, Seoul; [7]Department of Biological Science, Dong-A University, Busan; [8]Korean Bioinformation Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon; and [9]Department of Physiology, Chonbuk National University Medical School and Hospital, Jeonju, Korea

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Ju-Seog Lee, Department of Systems Biology, Division of Cancer Medicine, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Unit 950, Houston, TX 77054. Phone: 713-834-6154; Fax: 713-563-4235. E-mail: jlee@mdanderson.org

doi: 10.1158/1078-0432.CCR-10-2180

©2011 American Association for Cancer Research.

Translational Relevance

Gastric cancer is the second leading cause of cancer-related death in the world, and prognosis is difficult to predict for individual patients. Most of gastric cancer patients receive similar treatments, typically surgery followed by chemotherapy because there are no reliable biomarkers to optimize therapy. Our study identified the prognostic gene expression signatures and limited number of prognostic biomarkers. We developed a score based on these 6 genes which significantly associated with survival and early relapse. This method requires the determination of only 6 genes by using simple reverse transcriptase PCR technology and easily accessible paraffin-embedded tissues, which are routinely acquired at diagnosis. This will open up new opportunities to optimize treatment of gastric cancer patients according to molecular subtypes of tumors.

Materials and Methods

Patients and samples

Tumor specimens and clinical data from 213 gastric cancer patients undergoing gastrectomy as a primary treatment option were obtained from Yonsei University Severance Hospital, Seoul, Korea. Sixty-five surgically removed frozen gastric adenocarcinoma tissues, with 19 normal surrounding tissue samples, from gastric cancer patients were used for microarray experiments [Yonsei gastric cancer (YGC) cohort]. In addition, 6 frozen tissue samples from gastrointestinal stromal tumor (GIST) patients were included in the microarray experiments as reference for distinct tumors resided in gastric tissues. To validate gene expression patterns found by microarray analysis, quantitative reverse transcriptase PCR (qRT-PCR) experiments were done with RNA from 96 formalin-fixed, paraffin-embedded (FFPE) tissues from a separate gastric adenocarcinoma patient group from Yonsei Gangnam Severance Hospital (GSH1 cohort). For validation of risk score, FFPE tissues of independent patient group from Yonsei Gangnam Severance Hospital (GSH2 cohort, $n = 52$) were used for qRT-PCR. Tissue specimens used in microarray and qRT-PCR were obtained from the surgical specimens. All samples were collected after obtaining written informed consent from patients, and the study was approved by the Institutional Review Board of The University of Texas M. D. Anderson Cancer Center (Houston, TX), the Yonsei University Severance Hospital (Seoul, Korea), and Yonsei Gangnam Severance Hospital (Seoul, Korea). Clinical data also were obtained retrospectively. All of the experiments and analyses were done in the Department of Systems Biology at M. D. Anderson Cancer Center.

Experimental procedures for microarray

Total RNA was extracted from the fresh-frozen tissues by using a mirVana RNA Isolation labeling kit (Ambion, Inc.). Five hundred nanograms of total RNA was used for labeling and hybridization, according to the manufacturer's protocols (Illumina). The microarray data were normalized using the quantile normalization method in the LIMMA (Linear Models for Microarray Data) package in the R language environment (17). The expression level of each gene was transformed into a $\log_2$ base before further analysis. Primary microarray data are available in NCBI's GEO (Gene Expression Omnibus) database (http://www.ncbi.nlm.nih.gov/geo/; microarray platform, GPL6884; microarray data, accession number GSE13861).

Statistical analysis of microarray data

BRB-ArrayTools were primarily used for all statistical analysis (18, 19). Gene expression differences were considered statistically significant if the P value was less than 0.001. Cluster analysis was done with Cluster and Treeview (20). Kaplan–Meier plots and the log-rank test were used to estimate patient prognosis. Multivariate Cox proportional hazards regression analysis was used to evaluate independent prognostic factors associated with survival, and gene signature, tumor stage, and pathologic characteristics were used as covariates. A P value of less than 0.05 was considered to indicate statistical significance, and all tests were 2-tailed.

Selection of genes for qRT-PCR assay and experimental procedures

To select the candidate genes during the course of validation experiments, we used receiver operating characteristic (ROC) curves from censored relapse-free survival (RFS) data using the nearest neighbor estimation method, with a cutoff value of 36 months and under the curve (AUC) were calculated with 95% CIs (21). First ROC models were constructed by using gene expression data from microarray experiments. From 2,755 gene features identified from microarray experiments, top 27 genes with highest AUC values (14 genes) or lowest AUC values (13 genes) were selected for validation with qRT-PCR experiments in the GSH1 cohort. Using qRT-PCR–based gene expression data from GSH1 cohort, we constructed second ROC curves to further select genes with AUCs of more than 0.55 as risk genes and less than 0.45 as protective genes. Of 27 candidate genes, only 6 genes were within the range of selection cutoff.

Total RNA was extracted from the FFPE sections according to the manufacturer's instruction manual (RecoverAll Total Nucleic Acid Isolation; Ambion, Inc.). Real-time RT-PCR amplification was done using the 7900HT Fast Real-Time PCR System with a 384-well block module (Applied Biosystems). Cycling conditions were 45°C for 10 minutes and 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Relative amounts of mRNA were calculated from the threshold cycle (C_t) number using expression of cyclophilin A (PPIA) as an endogenous control. All PCR experiments were duplicated and the values averaged.

Development of 6-gene risk scoring system

To generate a risk score with 6 genes, we adopted a previously established strategy by using the Cox regression coefficient of each gene (22, 23). The risk score of each patient was derived from sum of multiplication of reference-normalized expression level of the gene by its corresponding coefficient: Risk score = (0.097 × CTNNB1 value) + (0.141 × EXOCS3 value) + (0.148 × TOP2A value) + (−0.0898 × LBA1 value) + (−0.0985 × CCL5 value) + (−0.0618 × LZTR1 value). GSH1 patients were dichotomized into both a high-risk group and a low-risk group, using the 50th percentile (median) cutoff of the risk score as the threshold value. Both the coefficient and the threshold value derived from the GSH1 cohort were directly applied to the gene expression data from the exploration data set (YGC cohort) and an independent test sets (GSH2 patient cohorts).

Results

Two major subclasses revealed by hierarchical clustering of gene expression patterns are highly associated with prognosis

We applied hierarchical clustering analysis to gene expression data from 65 human primary tumor tissue samples (YGC cohort in Table 1) and 6 GIST tissue samples. Unsupervised clustering revealed 3 distinctive subtypes with clear differences in overall gene expression patterns (Fig. 1A). Most of the gastric cancer tissues were subdivided into 1 of 2 subgroups (C1 and C2). Intriguingly, a few gastric cancer tumors (C3) were coclustered with the GIST tissues, indicating that a small percentage of gastric cancers may acquire sarcomatoid features during progression. When clinical relevance was examined, RFS (systemic) was found to differ significantly between the 2 major clusters (C1 and C2). Kaplan–Meier plots and log-rank tests indicated that C2 patients had a significantly better RFS than C1 or C3 patients ($P = 0.001$ by the log-rank test; Fig. 1B). When only patients with stage III tumors were considered for analysis, the differences in prognosis between C1 and C2 patients were still significant ($P = 0.005$ by the log-rank test; Fig. 1C), indicating that the molecular features of these tumors reflected in gene expression patterns might be strong independent predictors of clinical outcomes. Because the number of gastric cancer patients in group C3 was too small ($n = 5$), these patients were removed from further analysis.

Prognostic gene expression signature in gastric cancer

Because the C1 subgroup was strongly associated with poor prognosis, we next sought to identify genes whose expression is unique to the C1 subgroup by cross-comparing gene lists from different statistical tests. We first generated 2 different gene lists by applying 2-sample t tests ($P < 0.001$). Gene list A represents the genes that were differentially expressed between C1 and C2. Gene list B represents the genes that were differentially expressed between C2 and normal gastric tissues (Supplementary Fig. S1). When gene expression patterns of all tissues were compared together, 3 different patterns were observed: A not B (2,755 genes), A and B (241 genes), and B not A (1,437 genes). Genes in the A not B category displayed a poor prognostic C1-specific gene expression pattern and are potential markers for predicting RFS (Fig. 2). Because the use of a complex algorithm with a long gene list from microarray data may not be practical in the clinic, we tried to identify a small number of genes whose expression patterns can still reliably predict RFS. Of 2,755 genes in A not B category, we further selected candidate genes on the basis of ROC model analysis. Top 27 genes with highest AUC values (14 genes) or lowest AUC values (13 genes) were selected for validation with qRT-PCR experiments. We next tested, using qRT-PCR, whether expression of these genes or their subsets could predict the RFS in an independent cohort (GSH1).

Table 1. Clinical characteristics of the patients

Characteristics	YGC ($n = 65$)	GSH1 ($n = 96$)
Age, y		
Median	63	60
Range	32–83	26–77
% Sex		
Male	46 (71%)	60 (62%)
Female	19 (29%)	36 (38%)
% Subsite of tumor		
Cardia	5 (8%)	11 (12%)
Body	24 (37%)	47 (49%)
Antrum	31 (48%)	28 (29%)
Diffuse	4 (6%)	10 (10%)
Unknown	1	0
% Histologic type of tumor		
Intestinal	23 (35%)	23 (24%)
Diffuse	32 (49%)	71 (74%)
Mixed	10 (16%)	2 (2%)
% Cancer stage, TNM class		
I	12 (18.5%)	0 (0%)
II	2 (3%)	36 (38%)
III	34 (52%)	59 (61%)
IV	12 (18.5%)	1 (1%)
Relapse and survival		
Relapse	27 (41%)	48 (50%)
Death	20 (30%)	33 (34%)
Adjuvant chemotherapy		
Not received	16 (25%)	10 (10%)
Received	49 (75%)	86 (90%)

Six-gene signature and risk score

We again constructed another ROC models, using gene expression data from qRT-PCR experiments with tissues from GSH1 cohort, to assess the prognostic relevance of gene expression in 96 GSH1 patients. Considering AUC

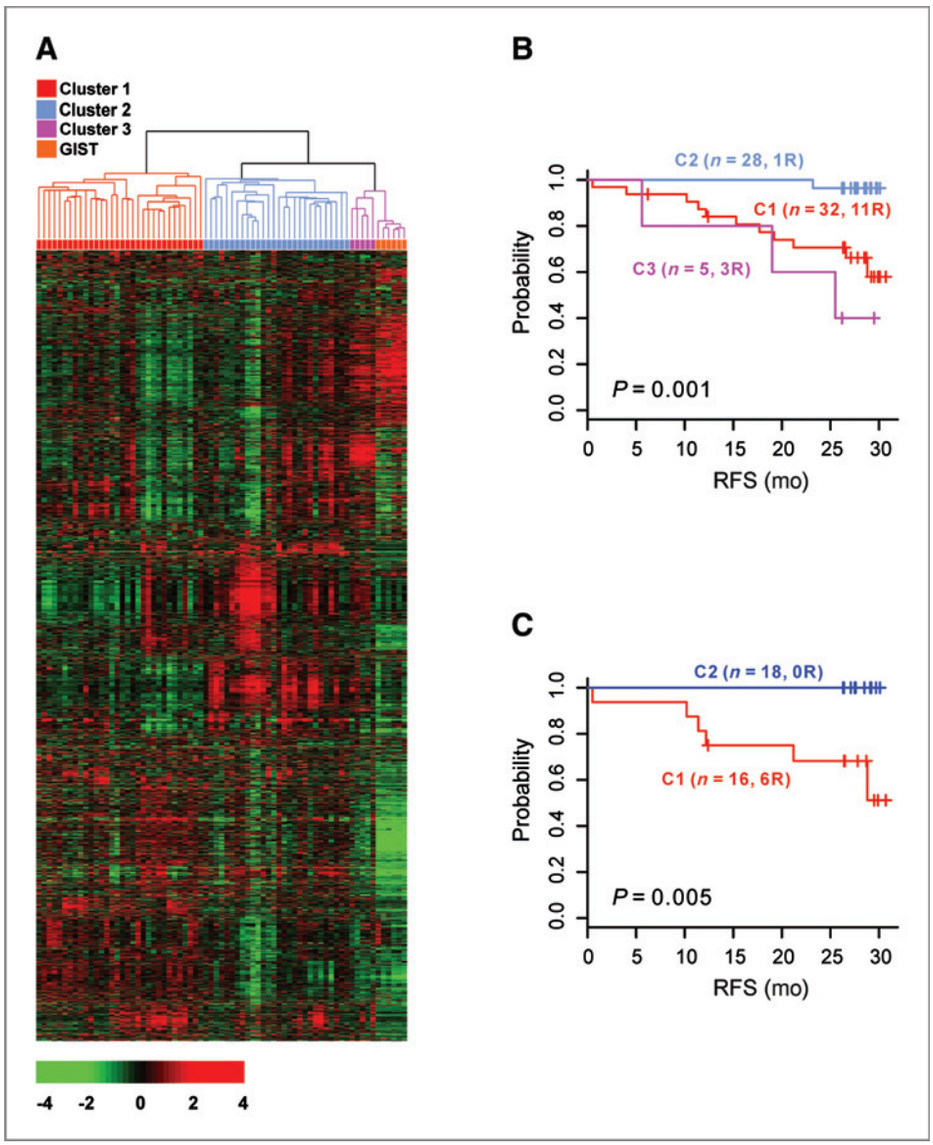

Figure 1. Hierarchical clustering analysis of gene expression data from the YGC cohort. A, hierarchical clustering of gene expression data from 65 gastric cancer and 6 GIST patients in the YGC cohort. Genes with expression levels that were at least 2-fold different in at least 15 tissues, relative to the median value across tissues, were selected for hierarchical clustering analysis (2,077 gene features). The data are given in matrix format, in which rows represent individual genes and columns represent each tissue. Each cell in the matrix represents the expression level of a gene feature in an individual tissue. The color red or green in cells reflects relative high or low expression levels, respectively, as indicated in the scale bar (log_2-transformed scale). B, Kaplan–Meier plots of 3 gastric cancer clusters in the YGC cohort. The 6 patients with GIST were excluded from the plotting. C, Kaplan–Meier plots of stage III patients in 2 clusters (C1 and C2) in the YGC cohort. (No stage III patients were identified in C3.)

over 0.55 and under 0.45 to be significant, we found that the expression of 6 genes (*CTNNB1*, *EXOSC3*, *TOP2A*, *LBA1*, *LZTR1*, and *CCL5*) had a nontrivial correlation with RFS. We next tested whether we could use expression of these 6 genes as a prognostic signature in the GSH1 cohort. When hierarchical clustering analysis was applied to the 6-gene expression data, the 96 patients were divided into 2 subgroups with significantly different RFS ($P = 0.017$ by the log-rank test; Supplementary Fig. S2).

Because the use of hierarchical clustering analysis methods in clinical practice has proven to be difficult (24), we developed risk score methods by using the Cox regression coefficient of each gene (Supplementary Table S1; refs. 22, 23). Patients in the GSH1 cohort were dichotomized according to their risk score, and the RFS rate was significantly lower in the patient group with the high risk score ($P = 0.048$ by the log-rank test; Fig. 3). Gene expression data from the YGC cohort were reanalyzed with the 6-gene–based risk score. With direct application of the Cox regression coefficient from the GSH1 cohort and the 50th percentile cutoff threshold, RFS in 2 patient groups differed significantly ($P = 0.04$ by the log-rank test; Supplementary Fig. S3).

In the GSH1 cohort, the prognostic association between our newly identified 6-gene signature and other known clinical and pathologic risk factors for gastric cancer progression was assessed by univariate and multivariate analyses. As expectedly, in addition to stage and lymph node status, which are already well-known risk factors, the 6-gene signature was a significant risk factor for shorter RFS in univariate analysis (Table 2). Multivariate analysis that included all relevant pathologic variables revealed that

the gene signature remained an independent prognostic risk factor for RFS.

Validation of risk score in independent cohorts

We next sought to validate risk score in another independent cohort, especially in stage III patients (GSH2 cohort, $n = 52$; Supplementary Table S2). Expression data of 6 genes from FFPE tissues were obtained by applying qRT-PCR and used to generate risk score. When patients in the GSH2 cohort were dichotomized according to their risk score, both of RFS and overall survival rate were significantly lower in the patient group with the high risk score ($P = 0.028$ and $P = 0.032$, respectively, by the log-rank test; Fig. 4A and D).

To test whether the 6-gene–based risk score is independent of American Joint Committee on Cancer (AJCC) stage, patients in GSH2 cohort were restratified according to substage and risk score. As expected, prognosis of stage IIIB is significantly worse than that of stage IIIA (Supplementary Fig. S4A and C). When the risk score was applied to stage IIIA and IIIB separately, the risk score successfully identified a population of high-risk patients in both subgroups (Fig. 4B, C, E, and F). In fact, when all stratifications were combined together, the risk score identified gastric cancer patients in IIIA subgroup whose risk of relapse was similar to or worse than that of IIIB (Supplementary Fig. S4B and D). Because nodal stage is best known predictor of relapse, we assessed the utility of the risk score in T3 patients who differed only with N stage (T3N1 and T3N2). Nodal stage is well associated with RFS (Supplementary Fig. S5A). Within these groups, the risk score clearly identified high-risk patients (Supplementary Fig. S5B–D). Taken together, these data indicate that the risk score provides information on the risk of relapse independent of current staging systems and shows that the risk score captures biological differences among gastric cancers that are not encompassed in the current staging criteria.

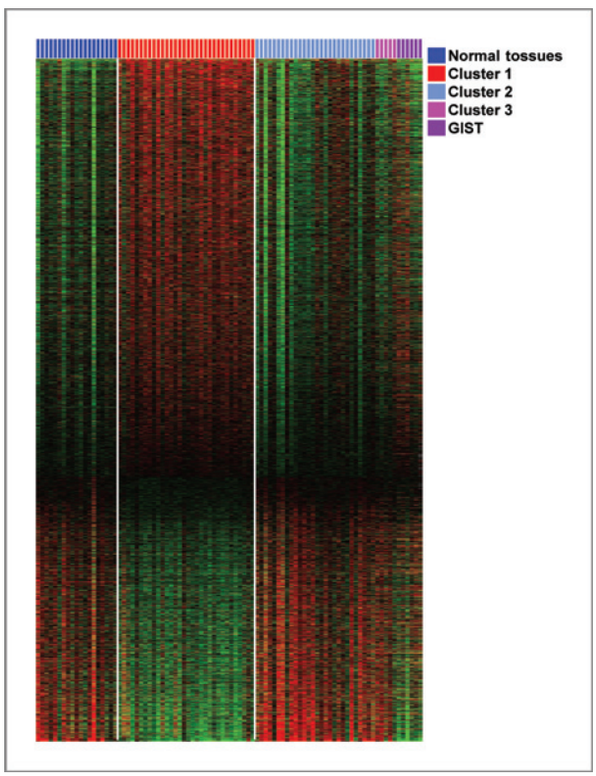

Figure 2. Gene expression signature unique to Cluster C1. Measured gene expression values were $\log_2$-transformed and median-centered across samples before generating heatmap.

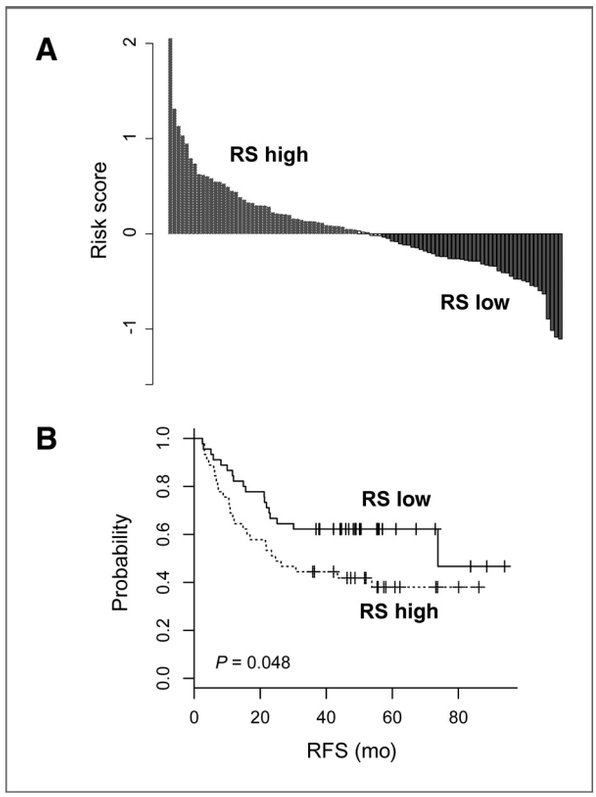

Figure 3. Risk score based on 6-gene signature and RFS of patients in GSH1. A, the relative risk score based on the 6-gene signature of each patient. (Each bar represents the risk score of an individual patient.) The regression coefficients of each gene were calculated by Cox regression analysis (Supplementary Table S1). The risk score was used to dichotomize patients into high- or low-risk groups, with the 50th percentile as the cutoff value. To avoid the ambiguity of a risk score near the median value, patients in the top and bottom 5th percentiles from the risk score median were removed from Kaplan–Meier plotting. Blank bars near the median indicate these patients. B, Kaplan–Meier plots of 2 risk score risk groups in the GSH1 cohort. *P* values were obtained from the log-rank test. RS, risk score.

Discussion

We identified and validated, using a series of independent experiments and complementary data analyses (Supplementary Fig. S6), robust prognostic markers in gastric cancer and developed a prognostic risk score that

Table 2. Univariate and multivariate Cox proportional hazard regression analyses of RFS

	Univariate		Multivariate	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Six-gene-based risk score (high or low)	1.81 (0.998–3.3)	0.0476	2.587 (1.351–4.953)	0.004
T (T2 or T3)	4.42 (2.21–8.81)	<0.001	3.969 (1.906–8.265)	<0.001
N (N1 or N2)	2.94 (1.65–5.22)	<0.001	3.389 (1.686–6.815)	<0.001
Age (>60 or not)	0.652 (0.368–1.16)	0.140	0.701 (0.354–1.390)	0.31
Gender (M or F)	0.56 (0.317–0.991)	0.043	0.567 (0.306–1.059)	0.075
Adjuvant chemotherapy (yes or no)	0.423 (0.189–0.947)	0.031	0.358 (0.142–0.904)	0.03
Lauren classification (intestinal or diffuse)	0.935 (0.475–1.84)	0.844	1.384 (0.612–3.131)	0.44

can be easily translated into the clinic. First, we used microarray technology to uncover potential prognostic subgroups of gastric cancer patients and identify candidate genes for prognostic markers. The association of gene expression patterns with prognosis was significant (Fig. 1), which suggests that our gene expression signature well reflects clinical differences between subgroups of patients with gastric cancer. However, the difficulty of acquiring fresh-frozen tissues from patients and the complexity of data analysis make it hard to use this approach in the clinic. To overcome this limitation, we switched from microarray-based technology to qRT-PCR technology, simpler and easily accessible technology in clinics, to measure gene expression and identified a small number of genes (*CTNNB1*, *EXOSC3*, *TOP2A*, *LBA1*, *LZTR1*, and *CCL5*) whose expression patterns can reliably predict the prognosis of gastric cancer patients. The robustness of the prognostic gene expression signature was validated in an independent cohort by using the reduced gene set. For easy translation of our finding to the clinic, we developed a risk

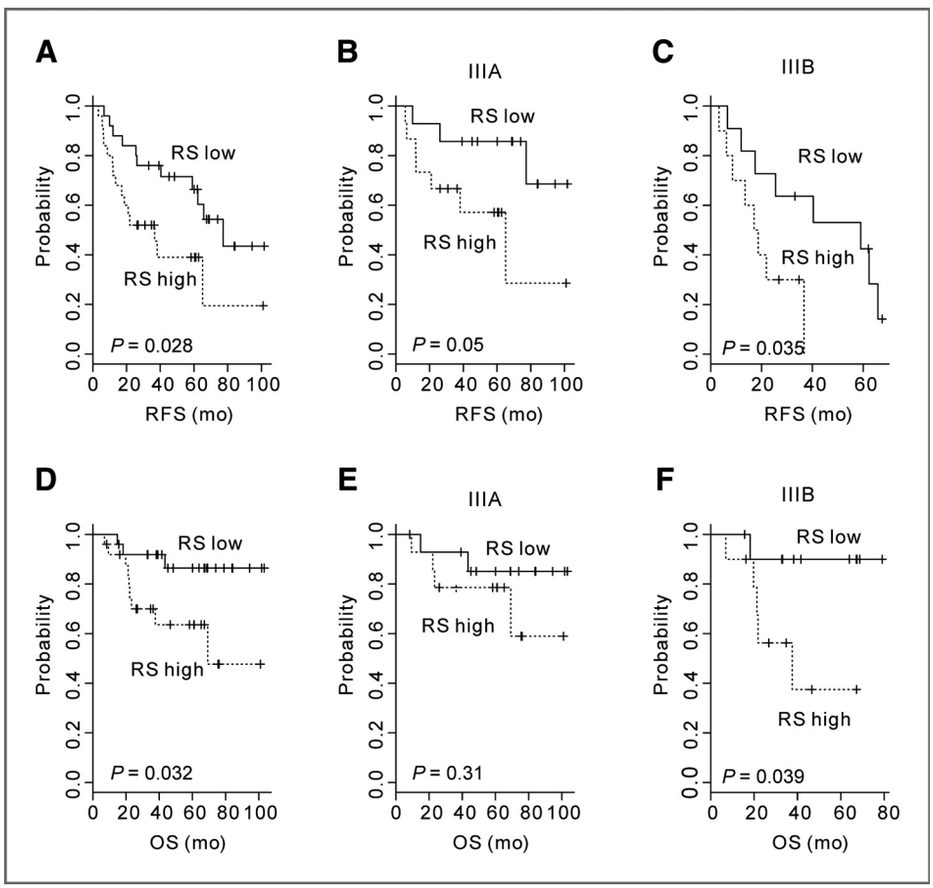

Figure 4. Kaplan–Meier survival plots of overall survival and RFS in AJCC stage III gastric cancer patients in the GSH2 cohort. Patients were stratified by risk score in all stage III (A and D), risk score in IIIA (B and E), in IIIB (C and F). *P* values were obtained from the log-rank test. RS, risk score.

score for relapse after curative resection of tumor (Fig. 3). Finally, the robustness of our risk score was validated in an independent cohort, especially in stage III patients who show most heterogeneous clinical outcome (Fig. 4 and Supplementary Fig. S5).

The unique molecular characteristics of each subgroup of gastric cancer may lead to new therapeutic strategies. *CTNNB1* is a key mediator of the *WNT* signaling pathway that regulates cell-fate decisions and cell proliferation during gut development (25). Activated mutation of *CTNNB1* was reported in gastric cancer, and abnormal expression of *CTNNB1* in gastric cancer was significantly associated with poorer survival (26, 27), supporting the notion that our gene expression data may well recapitulate the molecular abnormality of gastric carcinogenesis. *TOP2A* encodes a DNA topoisomerase II, an enzyme that controls the topologic state of DNA during transcription. This gene is currently the target of several anticancer agents, and a variety of its mutations have been associated with the development of drug resistance (28–30).

The development of the 6-gene–based risk score has strong clinical implications. We could identify gastric cancer patients at higher risk by using simple qRT-PCR technology and paraffin-embedded tissues, which are routinely acquired at diagnosis. One of limitations of our study is its retrospective character. Thus, to validate its true clinical relevance of the risk score, it will be necessary that 6-gene–based risk score is integrated into prospective randomized trials in the form of a biologic stratification criterion. In addition, because this new approach has not been applied to samples from small pretreatment biopsies, the reliability of the new approach should be extensively tested before its use in clinical trials.

Prognostic characteristics of the risk score may not be sufficient to change current clinical practice because they provide only information on probable course of the disease and not the probable response to treatments. However, biomarker study in breast cancer showed that 21-gene prognostic marker could also be used as predictive marker for standard adjuvant chemotherapy (23). Thus, in future study, it will be interesting to test whether the risk score can also be good predictive marker for response to adjuvant chemotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This study was supported by NIH grants (CA127672, CA129906, CA138671, and CA150229); Multidisciplinary Research Program grants from The University of Texas M. D. Anderson Cancer Center; Institute of Personalized Cancer Treatment grant from The University of Texas M. D. Anderson Cancer Center faculty startup fund from The University of Texas M. D. Anderson Cancer Center; Dallas, Cantu, Smith, Park, and Myer Families; the Kevin Fund; the River Creek Foundation; and 2009 Academic faculty research fund of Yonsei University College of Medicine.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 13, 2010; revised October 2, 2010; accepted October 27, 2010; published online March 29, 2011.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
2. Hundahl SA, Phillips JL, Menck HR. The National Cancer Data Base report on poor survival of U.S. gastric carcinoma patients treated with gastrectomy: Fifth Edition American Joint Committee on Cancer Staging, Proximal Disease, and the "Different Disease" Hypothesis. Cancer 2000;88:921–32.
3. Kovoor PA, Hwang J. Treatment of resectable gastric cancer: current standards of care. Expert Rev Anticancer Ther 2009;9:135–42.
4. Moriguchi S, Maehara Y, Korenaga D, Sugimachi K, Nose Y. Risk factors which predict pattern of recurrence after curative surgery for patients with advanced gastric cancer. Surg Oncol 1992;1:341–6.
5. Landry J, Tepper JE, Wood WC, Moulton EO, Koerner F, Sullinger J. Patterns of failure following curative resection of gastric carcinoma. Int J Radiat Oncol Biol Phys 1990;19:1357–62.
6. Roukos DH, Kappas AM. Perspectives in the treatment of gastric cancer. Nat Clin Pract Oncol 2005;2:98–107.
7. Maruyama K, Gunven P, Okabayashi K, Sasako M, Kinoshita T. Lymph node metastases of gastric cancer. General pattern in 1931 patients. Ann Surg 1989;210:596–602.
8. Hermanek P. Prognostic factors in stomach cancer surgery. Eur J Surg Oncol 1986;12:241–6.
9. 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530–6.
10. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503–11.
11. Lee JS, Chu IS, Mikaelyan A, Calvisi DF, Heo J, Reddy JK, et al. Application of comparative functional genomics to identify best-fit mouse models to study human cancer 66. Nat Genet 2004;36:1306–11.
12. Lee JS, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells 197. Nat Med 2006;12:410–6.
13. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002;347:1999–2009.
14. Tay ST, Leong SH, Yu K, Aggarwal A, Tan SY, Lee CH, et al. A combined comparative genomic hybridization and expression microarray analysis of gastric cancer reveals novel molecular subtypes. Cancer Res 2003;63:3309–16.
15. Weiss MM, Kuipers EJ, Postma C, Snijders AM, Siccama I, Pinkel D, et al. Genomic profiling of gastric cancer predicts lymph node status and survival. Oncogene 2003;22:1872–9.
16. Myllykangas S, Junnila S, Kokkola A, Autio R, Scheinin I, Kiviluoto T, et al. Integrated gene copy number and expression microarray analysis of gastric cancer highlights potential target genes. Int J Cancer 2008;123:817–25.
17. Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 2003;19:185–93.
18. Simon R, Lam A, Li M-C, Ngan M, Menenzes S, Zhao Y. Analysis of gene expression data using BRB-array tools. Cancer Informatics 2007;3:11–7.

19. Simon R, Radmacher MD, Dobbin K, McShane LM. Pitfalls in the use of DNA microarray data for diagnostic and prognostic classification. J Natl Cancer Inst 2003;95:14–8.
20. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 1998;95:14863–8.
21. Heagerty PJ, Zheng Y. Survival model predictive accuracy and ROC curves. Biometrics 2005;61:92–105.
22. Chen HY, Yu SL, Chen CH, Chang GC, Chen CY, Yuan A, et al. A five-gene signature and clinical outcome in non-small-cell lung cancer. N Engl J Med 2007;356:11–20.
23. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;351:2817–26.
24. Abdullah-Sayani A, Bueno-de-Mesquita JM, van de Vijver MJ. Technology Insight: tuning into the genetic orchestra using microarrays—limitations of DNA microarrays in clinical practice. Nat Clin Pract Oncol 2006;3:501–16.
25. Lickert H, Kispert A, Kutsch S, Kemler R. Expression patterns of Wnt genes in mouse gut development. Mech Dev 2001;105:181–4.
26. Clements WM, Wang J, Sarnaik A, Kim OJ, MacDonald J, Fenoglio-Preiser C, et al. beta-Catenin mutation is a frequent cause of Wnt pathway activation in gastric cancer. Cancer Res 2002;62:3503–6.
27. Jawhari A, Jordan S, Poole S, Browne P, Pignatelli M, Farthing MJ. Abnormal immunoreactivity of the E-cadherin-catenin complex in gastric carcinoma: relationship with patient survival. Gastroenterology 1997;112:46–54.
28. Maqani N, Belkhiri A, Moskaluk C, Knuutila S, Dar AA, El-Rifai W. Molecular dissection of 17q12 amplicon in upper gastrointestinal adenocarcinomas. Mol Cancer Res 2006;4:449–55.
29. Kanta SY, Yamane T, Dobashi Y, Mitsui F, Kono K, Ooi A. Topoisomerase II alpha gene amplification in gastric carcinomas: correlation with the HER2 gene. An immunohistochemical, immunoblotting, and multicolor fluorescence *in situ* hybridization study. Hum Pathol 2006;37:1333–43.
30. Tanaka T, Tanimoto K, Otani K, Satoh K, Ohtaki M, Yoshida K, et al. Concise prediction models of anticancer efficacy of 8 drugs using expression data from 12 selected genes. Int J Cancer 2004;111:617–26.