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Predictive Biomarkers and Personalized Medicine

Clinical Cancer Research

Prognostic and Predictive Role of JWA and XRCC1 Expressions in Gastric Cancer

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Abstract

Purpose: To investigate the expression pattern and significance of DNA repair genes *JWA* and X-ray repair cross complement group 1 (*XRCC1*) in gastric cancer.

Experimental Design: Expressions of JWA and XRCC1 were assessed by immunohistochemistry in a training cohort and they went into a second testing cohort and finally to a validating cohort. Prognostic and predictive role of JWA and XRCC1 expression status in cases treated with surgery alone or combined with adjuvant chemotherapy was evaluated, respectively.

Results: JWA and XRCC1 protein levels were significantly downregulated in gastric cancer lesions compared with adjacent noncancerous tissues. Low tumoral JWA or XRCC1 expression significantly correlated with shorter overall survival (OS), as well as with clinicopathologic characteristics in patients without adjuvant treatment. Multivariate regression analysis showed that low JWA and XRCC1 expressions, separately and together, were independent negative markers of OS. Adjuvant fluorouracil-leucovorin-oxaliplatin (FLO) significantly improved OS compared with surgery alone (log-rank test, $P = 0.01$). However, this effect was evident only in the JWA or XRCC1 low expression group (HR = 0.44; 95% CI: 0.26–0.73; $P = 0.002$, and HR = 0.44, 95% CI: 0.26–0.75; $P = 0.002$, respectively); Adjuvant fluorouracil-leucovorin-platinol (FLP) did not improve OS, except in the patients with low JWA and XRCC1 expressions ($P = 0.010$ for JWA and 0.024 for XRCC1, respectively).

Conclusions: JWA and XRCC1 protein expressions in tumor are novel candidate prognostic markers and predictive factors for benefit from adjuvant platinum-based chemotherapy (FLO or FLP) in resectable human gastric carcinoma. *Clin Cancer Res; 18(10); 2987–96. ©2012 AACR.*

Introduction

Gastric cancer is the fourth most common cancer and the second leading cause of cancer-related death worldwide (1). Despite the improved prognosis of patients with gastric cancer resulting from earlier diagnosis, radical surgery, and the development of adjuvant therapy, the 5-year survival rate across all stages is only about 20%. Chemotherapy both in resectable and advanced disease has only limited efficacy (2, 3). New molecular markers pivotal to tumor biology, to improve prognosis and prediction of the adjuvant treatment regimens are urgently needed.

DNA repair systems have been increasingly implicated both in carcinogenesis and treatment resistance (4–6). Reactive oxygen species–induced oxidative base lesions are important to carcinogenesis (7). Base excision repair (BER) pathway is the primary mechanism for repair of these lesions (8), and abnormal expression of the molecular targets in the BER pathway has also been associated with carcinogenesis (9). X-ray repair cross complement group 1 (XRCC1) protein, acts as a scaffold in the process of BER. It recognizes DNA breaks, binds the DNA and recruits other components of the repair machinery (10–12). Molecular epidemiologic studies indicate that single-nucleotide polymorphisms of XRCC1 were associated with the risk of various cancers including gastric cancer as well as being predictive for chemotherapy outcome (13–15). Few studies,

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Translational Relevance

This is the first report that has examined expression of the JWA and X-ray repair cross complement group 1 (XRCC1) and their prognostic and predictive significance in human gastric carcinoma. The expressions of JWA and XRCC1 were reduced in gastric cancer tissues and significantly correlated with shorter overall survival, as well as with advanced clinicopathologic features in patients. Moreover, resectable gastric cancer patients with low JWA and XRCC1 expressions could have a survival benefit from adjuvant platinum-based chemotherapy (FLO or FLP). Our findings indicate that JWA and XRCC1 may be candidate prognostic and predictive biomarkers and potentially interesting for the personalized chemotherapy of gastric cancer patients.

however, have investigated XRCC1 expression in human tumors. Low XRCC1 expression was reported in pancreatic cancer versus adjacent tissue as well as in predicting the outcome after bladder cancer radiotherapy (16, 17).

We recently showed that JWA, also called ADP ribosylation–like factor 6 interacting protein 5 (ARL6ip5), may serve as a novel regulator of XRCC1 in the BER protein complex to facilitate repair of DNA damage (18). In addition, we showed that JWA is a novel microtubule-associated protein, which regulates cancer cell migration via mitogen-activated protein kinase cascades (19) and inhibits cell adhesion, invasion, and the metastasis of melanoma cells by suppressing integrin-αVβ3 signaling (20). Our group also showed that polymorphisms in the *JWA* gene are associated with increased susceptibility to gastric cancer in a Chinese population (21).

In this context, we aimed to address this paucity of translational information and identify the expression patterns of JWA and XRCC1 in 3 large independent cohorts of gastric cancer patients and to examine the possible prognostic and predictive role of these markers.

Materials and Methods

Patients and specimens

Three independent retrospective patient cohorts were studied. The training cohort and testing cohort were recruited in Nantong Cancer Hospital, Nantong City, in the North of Jiangsu Province, China. The validation cohort was recruited in Yixing People's Hospital, Yixing City, in the South of Jiangsu Province, China. The tissues were obtained from the respective pathology departments. Inclusion criteria were gastric carcinoma treated with radical gastrectomy with or without adjuvant chemotherapy. Exclusion criteria were previous gastric cancer or active nongastric cancer, preoperative chemotherapy or radiotherapy.

The training cohort included 103 patients who underwent radical gastrectomy at Nantong Cancer Hospital from May 1, 1990 to June 1, 1995. A tissue microarray (TMA) was constructed, including the gastric cancer samples and matched noncancerous gastric mucosa more than 5 cm from the tumoral margins. Two more independent tumor TMAs were constructed to validate training cohort data, all patients operated before 2006 to evaluate at least 5-year survival. The testing cohort consisted of all 640 surgical cases from the Nantong Cancer Hospital from December 1, 2000 to April 1, 2005 and the validation cohort included all 1,022 surgical cases in Yixing People's Hospital from January 1, 1999 to December 31, 2006. These patients were treated with surgery only or with postoperative adjuvant chemotherapy (for details, see the Supplementary Methods and Supplementary Fig. S1). As shown in Supplementary Table S1, the distributions of demographic characteristics and the selected clinicopathologic variables of patients between the 2 districts (Nantong and Yixing) were significantly different (all of $P < 0.05$). However, the distributions of these variables of patients between the training cohort and testing cohort in Nantong district were mostly matched, except depth of invasion and distant metastasis ($P = 0.005$ and 0.003, respectively; data not shown). For the resectable gastric cancer patients with chemotherapy, the distributions of demographic characteristics and the selected clinicopathologic variables of patients between fluorouracil-leucovorin-oxaliplatin (FLO) and fluorouracil-leucovorin-platinol (FLP) groups were similar (all of $P > 0.05$), except histologic type ($P = 0.003$; Supplementary Table S2). In addition, 11 pathologically confirmed gastric cancer and respective noncancerous fresh-frozen gastric mucosa tissues from recent patients from Nantong Cancer Hospital were obtained for Western blot analysis after signed informed consent. Institutional approval was obtained from the Review Board of the respective institutions before this study.

Overall survival (OS) was the primary endpoint of this analysis. Survival time was calculated from the date of surgery to the date of death or to the last follow-up. Date of death was obtained from patient records or patients' families through follow-up telephone calls. Date of death for each case was double verified by local civil affairs department and public security department. Detailed clinicopathologic information was obtained. The Lauren criteria were used to classify the tumors into intestinal or diffuse types (22) and staged according to the tumor-node-metastasis (TNM) guidelines (23).

TMA construction and immunohistochemistry

The gastric cancer TMAs were created by contract service at the National Engineering Center for Biochip, Shanghai, China. Duplicate 1.0-mm diameter cores of tissue from each sample were punched from paraffin tumor block and corresponding nontumoral tissues in the training cohort or from cores of primary tumor biopsies in the validation cohorts. As a tissue control, the biopsies of normal gastric epithelium tissues were inserted in the 4 angles and the center of each slide.

A standard protocol was used for the immunostaining of the TMAs (for details, see Supplementary Methods). The polyclonal rabbit anti-JWA antibody (1:200 dilution;

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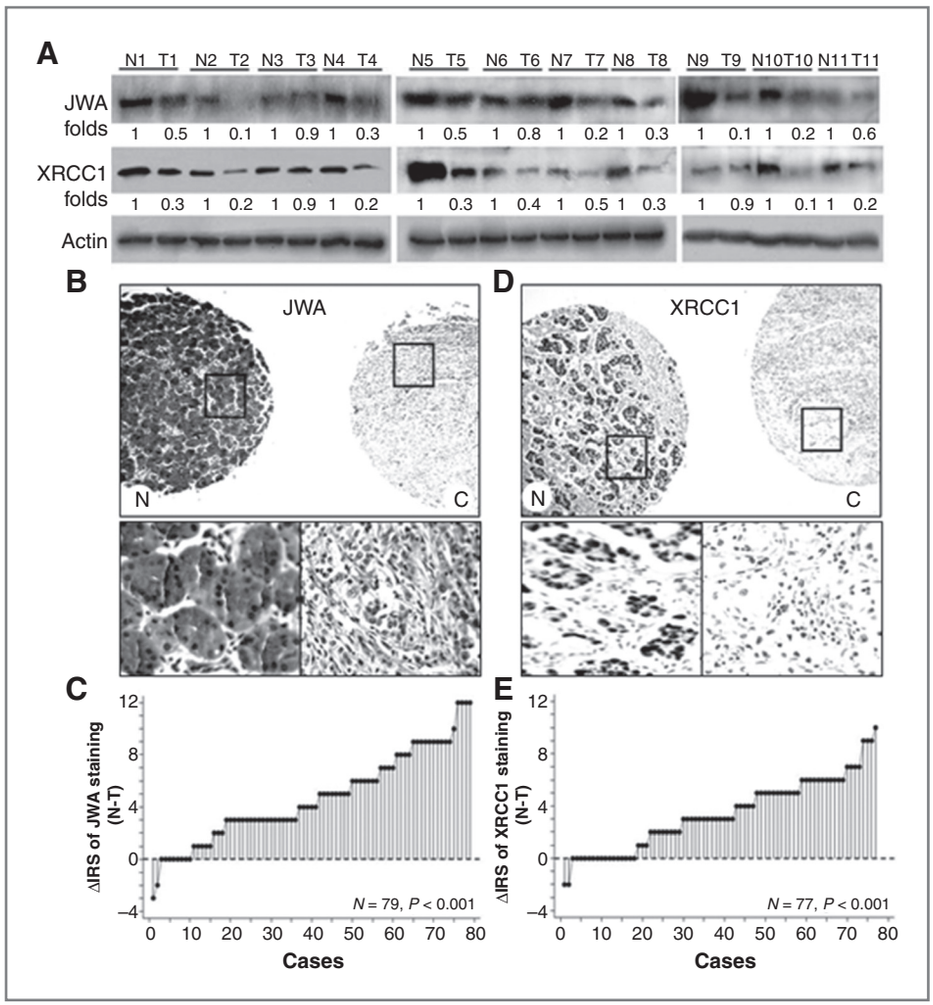

Figure 1. Correlation of JWA and XRCC1 expressions in primary tumors and corresponding nontumors in gastric cancer patients. A, JWA and XRCC1 protein levels in 11 cancer tissues and paired noncancerous normal tissues of gastric cancer patients were analyzed by Western blotting. The level of each protein was normalized against β-actin, and the protein levels in cancer tissues indicated as a ratio to paired noncancerous normal tissues. N, noncancerous normal tissue; T, tumor tissue. B and D, immunohistochemical staining for JWA and XRCC1 in TMA, respectively. C, gastric cancerous tissues; N, paired noncancerous gastric tissues. Top, original magnification: ×40; bottom, magnification: ×200. C and E, the distribution of the difference of JWA and XRCC1 staining (Δ IRS = IRS_N-IRS_T), respectively. *P* values were calculated with the Wilcoxon test.

Research Genetics Inc.) and monoclonal mouse anti-XRCC1 antibody (1:300 dilution; Abcam), were used as described previously (18). The omission of the primary antibody served as negative control. The staining scores of the tissue controls in each microarray slide were preevaluated as a quality control of the immunostaining.

Assessment of immunohistochemistry

At first, staining of JWA and XRCC1 in the tissue was scored independently by 2 pathologists blinded to the clinical data, by applying a semiquantitative immunoreactivity score (IRS) in the training cohort, as reported elsewhere (24). Category A documented the intensity of immunostaining as 0–3 (0, negative; 1, weak; 2, moderate; 3, strong; Supplementary Fig. S2). Category B documented the percentage of immunoreactive cells as 1 (0%–25%), 2 (26%–50%), 3 (51%–75%), and 4 (76%–100%). Multiplication of category A and B resulted in an IRS ranging from 0 to 12 for each tumor or nontumor. The concordance for IRS staining score of JWA and XRCC1 between the 2 pathologists was 73 (91%) and 71 (89%) in 80 tumors of the training set, respectively; the few discrepancies were resolved by consensus using a multihead microscope. The variability in JWA and XRCC1 staining was 3 (4%) and 2 (3%) in the duplicate cores of 80 tumors, respectively. These cases were stained by whole-slide immunohistochemistry and further scored.

The optimum cutoff value of IRS is obtained by receiver-operator characteristic (ROC) analysis, the area under the curve (AUC) at different cutoff values of JWA or XRCC1 IRS for 1, 3, and 5 years of OS time was calculated. The optimal value of cutoff points of the JWA or XRCC1 IRS in Nantong district cohort (combined training cohort and testing cohort) was 4 or 3 due to the predictive value of this cutoff point for death was the best (Supplementary Fig. S3). Under these conditions, samples with IRS 0–4 and IRS 5–12 or IRS 0–3 and IRS 4–12 were classified as low and high expression of JWA or XRCC1 in tumors, respectively. After establishing the immunohistochemical assessment criteria in the Nantong district cohort, the expressions of JWA and XRCC1 in the Yixing district cohort (validation cohort) were scored by the same pathologists with the same procedure.

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Table 1. Relationship between expression levels of JWA and clinicopathologic features of the individuals in 3 cohorts of gastric cancers treated with surgery alone

Variables	Training cohort ($n = 80$ cases)			Testing cohort ($n = 374$ cases)			Validation cohort ($n = 385$ cases)		
	Low (%)	High (%)	P^a	Low (%)	High (%)	P^a	Low (%)	High (%)	P^a
All patients	50 (62.5)	30 (37.5)		166 (44.4)	208 (55.6)		195 (50.6)	190 (49.4)	
Age, y			0.051			0.331			0.182
≤65	43 (86.0)	20 (66.7)		111 (66.9)	129 (62.0)		92 (47.2)	76 (40.0)	
>65	7 (14.0)	10 (33.3)		55 (33.1)	79 (38.0)		103 (52.8)	114 (60.0)	
Gender			0.595			0.442			0.225
Males	36 (72.0)	24 (80.0)		112 (67.5)	148 (71.2)		145 (74.4)	152 (80.0)	
Females	14 (28.0)	6 (20.0)		54 (32.5)	60 (28.8)		50 (25.6)	38 (20.0)	
			0.063			0.004			0.040
Depth of invasion									
T1/T2	1 (2.0)	4 (13.3)		21 (12.7)	51 (24.5)		55 (28.2)	73 (38.4)	
T3/T4	49 (98.0)	26 (86.7)		145 (87.3)	157 (75.5)		140 (71.8)	117 (61.6)	
			<0.001			<0.001			0.003
Lymph node metastasis									
N0	0 (0.0)	20 (66.7)		21 (12.7)	83 (39.9)		60 (30.8)	87 (45.8)	
N1/N2/N3	50 (100.0)	10 (33.3)		145 (87.3)	125 (60.1)		135 (69.2)	103 (54.2)	
			0.001			0.820			0.259
Distant metastasis									
M0	35 (70.0)	30 (100.0)		158 (95.2)	199 (95.7)		186 (95.4)	186 (97.9)	
M1	15 (30.0)	0 (0.0)		8 (4.8)	9 (4.3)		9 (4.6)	4 (2.1)	
TNM stage			<0.001			<0.001			0.001
I	0 (0.0)	9 (30.0)		5 (3.0)	35 (16.8)		38 (19.5)	56 (29.5)	
II	3 (6.0)	15 (50.0)		25 (15.0)	56 (26.9)		38 (19.5)	51 (26.8)	
III	29 (58.0)	4 (13.3)		104 (62.7)	94 (45.2)		110 (56.4)	82 (43.2)	
IV	18 (36.0)	2 (6.7)		32 (19.3)	23 (11.1)		9 (4.6)	1 (0.5)	
			0.495			<0.001			0.124
Tumor diameter									
≤5 cm	24 (48.0)	17 (56.7)		47 (28.3)	104 (50.0)		101 (51.8)	114 (60.0)	
>5 cm	26 (52.0)	13 (43.3)		119 (71.7)	104 (50.0)		94 (48.2)	76 (40.0)	
			0.021			<0.001			<0.001
Histologic type[b]									
Intestinal	21 (42.0)	21 (70.0)		71 (42.8)	141 (67.8)		58 (29.7)	100 (52.6)	
Diffuse	29 (58.0)	9 (30.0)		95 (57.2)	67 (32.2)		135 (69.2)	89 (46.8)	
			<0.001			<0.001			<0.001
XRCC1 expression									
Low	44 (88.0)	7 (23.3)		143 (86.1)	34 (16.3)		113 (57.9)	70 (36.8)	
High	6 (12.0)	23 (76.7)		23 (13.9)	174 (83.7)		82 (42.1)	120 (63.2)	

[a]Two-sided Fisher exact tests.

[b]Excluded 3 patients with mixed intestinal and diffuse types in validation cohort.

Western blotting

Total 11 pairs of recently collected fresh tissues were ground in liquid nitrogen and washed 3 times with phosphate-buffered saline. Tissue extracts were made with a detergent lysis buffer [50 mmol/L Tris (pH 7.4); 150 mmol/L NaCl; 1% NP-40; 0.5% sodium deoxycholate; 0.1% SDS; and the protease inhibitor, 1 mmol/L phenylmethanesulfonyl fluoride). Protein (60 μg) was run on a 12.5% or 7.5% PAGE and transferred to a nitrocellulose membrane (Hybond-ECL; Amersham Pharmacia Biotech). The membrane was blocked with Tris-buffered saline containing 0.1% Tween 20 and 5% nonfat milk (w/v; TBSTM) for 2 hours at room temperature and then incubated overnight at 4°C with primary antibody diluted in TBSTM, which included the polyclonal rabbit anti-JWA antibody (1:1,000 dilution, Research Genetics Inc.), monoclonal mouse anti-XRCC1 antibody (1:1,000 dilution; Abcam), and monoclonal mouse anti–β-actin antibody (1:2,000 dilution; Boster Biotechnology). Immunoreactive bands were detected with a Phototope-horseradish peroxide

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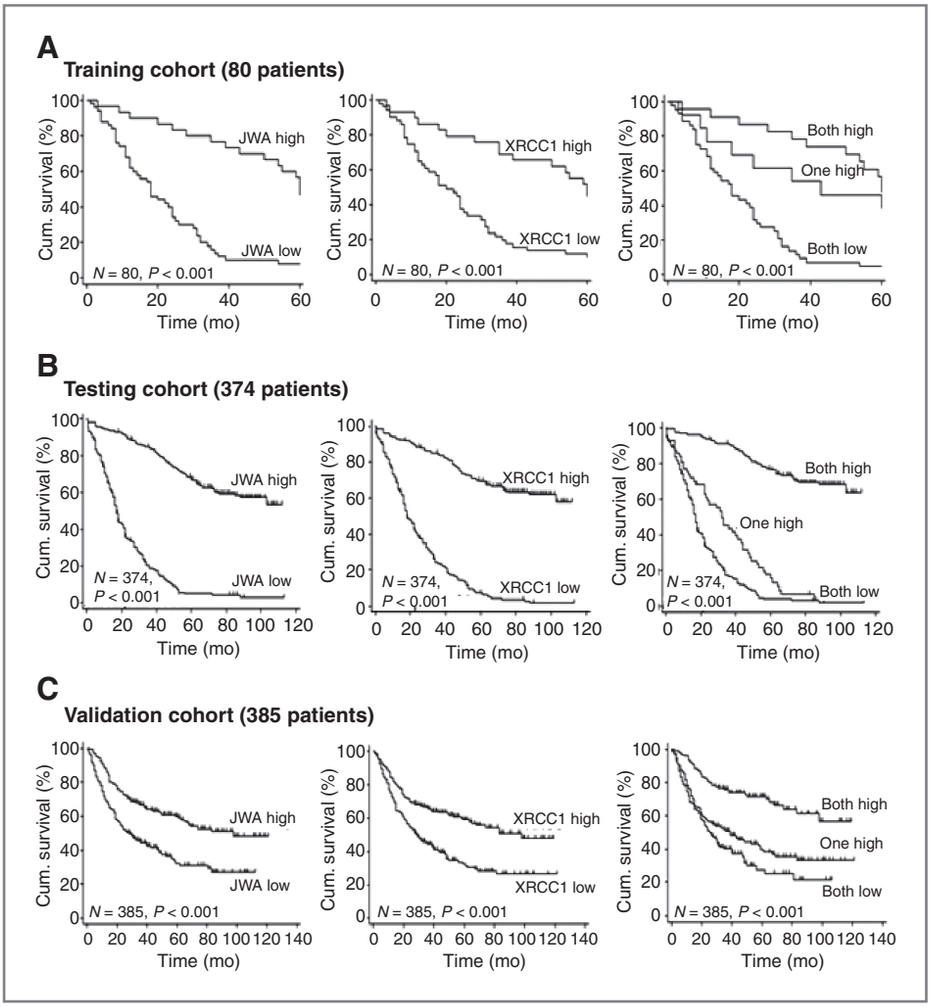

Figure 2. Kaplan–Meier curves depicting OS according to expression patterns of JWA, XRCC1, and combined with JWA/XRCC1 expression in training cohort (A), testing cohort (B), and validation cohort (C). *P* values were calculated with the log-rank test.

Western blot detection kit (Cell Signaling Technology Inc.). The intensity of the JWA and XRCC1 protein bands were analyzed by densitometry, after normalization to the corresponding β-actin level.

Statistical analysis

The associations between JWA or XRCC1 expression and clinicopathologic parameters were evaluated by Fisher exact test. The significance of correlations of JWA or XRCC1 staining in primary tumors and their corresponding nontumors were assessed by the Wilcoxon test (grouped) and by Spearman rank-order correlation (raw scores). The correlation of the expressions of JWA and XRCC1 was established by Spearman rank-order correlation (raw scores) and Fisher exact test (grouped). Probability of differences in OS as a function of time was ascertained by use of the Kaplan–Meier method, with a log-rank test probe for significance. Univariate or multivariate Cox regression analysis was conducted to estimate the crude HRs, adjusted HRs and their 95% CIs, with adjustment for potential confounders. Based on the sample size in the testing (374 cases) and validation cohorts (385 cases), 0.51 difference HR (upper limit of CI in the training cohort) between JWA low and high expression levels with both power of 1.0, and 0.69 difference HR between XRCC1 low and high expression levels with both power of 0.95 could be determined. We analyzed the predictive value of the parameters using time-dependent ROC curve analysis for censored data and calculated AUC of the ROC curves (25). We evaluated the performances of different scores by plotting (t, AUC [t]) for different values of follow-up time (t). All the statistical analyses were conducted by Statistical Analysis System software (version 9.1.3; SAS Institute), STATA statistical software (version 10.1; StataCorp), and R software (version 2.10.1; The R Foundation for Statistical Computing). $P < 0.05$ was deemed statistically significant.

Results

Reduced JWA and XRCC1 expressions in gastric cancer versus noncancer tissues

Eleven pairs of human gastric cancer samples, including primary gastric cancer tissues and matched normal gastric mucosa were selected to test JWA and XRCC1 protein expressions by Western blot. Decreased expressions of JWA

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Table 2. Multivariate Cox regression analysis of JWA, XRCC1, or JWA/XRCC1 expression and clinicopathologic variables predicting survival in 3 cohorts of gastric cancers treated with surgery alone

	Training cohort ($n = 80$ cases)		Testing cohort ($n = 374$ cases)		Validation cohort ($n = 385$ cases)	
Variables	**HR (95% CI)**	***P*[a]**	**HR (95% CI)**	***P*[a]**	**HR (95% CI)**	***P*[a]**
JWA						
Age (≤65 vs. >65)	1.53 (0.76–3.08)	0.238	1.16 (0.90–1.51)	0.250	0.81 (0.61–1.06)	0.125
Gender (male vs. female)	1.69 (0.95–3.02)	0.076	0.95 (0.73–1.25)	0.733	1.04 (0.75–1.45)	0.801
Histologic type (diffuse vs. intestinal)	0.92 (0.54–1.56)	0.757	0.94 (0.72–1.21)	0.614	1.35 (0.99–1.84)	0.058
Tumor diameter (≤5cm vs. >5cm)	1.29 (0.77–2.17)	0.330	1.58 (1.18–2.14)	0.003	1.66 (1.25–2.21)	0.001
TNM stage (I–II vs. III/IV)	1.09 (0.42–2.86)	0.859	1.41 (1.01–1.97)	0.044	4.07 (2.86–5.80)	<0.001
JWA expression (low vs. high)	0.20 (0.08–0.51)	0.001	0.16 (0.11–0.21)	<0.001	0.67 (0.50–0.89)	0.006
XRCC1						
Age (≤65 vs. >65)	1.69 (0.85–3.35)	0.135	1.14 (0.88–1.48)	0.332	0.80 (0.61–1.05)	0.111
Gender (male vs. female)	1.52 (0.86–2.69)	0.153	1.02 (0.78–1.34)	0.870	1.05 (0.75–1.46)	0.782
Histologic type (diffuse vs. intestinal)	0.94 (0.54–1.63)	0.824	0.90 (0.69–1.17)	0.425	1.35 (1.00–1.85)	0.057
Tumor diameter (≤5cm vs. >5cm)	1.01 (0.59–1.70)	0.983	1.49 (1.11–2.01)	0.008	1.62 (1.22–2.16)	0.001
TNM stage (I–II vs. III/IV)	2.80 (1.37–5.71)	0.005	1.40 (1.00–1.94)	0.049	4.15 (2.92–5.90)	<0.001
XRCC1 expression (low vs. high)	0.36 (0.19–0.69)	0.002	0.16 (0.12–0.22)	<0.001	0.72 (0.54–0.96)	0.025
JWA/XRCC1						
Age (≤65 vs. >65)	1.53 (0.76–3.08)	0.237	1.18 (0.91–1.54)	0.215	0.81 (0.61–1.07)	0.138
Gender (male vs. female)	1.57 (0.88–2.79)	0.128	0.96 (0.73–1.26)	0.766	1.07 (0.77–1.49)	0.692
Histologic type (diffuse vs. intestinal)	0.88 (0.51–1.52)	0.648	0.83 (0.64–1.07)	0.156	1.31 (0.96–1.79)	0.088
Tumor diameter (≤5cm vs. >5cm)	1.04 (0.62–1.76)	0.871	1.45 (1.08–1.96)	0.015	1.61 (1.20–2.14)	0.001
TNM stage (I–II vs. III/IV)	1.49 (0.63–3.52)	0.364	1.27 (0.90–1.78)	0.170	3.97 (2.79–5.64)	<0.001
JWA/XRCC1 expression (both low vs. one high)	0.34 (0.13–0.86)	0.023	0.55 (0.40–0.76)	<0.001	0.90 (0.66–1.23)	0.495
(both low vs. both high)	0.21 (0.08–0.50)	<0.001	0.09 (0.06–0.13)	<0.001	0.51 (0.34–0.75)	0.001

[a]Multivariate Cox regression analysis including age, gender, TNM stage, tumor diameter, histologic type, JWA or XRCC1 or combined 2 proteins expression status.

and XRCC1 occurred in all of gastric tumors compared with the paired normal gastric mucosa (Fig. 1A). Immunohistochemical staining of the gastric TMA was used to further investigate JWA and XRCC1 expressions in 80 gastric cancer patients in the training cohort. Because some samples were lost during antigen retrieval or with no relevant cells present in the core, JWA and XRCC1 expressions were examined in 79 and 77 gastric cancer patients having both of gastric cancerous tissues and matched normal gastric mucosa. It was shown that JWA staining was mainly localized in the cytoplasm, whereas XRCC1 was exclusively expressed in the nuclei (Fig. 1B). The distribution of the differences of IRS for JWA and XRCC1 expressions in nontumors and matched tumors was shown in Fig. 1C. Moreover, JWA and XRCC1 expressions were significantly decreased in 69 of 79 (87.3%) and 59 of 77 (76.6%) of gastric cancers compared with the matched normal gastric tissues (both $P < 0.001$, Wilcoxon test; Fig. 1C).

In all 3 independent cohorts of patients treated only with surgery, the expression of JWA correlated significantly with XRCC1 expression in the cancerous tissues ($P < 0.001$ for all correlations; Table 1).

Association of JWA and XRCC1 expressions with clinicopathologic characteristics in patients treated only with surgery

Protein expressions of JWA and XRCC1 in the cancerous tissues of all 3 cohorts were significantly associated with clinicopathologic features, such as lymph node metastasis (N-category), and TNM stage (Table 1 and Supplementary Table S3). Significantly more low JWA expression was seen in diffuse type in all 3 cohorts, but low XRCC1 was significant in the 2 larger cohorts. However, there was no correlation between JWA or XRCC1 protein expression in noncancerous tissues and clinicopathologic features in the training cohort (Supplementary Table S4).

Correlation of JWA, XRCC1 expression, and OS in patients treated only with surgery

In the training cohort, 80 primary tumor samples eligible for analysis showed a statistically significant positive correlation between JWA or XRCC1 expression and overall 5-year survival using Kaplan–Meier survival curves ($P < 0.001$ for both). These findings were confirmed in 2 independent and larger ($n = 374$ and 385, respectively)

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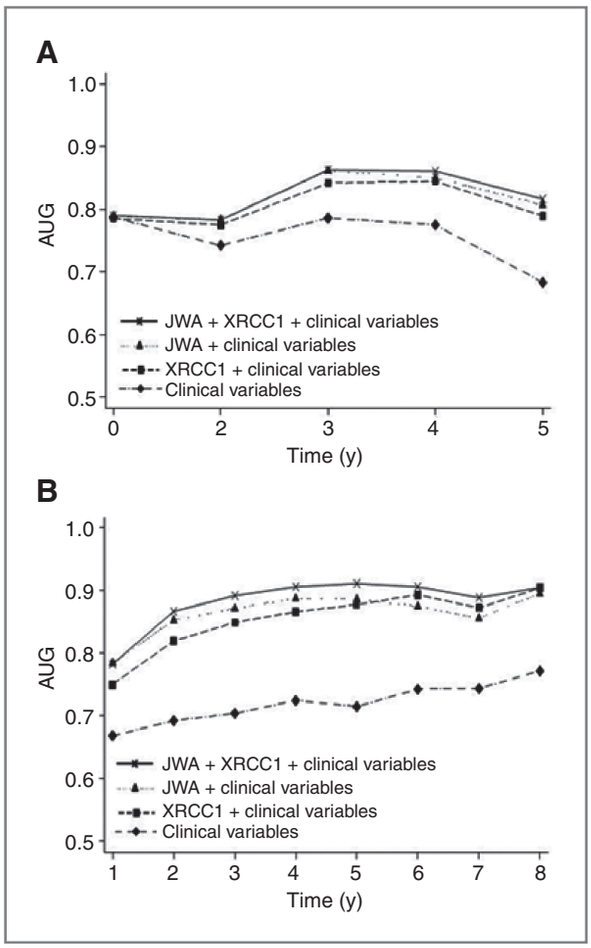

Figure 3. Time-dependent ROC analyses for the clinical risk score (TNM stage, histologic type, and tumor diameter), the combined JWA, XRCC1, or JWA plus XRCC1 and clinical risk score in the training cohort (A) and testing cohort (B).

cohorts of gastric cancer patients with minimum 5-year follow-up (Fig. 2A–C). The patients were then stratified into 3 distinct groups depending on staining for JWA and XRCC1: both high, 1 high (JWA or XRCC1 high), and both low. It was shown that patients with both high had a better outcome of survival than in the 2 other groups ($P < 0.001$, log-rank test; Fig. 2). Other significant negative predictors for survival by univariate analysis in the 3 independent cohorts were lymph node metastasis (N-category; $P < 0.01$ for all) and clinical TNM stage ($P < 0.001$ for all; data not shown). Nontumoral JWA or XRCC1 expression was not correlated with OS (Supplementary Fig. S4A and B).

The multivariate Cox regression analysis indicated that high JWA and XRCC1 expressions were independent positive prognostic factors separately or together for gastric cancer in all 3 cohorts ($P < 0.05$ for all, Table 2).

To further evaluate the prognostic efficacy of JWA and XRCC1 expressions, we conducted a time-dependent ROC analysis for the censored data, which indicated that the combination of the clinical risk score (TNM stage, histologic type, and tumor diameter) and JWA or XRCC1 or JWA plus XRCC1 contributed much more than either one alone in both of training and testing cohorts (Fig. 3). For example, in the testing cohort, the AUC at year 5 was 0.715 (95% CI: 0.662–0.769) for clinical risk score, whereas it was significantly increased to 0.912 (95% CI: 0.881–0.942) when combination of the clinical risk score with JWA plus XRCC1 risk score. However, this effect was not significant in the validation cohort due to the relatively higher AUC (about 0.8) of clinical predictors (Supplementary Fig. S5).

Correlation between JWA or XRCC1 expressions and OS in patients with adjuvant chemotherapy

In the testing and validation cohorts, OS was analyzed between the patients who received adjuvant chemotherapy versus those who did not. Data showed no difference in OS between the surgery only group and any regimen of postoperative adjuvant chemotherapy (data not shown) except in the group receiving FLO ($n = 93$, log-rank test, $P = 0.01$; Fig. 4). A multivariate Cox regression analysis including 6 variables (age, gender, TNM stage, histologic types, tumor diameter, and chemotherapy treatment) was conducted to indicate the benefit of chemotherapy on OS. There was a statistically significant benefit only of FLO chemotherapy over surgery alone (HR = 0.50, 95% CI: 0.34–0.73, data not shown). Conspicuously, this effect was only found in low JWA or XRCC1 expression patients in which adjuvant FLO significantly increased OS as compared with surgery alone (for JWA, HR = 0.44, 95% CI: 0.26–0.73, Supplementary Table S4; log-rank test, $P = 0.002$; Fig. 4; for XRCC1, HR = 0.44, 95% CI: 0.26–0.75, Supplementary Table S4; log-rank test, $P = 0.02$; Fig. 4). Moreover, patients with high JWA or XRCC1 expression in tumors had no additional survival benefit from adjuvant chemotherapy (Supplementary Table S4 and Fig. 4).

We also analyzed the significance of another platinum-based chemotherapy, FLP regimen ($n = 78$) in resectable gastric cancer. The results did not show a significant survival difference (log-rank test, $P = 0.364$; Supplementary Fig. S6), whereas low JWA or XRCC1 expression patients receiving FLP regimen showed a trend of longer survival compared with those with surgery only (Supplementary Fig. S6). In contrast, high JWA or XRCC1 expression patients with FLP regimen had significantly shorter survival compared with those with surgery only (JWA, $P < 0.001$; XRCC1, $P = 0.003$; Supplementary Fig. S6). Further multivariate analysis indicated that lower risk for mortality was observed in those with FLP chemotherapy compared with surgery only (HR = 0.55, 95% CI: 0.35–0.86 for JWA; HR = 0.58, 95% CI: 0.36–0.93 for XRCC1; Supplementary Table S6).

Discussion

One of the most challenging problems in oncology is that we know that a large percentage of the cancer patients are treated unnecessary, but we do not know how to select them. Even in patients with similar clinical or pathologic

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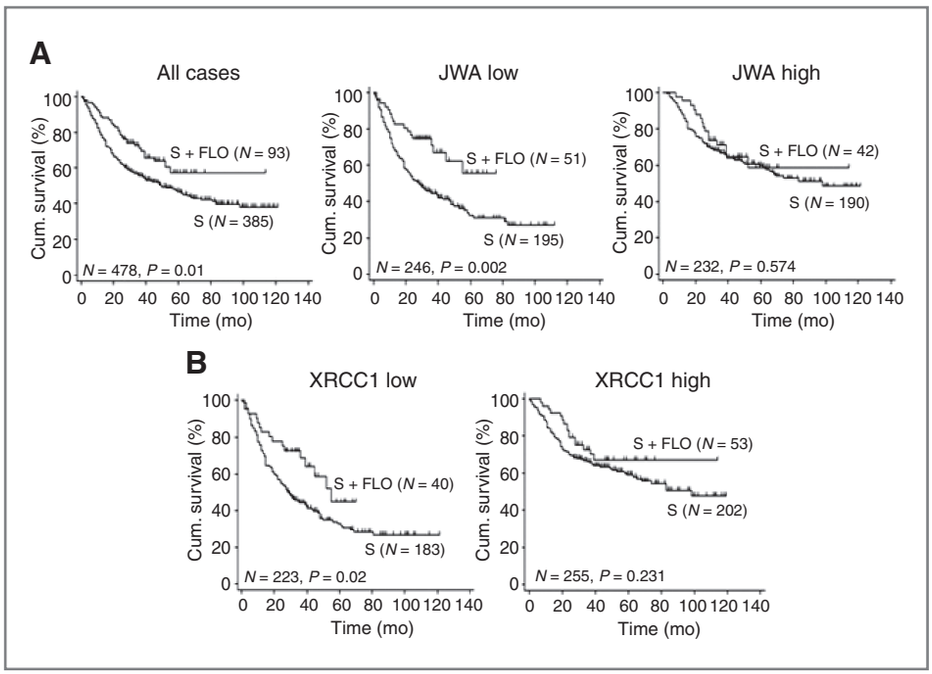

Figure 4. Kaplan–Meier curves depicting OS according to JWA (A) or XRCC1 (B) expression patterns in validation cohort patients treated with or without FLO. *P* values were calculated with the log-rank test. S, surgery alone.

features, their survival outcomes vary. Discovery of prognostic and predictive biomarkers may enable personalized cancer therapies. In this study, we recorded and independently confirmed that low expression of JWA and XRCC1 were significantly associated with unfavorable clinicopathologic parameters and decreased overall patient survival. Moreover, patients with low JWA or XRCC1 expression in tumors had significant survival benefit from adjuvant first-line platinum-based-chemotherapy (FLO or FLP).

In this study, we show significant underexpression of both JWA and XRCC1 proteins in gastric cancer cells versus paired normal tissue, indicating a potentially important role of these genes in gastric carcinogenesis. These data were consistent with previous studies, which reported that normal nevi and normal melanocytes had high JWA expression and low expression in dysplastic nevi and malignant melanoma tissues and cell lines (20). Moreover, XRCC1 expression was downregulated in pancreatic cancer versus normal cells (16). To study the possible tumor suppressor effect of JWA, we recently constructed conditional JWA knockout mice. However, spontaneous tumors of these mice were not observed (Wang and colleagues; unpublished data), indicating that environment–gene interactions in tumorigenesis should be considered. The exact mechanisms of XRCC1 in tumorigenesis are formidable to study in animal models due to the embryonic lethality in XRCC1 knockout mice (26).

An interesting question that arises in this study is, how can low expression of the same protein be both a negative prognostic factor and a positive predictive factor? These Janus-like properties in cancer biology and treatment resistance has been documented for the nucleotide excision repair (NER) protein ERCC1 in which a high ERCC1 expression in resectable lung cancer was a positive prognostic factor, but a negative predictive factor for platinum treatment (27–29). *In vitro* and animal studies indicated that the loss of JWA decreased cell differentiation (30) and increased cell migration and metastasis (19, 20). Similarly, in our study, low JWA expression in gastric tumors correlated with unfavorable TNM stage and diffuse type, thus to a more malignant, aggressive phenotype with a negative prognosis when untreated. Conversely, the positive predictive effect of low JWA and XRCC1 on survival in both platinum treated patients was highly significant. This may point to a role of both proteins in chemoresistance, probably related to platinum. Dysregulation of several DNA repair mechanisms is an important modulator of platinum effect (31). The role of JWA in these DNA repair mechanisms remains to be elucidated, but lowering of JWA was shown to increase susceptibility to DNA-damaging agents (18). Moreover, JWA was shown to play a role in transcriptional and translational regulation of XRCC1 levels (18). XRCC1 is a critical component of the BER pathway, and downregulation of BER sensitized cancer to cisplatin or oxaliplatin (32, 33). XRCC1 is also involved in NER (34), homologous recombination, and nonhomologous end joining (35, 36), which are involved in platinum resistance (31). However, the direct roles of JWA and XRCC1 in platinum treatment need to be further provided.

In interpreting our results, several issues need to be considered and clarified. First, a timely question about this type of study is how reliable is the interobserver concordance on scoring the immunohistochemical expression. IRS was a reliable tool in our hands and reported to be robust for other antibodies, even with basic training only (37).

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Moreover, we applied ROC analysis to exclude the subjective division of IRS. Second, the use of only duplicate 1.0-mm diameter cores of tissue from each sample when preparing TMA might lead to a limitation of reprehensive samples due to tumor heterogeneity. However, 3 relatively large independent TMA were used to minimize the impact of tumor heterogeneity and determine a reliable value of our studies. Third, it must be noted that the patients received more survival benefit from FLO than from FLP, which may be partially due to that FLO reduces toxicity as compared with FLP (38) and oxaliplatin has recently been shown to induce immunogenic cell death *in vivo* (39), while cisplatin fails to do so (40). As the study is retrospective in nature, and the number of patients receiving first-line oxaliplatin or cisplatin-based adjuvant chemotherapy is relatively small, large efforts were done to obtain correct clinical and survival data (see Materials and Methods). The database built upon this information is thus as complete as possible. Nevertheless, despite highly significant results in such a large patient material, these markers should be validated in different ethnic population and prospective studies are warranted before using these markers in the clinic.

Taken together, we found that 2 BER proteins, JWA and XRCC1 were higher expressed in noncancerous gastric mucosa than in gastric cancer tissues. We report for the first time that JWA and XRCC1 are potential prognostic biomarkers and predictors for adjuvant chemotherapy with platinum-based regimen (FLO or FLP) in resectable gastric cancer patients. The simplicity of immunostaining and assessment by IRS makes these proteins as interesting candidates for personalizing gastric cancer treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Wang, X. Wu, J. Ding, Y. Tan, G. Li, F. Qiang, J. Zhou
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Acquisition of data: X. Wu, J. Ding, Y. Zhou, Y. Tan, J. Zhang, S. He, F. Qiang, J. Zhou
Analysis and interpretation of data: S. Wang, Y. Chen, J. Ding, O. Dimitri
Writing, review, and/or revision of the manuscript: S. Wang, G. Li, O. Dimitri, J. Zhou
Administrative, technical, or material support: S. Wang, J. Bai, J. Zeng, Z. Gong, A. Li, J. Zhou
Study supervision: S. Wang, J. Zhou

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