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Original Article

Expression of JWA and XRCC1 as prognostic markers for gastric cancer recurrence

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Abstract: Gastric cancer is one of the common gastrointestinal tumors. Tumor recurrence leads to a high death rate of gastric cancer. It is very important to find markers to effectively predict gastric cancer recurrence. We constructed a gastric cancer tissue microarray containing 89 tumors and corresponding normal tissues to explore the relationship between some proteins' expression and gastric cancer recurrence. The expression of JWA, Cullin1, p53, XRCC1, CHIP, FAK, MMP-2, MDM2 and p21 was determined on the microarray by immunohistochemistry. The relationship between the expression of these proteins and gastric cancer recurrence was analyzed. Tumor diameter, lymph node metastasis, and TNM stage were closely related with gastric cancer recurrence by Fisher's exact test ($P<0.05$). We used the univariate Cox regression analysis to find that JWA, XRCC1 were related to gastric cancer recurrence ($P<0.05$); Lymph node metastasis and TNM stage were closely related to gastric cancer recurrence ($P<0.05$). Multivariate Cox regression analysis revealed that XRCC1 or lymph node metastasis were independent risk factors of gastric cancer recurrence ($P<0.05$). Kaplan-Meier survival curve assay indicated that patients with low JWA or XRCC1 expression in gastric cancer had significantly shorter DFS than those with high-expressed proteins ($P<0.05$). JWA or XRCC1 may be effective markers to predict gastric cancer recurrence.

Keywords: Gastric cancer, recurrence, prognosis, JWA, XRCC1

Introduction

Gastric cancer is a cause of high morbidity and mortality worldwide [1], with a higher incidence rate in East Asia, especially in China, Korea, and Japan [2]. More than 80% of gastric cancer patients are in the advanced stage at the initial diagnosis. Some postoperative patients with gastric cancer may still recur even after complete adjuvant chemotherapy [3, 4]. Although the incidence of gastric cancer has declined in recent years, the high recurrence rate and high death rate remain severe problems [5].

Although chemotherapy, radiotherapy, and immunotherapy have become main treatment methods for gastric cancer, surgery is still the most important treatment method [6, 7]. In recent years, the treatment of gastric cancer has significantly improved but the rates of treatment failure and patient death are still high because of cancer recurrence [8]. Recurrence includes local recurrence and distant metastasis. Metastasis is a continuous complex, multi-step, multi-factor, polygene process. The process requires that tumor cells fall off from the primary foci, invade connective tissue, blood vessels, or lymphatics, and then deposit in the target organs [9]. Clinically, imaging diagnosis and hematology indexes are used to determine whether there is tumor recurrence in gastric cancer. However, these traditional methods have many drawbacks and cannot identify the micrometastases in the tumor, which misses recurrence of gastric cancer [10]. Therefore, looking for efficient markers to predict the recurrence of gastric cancer could save time to treatment.

Table 1. Relationship between gastric cancer recurrence and clinicopathological features in gastric cancer patients

Clinical variables	Recurrence group (n = 62, %)	No recurrence group (n = 27, %)	X^2	P[a]
Age (years)			0.233	0.812
≤65	38 (67.86)	18 (32.14)		
>65	24 (72.73)	9 (27.27)		
Gender			0.348	0.783
Male	47 (68.12)	22 (31.88)		
Female	15 (75.00)	5 (25.00)		
Tumor diameter (cm)			6.641	0.011
<5	30 (58.82)	21 (41.18)		
≥5	32 (84.21)	6 (15.79)		
Pathological classification			4.722	0.233
I	10 (50.00)	10 (50.00)		
II	18 (75.00)	6 (25.00)		
III	34 (75.56)	11 (24.44)		
Depth of invasion			1.548	0.488
T1	3 (60.00)	2 (40.00)		
T2	10 (58.82)	7 (41.48)		
T3	49 (73.13)	18 (26.87)		
Lymph node metastasis			13.376	0.001
N0	11 (42.31)	15 (57.69)		
N1	33 (75.00)	11 (25.00)		
N2	17 (94.44)	1 (5.56)		
N3	1 (100.00)	0 (0.00)		
TNM stage			8.616	0.010
I	5 (62.50)	3 (37.50)		
II	14 (50.00)	14 (50.00)		
III	43 (81.13)	10 (18.87)		

[a]Two-sided Fisher's exact tests.

The clinical diversity of gastric cancer is derived from its molecular biologic diversity, which includes functional changes of various proteins in the development of gastric cancer. Therefore, molecular markers can effectively predict the recurrence of postoperative gastric cancer patients [11]. In this study, we evaluated the correlation between expression of JWA, Cullin1, p53, XRCC1, CHIP, FAK, MMP-2, MDM2 and p21 with the recurrence of gastric cancer by tissue microarray, in order to provide a scientific basis for early prediction of gastric cancer recurrence.

Materials and methods

Patients and samples

89 gastric cancer patients were pathologically diagnosed, which were enrolled between 1999.01 and 2006.12 at Yixing Hospital Affiliated with Medical College of Yangzhou University. Inclusion criteria were treatment with radical operation andadjuvant chemotherapy. The regimen of LFP (Oxaliplatin + Tetrahydrofolic acid + 5-fluorouracil) was 4-6 periods after surgery. The clinicopathologic features are summarized in **Table 1**. The survival follow-up time was recorded. After the chemotherapy was completed, all patients were regularly checked by CT, type-B ultrasound, tumor related index, etc. Disease-free survival (DFS), the primary time-point, was calculated from the time of diagnosis to the date of gastric cancer recurrence. The last follow-up time was 2011.12.

Establishment of gastric cancer tissue microarray to examine the expression of JWA, Cullin1, p53, XRCC1, CHIP, FAK, MMP-2, MDM2 and p21 proteins

The gastric cancer tissue microarray was established by State Engineering Research Center of Shanghai. Tumor tissues of 1.0 mm were obtained from the center of paraffin-embedded tumor mass and the respective para-carcinoma tissues, which were used for tissue controls. As a negative control, normal gastric epithelial cell biopsies were inserted into the corners and centers of each chip. The expression of JWA, Cullin1, p53, XRCC1, CHIP, FAK, MMP-2, MDM2 and p21 were determined by standard procedures. The tissue chips were incubated at 55°C for 20 min and then washed with xylene for 3 times (5 min each time) to remove paraffin. Subsequently, the chips were washed with absolute ethyl alcohol, 950 mL/L ethyl alcohol, 800 mL/L ethyl alcohol, and distilled water respectively for 5 min. Antigen retrieval was then performed with samples in 10 mmol/L sodium citrate (pH 6.0) at 95°C for 30 min. The samples were incubated with hydrogen peroxide to block the activity of endogenous peroxidase. The samples were blocked with serum for 30 min. The antibodies:

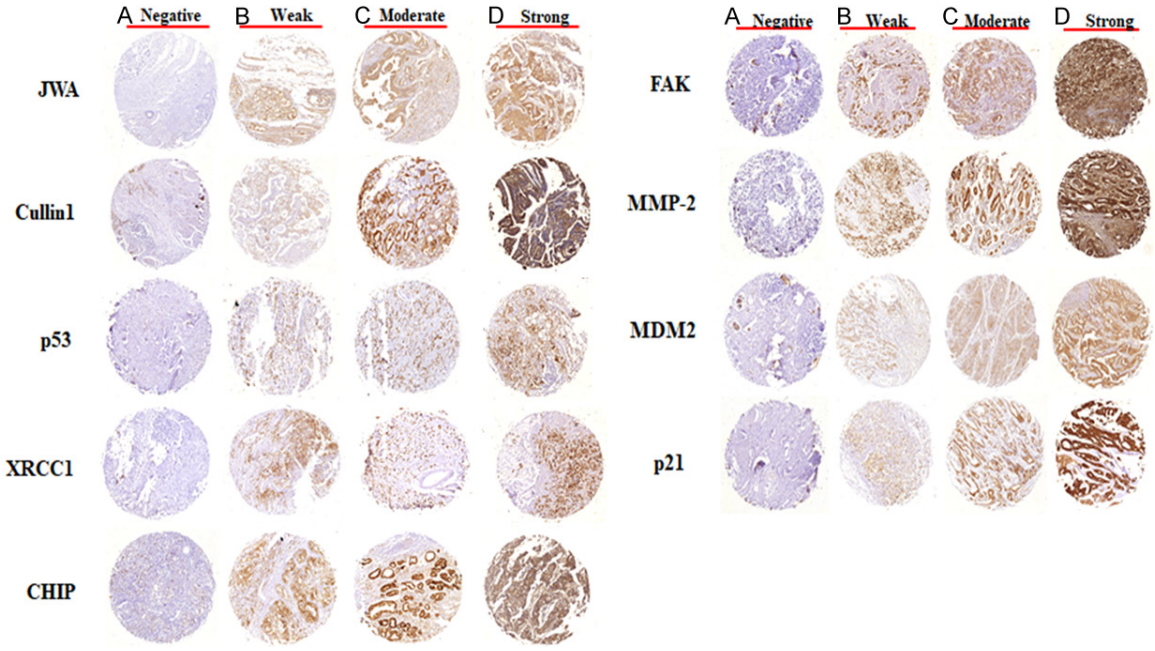

Figure 1. Representative images of all proteins' immunohistochemical staining in gastric cancer. A. Negative staining. B. Weak staining. C. Moderate staining. D. Strong staining. All panels, original magnification, ×50.

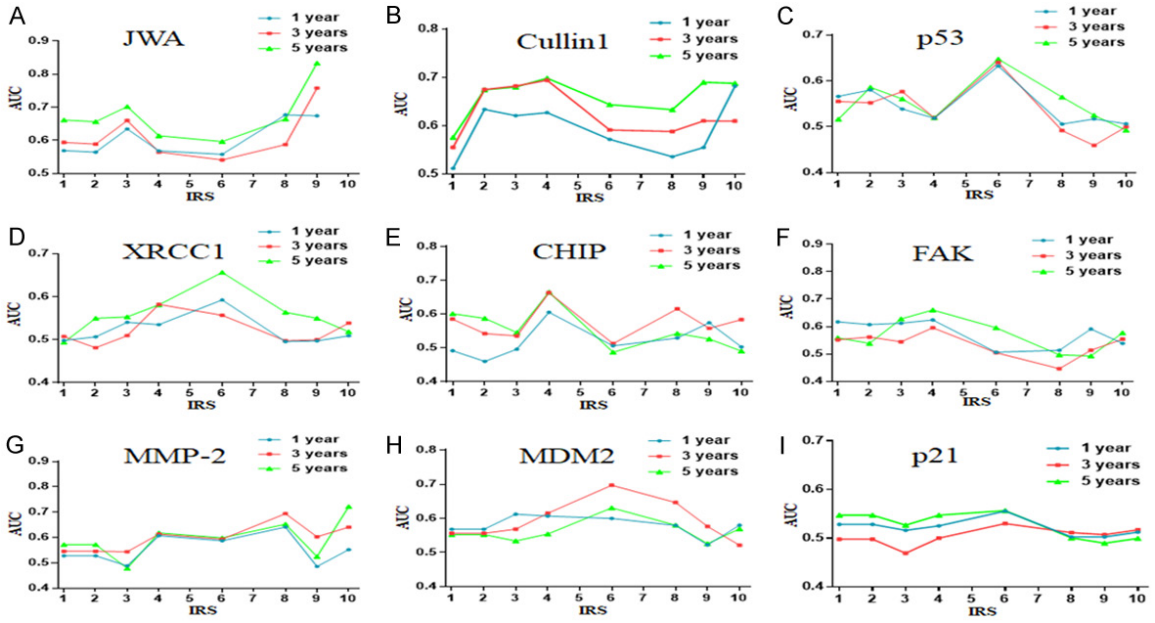

Figure 2. Area under the curve (AUC) at different cut-off values for all proteins' immunoreactivity score (IRS) for 1, 3, and 5 years of DFS time. A. JWA. B. Cullin1. C. p53. D. XRCC1. E. CHIP. F. FAK. G. MMP-2. H. MDM2. I. p21.

monoclonal rabbit anti-JWA (1:100, Epitomics, California, USA), monoclonal rabbit anti-Cullin1 (1:200, Epitomics, California, USA), monoclonal rabbit anti-p53 (1:200, Cell Signaling Technology, MA, USA), monoclonal rabbit anti-XRCC1 (1:200, Epitomics, California, USA), monoclonal rabbit anti-CHIP (1:200, Epitomics, California, USA), monoclonal rabbit anti-FAK (1:200, Epitomics, California, USA), monoclonal rabbit anti-MMP-2 (1:200, Epitomics, California, USA), monoclonal rabbit anti-MDM2 (1:200, Epitomics, California, USA), monoclonal

Table 2. Expression of proteins in gastric cancer recurrence group and no-recurrence group

Variable	Recurrence group (n = 62, %)	No recurrence group (n = 27, %)	P
JWA			0.037
High expression	14 (22.58)	12 (44.44)	
Low expression	48 (77.41)	15 (55.56)	
Cullin1			0.148
High expression	22 (35.48)	14 (51.85)	
Low expression	40 (64.52)	13 (48.15)	
P53			0.921
High expression	42 (67.74)	18 (66.67)	
Low expression	20 (32.26)	9 (33.33)	
XRCC1			0.01
High expression	23 (37.09)	18 (66.67)	
Low expression	39 (62.91)	9 (33.33)	
CHI			0.184
High expression	25 (40.32)	15 (55.56)	
Low expression	37 (59.68)	12 (44.44)	
FAK			0.455
High expression	44 (70.97)	17 (62.96)	
Low expression	18 (29.03)	10 (37.04)	
MMP2			0.403
High expression	41 (66.13)	19 (70.37)	
Low expression	11 (33.87)	8 (29.63)	
MDM2			0.717
High expression	37 (59.68)	15 (55.56)	
Low expression	25 (40.32)	12 (44.44)	
P21			0.688
High expression	27 (43.55)	13 (48.15)	
Low expression	35 (56.45)	14 (51.85)	

rabbit anti-p21 (1:200, Epitomics, California, USA) were incubated with the sample sections at 4°C overnight. The respective second antibody was incubated for 30 min followed by hematoxylin staining by 3, 3'-diamido-plate. For the sample sections in each chip, the quality standard of staining was based on staining of normal gastric mucosa epithelial tissue.

Evaluation of immunostaining

Staining of JWA, Cullin1, p53, XRCC1, CHIP, FAK, MMP-2, MDM2 and p21 in the tissues were scored independently by two pathologists blinded to the clinical data. The intensity of immunostaining is shown in **Figure 1**. Each protein staining grade was calculated using the immunostaining score (IRS). According to IRS, these proteins' staining was divided into different levels: negative (IRS: 0), weak (IRS: 1-2), moderate (IRS: 3-6) and strong (IRS: 8-12) [12, 13]. The optimum cutoff value of IRS was obtained by receiver operator characteristic (ROC) analysis. The area under the curve (AUC) at different cutoff values of these proteins' IRS for 1, 3 and 5 years of DFS time were calculated. The optimum value of cutoff points of these proteins' IRS was shown to be 2, 3, 4, 4, 3, 3, 6, 4, 4 respectively since these had the best predictive value for survival (**Figure 2**). Then, these proteins were classified as low or high expression according to the cutoff points.

Statistical analysis

For TMAs, statistical processing was performed with SPSS 20.0 software (SPSS, Inc, Chicago, IL). Fisher's exact test was used to evaluate the association between gastric cancer recurrence and clinicopathologic data. Proteins' staining scores in primary tumors were assessed by IRS. Probability of differences in DFS as a function of time was ascertained by the Kaplan-Meier method, with a log-rank test probe for significance. Univariate and multivariate Cox proportional hazards regression analysis were performed to estimate the crude hazard ratios (HRs), adjusted HRs, and 95% CI of HRs. All the statistical analyses were performed by STATA statistical software (version 10.1; StataCorp, College Station, TX). A P value of <0.05 was deemed significant, and all tests were two-sided.

Results

Clinicopathological data in gastric cancer recurrent group or no-recurrence group

A database including 89 postoperative patients with gastric cancer was established, which contains 62 cases of postoperative recurrence and 27 cases of non-recurrence. Fisher's test was used to compare the clinicopathologic characteristics of the two groups. The results showed that tumor diameter, lymph node metastasis, and TNM stage were closely related to the recurrence of gastric cancer (**Table 1**, P<0.05).

Table 3. Univariate Cox regression analysis of protein expression and clinicopathologic variables predicting recurrence in gastric cancer patients

	P-value	HR	95% CI	
			Lower	Upper
Protein				
JWA	0.003	0.848	0.761	0.944
Cullin1	0.658	0.984	0.915	1.057
P53	0.293	1.033	0.972	1.098
XRCC1	0.037	0.906	0.826	0.994
CHIP	0.81	1.011	0.926	1.104
FAK	0.122	1.08	0.98	1.189
MMP2	0.602	0.976	0.892	1.068
MDM2	0.931	0.997	0.924	1.075
P21	0.533	0.974	0.897	1.058
Pathologic features				
Age (≤65 years vs. >65 years)	0.935	1.019	0.649	1.601
Gender	0.133	1.503	0.883	2.561
Tumor diameter (<5 cm vs. ≥5 cm)	0.035	1.503	1.034	2.516
Differentiation	0.796	0.987	0.893	1.091
Infiltration depth	0.19	1.319	0.872	1.994
TNM stage	0.018	1.605	1.083	2.378

Table 4. Multivariate Cox regression analysis of JWA and XRCC1 expression, clinicopathologic features, and DFS in postoperative patients with gastric cancer

Variable	P	HR	95% CI	
			Lower	Upper
Age (≤65 y vs. >65 y)	0.199	0.654	0.342	1.25
Gender	0.068	2.382	0.939	6.04
Tumor diameter (<5 cm vs. ≥5 cm)	0.478	1.267	0.659	2.437
Differentiation	0.697	0.924	0.623	1.372
Infiltration depth	0.613	1.186	0.612	2.299
Lymph node metastasis	0.022	2.302	1.127	4.701
TNM stage	0.310	0.669	0.308	1.455
XRCC1	0.042	0.854	0.734	0.994
JWA	0.368	0.931	0.796	1.088

The expression of JWA, Cullin1, p53, XRCC1, CHIP, FAK, MMP-2, MDM2 and p21 in recurrent group and no-recurrence group

We detected the expression of these proteins by immunohistochemistry in 89 gastric cancer patients. Every protein's staining score could be recorded by IRS standard. We also estimated high or low expression of these proteins according to their cutoff value respectively. We used Fisher's exact test to analyze these proteins' expression in the gastric cancer recurrence group and no-recurrence group. The results indicated that only JWA or XRCC1 expression were closely related to the recurrence of gastric cancer (**Table 2**, P<0.05).

Univariate Cox regression analysis of protein expression, clinicopathologic features, and disease-free survival (DFS) in gastric cancer patients

The average DFS of postoperative recurrent patients with gastric cancer was 22.7 months (range: 0-87 months). We used univariate Cox regression to analyse the correlation of every protein expression or clinicopathologic features and DFS. Our results showed that JWA or XRCC1 expression was closely related to postoperative recurrence of gastric cancer (**Table 3**, P<0.05, respectively); the tumor diameter, lymph node metastasis or TNM stage were associated with postoperative recurrence of gastric cancer (**Table 4**, P<0.05, respectively).

Multivariate Cox regression analysis of JWA, XRCC1 expression, clinicopathologic features and postoperative DFS in gastric cancer patients

As in **Table 3**, we concluded that only JWA or XRCC1 had statistical differences (P<0.05). Similarly, we analyzed the relationship between the expressions of JWA, XRCC1, and clinicopathologic features by multivariate Cox regression analysis. Our data indicated that XRCC1 or lymph node metastasis was one of the independent factors for postoperative recurrence of gastric cancer (**Table 4**, XRCC1: HR 0.854, 95% CI 0.734-0.994, P<0.05; lymph node metastasis: HR 2.302, 95% CI 1.127-4.701, P<0.05).

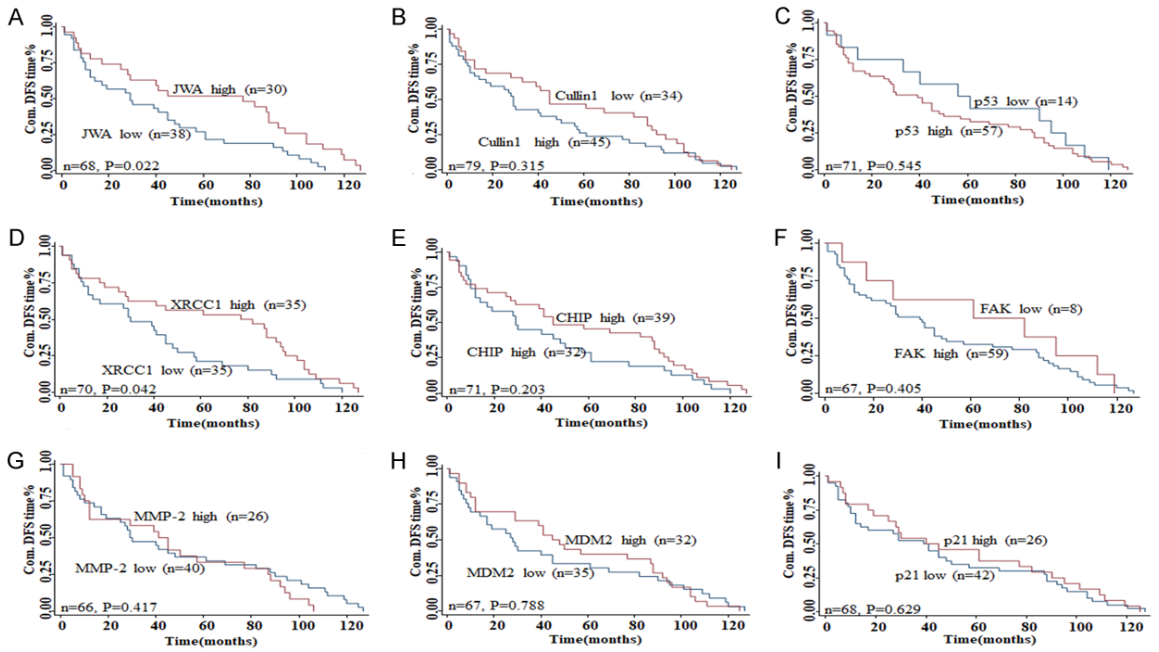

Figure 3. Kaplan-Meier curves for DFS time. A. JWA. B. Cullin1. C. p53. D. XRCC1. E. CHIP. F. FAK. G. MMP-2. H. MDM2. I. p21. *P*-values were calculated with the log-rank test.

High JWA or XRCC1 expression correlates with better DFS for gastric cancer patients

To further study whether these proteins' expression was correlated with DFS of gastric cancer patients. Kaplan-Meier survival curves were used for DFS to compare the patients with high-expressed proteins to those with low-expressed proteins respectively. Our data revealed that only high JWA or XRCC1 expression correlated with a better DFS; other proteins that we detected had no relation to the DFS in gastric cancer patients (**Figure 3**, JWA P = 0.022 and XRCC1 P = 0.042). We concluded that JWA or XRCC1 could predict the recurrence of gastric cancer.

Discussion

Gastric cancer has high postoperative recurrence and poor prognosis because of its anatomical location and adverse biologic behavior [14, 15]. Although the new treatment model that contains surgery and multidisciplinary treatment has been widely used in the clinic, the recurrence rate of gastric cancer remains as high as 21.8%~49.5% [16-18]. In recent years, treatment is insufficient once the gastric cancer has recurred and 24.0%~28.9% of gastric cancer patients die from recurrence [19, 20]. Predicting early gastric cancer postoperative recurrence and reducing risk factors of recurrenceshould help formulate a detailed treatment plan after surgery to reduce the chances of postoperative recurrence [21].

Some studies have found that lymph node metastasis and depth of infiltration were closely related to postoperative recurrence of gastric cancer. Through univariate and multivariate Cox regression analysis, only lymph node metastasis was an independent risk factor for gastric cancer recurrence [22]. Lymph node metastasis is not effective in predicting gastric cancer recurrence. Thus new biomarkers are needed.

In the occurrence and development of gastric cancer, the abnormal expression of oncogenes and tumor suppressor genes play an important role. In recent years, many researchers have revealed biomarkers that could predict postoperative recurrence of gastric cancer [23-27]. In this study, we used tissue microarray to detect the expression of JWA, Cullin1, p53, XRCC1, CHIP, FAK, MMP-2, MDM2 and p21 by immunohistochemistry. Simultaneously, we evaluated the correlation between these proteins' expression with postoperative recurrence of gastric cancer. Our results showed that

tumor diameter, lymph node metastasis, TNM stage, JWA, or XRCC1 expression were closely related with gastric cancer recurrence ($P< 0.05$, respectively). XRCC1 or lymph node metastasis was one of the independent risk factors of gastric cancer recurrence ($P<0.05$). Kaplan-Meier survival curve assay indicated that the high JWA or XRCC1 expression in gastric cancer patients predicted significantly longer DFS than low expression ($P<0.05$).

JWA gene is a new cytoskeleton-related gene induced by all-trans retinoic acid. Recent studies have shown that JWA has a variety of inhibitory effects in malignant tumor metastasis. XRCC1 acts as a scaffold protein in base excision repair (BER) and single-strand broken chain damage repair (SSBR). It can interact with multiple DNA repair-related proteins to complete DNA repair. XRCC1 can change the stability of the genome, which leads to the occurrence of malignant tumors. JWA or XRCC1 play a role of tumor suppressor genes in the development of gastric cancer, which is related to DNA repair. Some studies indicated that JWA or XRCC1 protein could be used as molecular markers for prognosis and chemotherapeutic effect of gastric cancer [12, 16]. However, whether these two proteins can predict the recurrence of gastric cancer was not clear. Our study mainly focused on molecular marker for postoperative recurrence. We concluded that JWA or XRCC1 expression could predict the recurrence of gastric cancer.

Conclusion

Our findings clearly indicated for the first time that JWA or XRCC1 expression could be used to evaluate the risk of gastric cancer recurrence. We may strengthen the intensity of adjuvant chemotherapy to reduce the probability of postoperative recurrence of gastric cancer according to JWA or XRCC1 expression levels. However, this study had several limitations. First, the data were from a single center. Second, this study was retrospective. These problems may be overcome in our next study.

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Disclosure of conflict of interest

None.

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