Original Article

Prognostic significance of *X-ray cross-complementing gene 1* expression in gastric cancer

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Abstract

Objective: The aim of this study is to identify the prognostic significance of *X-ray cross-complementing gene 1* (*XRCC1*) in patients with gastric cancer undergoing surgery and platinum-based adjuvant chemotherapy.

Methods: Immunohistochemistry (IHC) was used to evaluate *XRCC1* protein expression profiles on surgical specimens of 612 gastric cancer patients. The relationship between *XRCC1* expression and existing prognostic factors, platinum-based adjuvant chemotherapy, disease-free survival (DFS) and overall survival (OS) were analyzed.

Results: Among 612 patients staged II/III in our study, 182 (29.74%) were evaluated as *XRCC1* IHC positive. *XRCC1* expression was not significantly related to OS (P=0.347) or DFS (P=0.297). Compared with surgery only, platinum-based adjuvant chemotherapy significantly improved the OS (P=0.031). And the patients with negative *XRCC1* expression benefited more from platinum-based adjuvant chemotherapy (P=0.049). Multivariate analysis demonstrated that tumor size, T category, N category, vascular or nerve invasion and platinum-based chemotherapy were good prognostic factors for OS (P<0.05). Though *XRCC1* plays an important role in DNA repair pathways, no significant relationship is found in *XRCC1* expression and OS among gastric cancer in our study.

Conclusions: *XRCC1* might be an alternative prognostic marker for the patients of gastric cancer after radical resection. The patients with negative *XRCC1* expression can benefit more from platinum-based adjuvant chemotherapy.

Keywords: Gastric cancer; *X-ray cross-complementing gene 1* (*XRCC1*); platinum drugs prognosis

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Introduction

Gastric cancer in spite of its decreasing incidence remains one of the most common causes of cancer-related deaths worldwide (1). Surgery is the preferred method for the treatment of gastric cancer. Meanwhile, platinum-based adjuvant chemotherapy after surgery has been widely accepted as a standard treatment for several decades. However, chemotherapy has limited efficacy in both resectable and unresectable gastric cancer cases (2,3). New molecular markers pivotal to tumor biology, to prediction of the prognosis and adjuvant treatment regimens are

urgently needed.

Since the antineoplastic mechanism of platinum agents is to cause DNA damage by forming intrastrand and interstrand platinum-DNA cross-links, DNA repair systems have been increasingly implicated in chemotherapy-resistance (4). Thus, proteins involved in the DNA repair pathways, such as the *excision repair cross-complementing 1* (*ERCC1*), the *breast and ovarian cancer susceptibility gene 1* (*BRCA1*) and *X-ray cross-complementing gene 1* (*XRCC1*) are probably related to platinum-based chemotherapy responsiveness and prognosis.

XRCC1 is located on chromosome 19q13.2-13.3 with a length of 33 kilobase. It plays an important role in DNA repair-pathways, acting as a scaffolding protein for the base excision repair (BER) and single-strand break repair (SSBR). *XRCC1* is the first gene to be isolated which is sensitive to ionizing radiation. It is one of the most important DNA repair genes, and interacts with at least three other proteins (poly-ADP-ribose polymerase, DNA ligase 3, and DNA polymerase β) to repair single-strand breaks in DNA (5). The current studies of *XRCC1* mainly focused on the relationship between gene polymorphism and cancer susceptibility. There are three polymorphisms in the *XRCC1* have been extensively investigated: codon 194 (Arg194Trp), codon 280 (Arg280His) and codon 399 (Arg399Gln) (6). However, few studies have investigated the relationship between the expression of *XRCC1* and the prognostic significance in tumors (7). Therefore, we evaluated the expression of *XRCC1* in the surgically resected gastric cancer tissues and tried to determine if *XRCC1* can provide any role in predicting the prognosis.

Materials and methods

Patients

A total of 612 patients with gastric cancer were included in our study, provided by the First Affiliated Hospital of Nanjing Medical University between January 2006 and December 2009. Eligibility criteria used for the patients selection were as follows: (I) diagnosis of gastric cancer; (II) staged II or III; (III) D2 surgical resection; (IV) received adjuvant chemotherapy after surgery based on cisplatin or oxaliplatin combined with 5-fluorouracil (5-FU); and (V) follow-up data was available, and the end of follow-up was May 2013. The study protocol was approved by The Ethical Committee of this hospital which is equivalent to IRB. Each subject had signed an informed consent before entry into the study. The details were shown in *Table 1*.

Immunohistochemistry (IHC)

Tissue samples were formalin-fixed and paraffin-embedded; 4-μm thick sections were cut and stained by using the avidin-biotin complex method. After that, the slides were pretreated with microwaves for antigen retrieval in 10 mM citrate buffer (pH 6.0) and incubated in the primary antibody at 4 ℃ overnight. The antibody used for the detection of *XRCC1* was monoclonal mouse anti-*XRCC1* antibody (1:300 dilution; Abcam). In addition, each case included a negative and a positive control. If the staining was uncertain, it was repeated to confirm it.

Scoring of XRCC1

The staining intensity of *XRCC1* expression was scored on a scale of 1–3 as follows: 0 score for no staining; 1 for weak staining; 2 for moderate staining; and 3 for strong staining. The percentage of positive cancer cells was scored as follows: 0 score for 0%; 0.1 for 1–9%; 0.5 for 10–49%; 1.0 for 50% or more. We multiplied the staining intensity by the proportion score of the percentage of positive cancer cells. Thus, we divided the patients into two groups: positive ones (the product >1) and negative ones (the product ≤1) (8).

Statistical analysis

χ^2 test was performed to evaluate the associations between *XRCC1* expression and the existing prognostic factors. Survival analysis was done by using the log-rank test and Kaplan-Meier curve. Cox proportional hazard model was applied to find predictors for the overall survivals (OS) and disease-free survivals (DFS). For these analyses, $P<0.05$ was considered to be statistically significant. All statistical analyses were performed using the SPSS 17.0 software package.

Results

Study population

In total 612 gastric cancer patients were enrolled into our study with median age of 61 years old (61±11.1, range, 24–93 years old). There are more male patients than female (male: 72.5%, female: 27.5%). The mean tumor size was 4.954 cm. Patients with stage III and poorly differentiated gastric cancer were more prevalent (put the 74.0% here);

Table 1 Patient characteristics and relationship between *XRCC1* expression and existing prognostic factors

Patient characteristics	No. of patients (%)	*XRCC1* expression (%)		P
		Negative	Positive	
Age				0.328
<60	268 (43.8)	194 (31.7)	74 (12.1)	
≥60	344 (56.2)	236 (38.6)	108 (17.6)	
Gender				0.488
Male	444 (72.5)	308 (50.3)	136 (22.2)	
Female	168 (27.5)	122 (20.0)	46 (7.5)	
Tumor size (cm)				0.426
<5	316 (51.6)	217 (35.5)	99 (16.1)	
≥5	296 (48.4)	213 (34.8)	83 (13.6)	
Differentiation				0.269
Well moderate	122 (19.9)	91 (14.9)	31 (5.0)	
Poorly	490 (80.1)	339 (55.4)	151 (24.7)	
T category				0.886
T1–T2	65 (10.6)	45 (7.4)	20 (3.2)	
T3–T4	547 (89.4)	385 (62.9)	162 (26.5)	
N category				0.291
N_0	106 (17.3)	68 (11.1)	38 (6.2)	
N_1	129 (21.1)	87 (14.2)	42 (6.9)	
N_2	174 (28.4)	128 (20.9)	46 (7.5)	
N_3	203 (33.2)	147 (24.0)	56 (9.2)	
Vascular or nerves invasion				0.130
Negative	269 (44.0)	198 (32.4)	71 (11.6)	
Positive	343 (56.0)	232 (37.9)	111 (18.1)	
Stage				0.129
II	158 (25.8)	103 (16.8)	55 (9.0)	
III	454 (74.2)	327 (53.4)	127 (20.8)	
Adjuvant chemotherapy				0.922
No	176 (28.8)	123 (20.1)	53 (8.7)	
Yes	436 (71.2)	307 (50.1)	129 (21.1)	

XRCC1, X-ray cross-complementing gene 1.

436 (71.2%) patients received platinum-based adjuvant chemotherapy. The clinical characteristics of 612 patients were presented in *Table 1*.

XRCC1 expression

In gastric cancer, *XRCC1* protein was mostly located in the cell nucleus (*Figure 1A*). The intensity of staining was varied from absent to strong (*Figure 1B-E*). Of 612 patients, 182 samples (29.7%) showed an IHC score of more than 1 point, and they were evaluated as *XRCC1* IHC positive. The other 430 samples were evaluated as *XRCC1* IHC negative.

Survival and XRCC1 expression

The time-to-event was defined as the time from the surgery to death caused by gastric cancer (for event) and from surgery to last follow-up (for censoring). At the end of follow-up, in May 2013, 250 (40.8%) patients were still

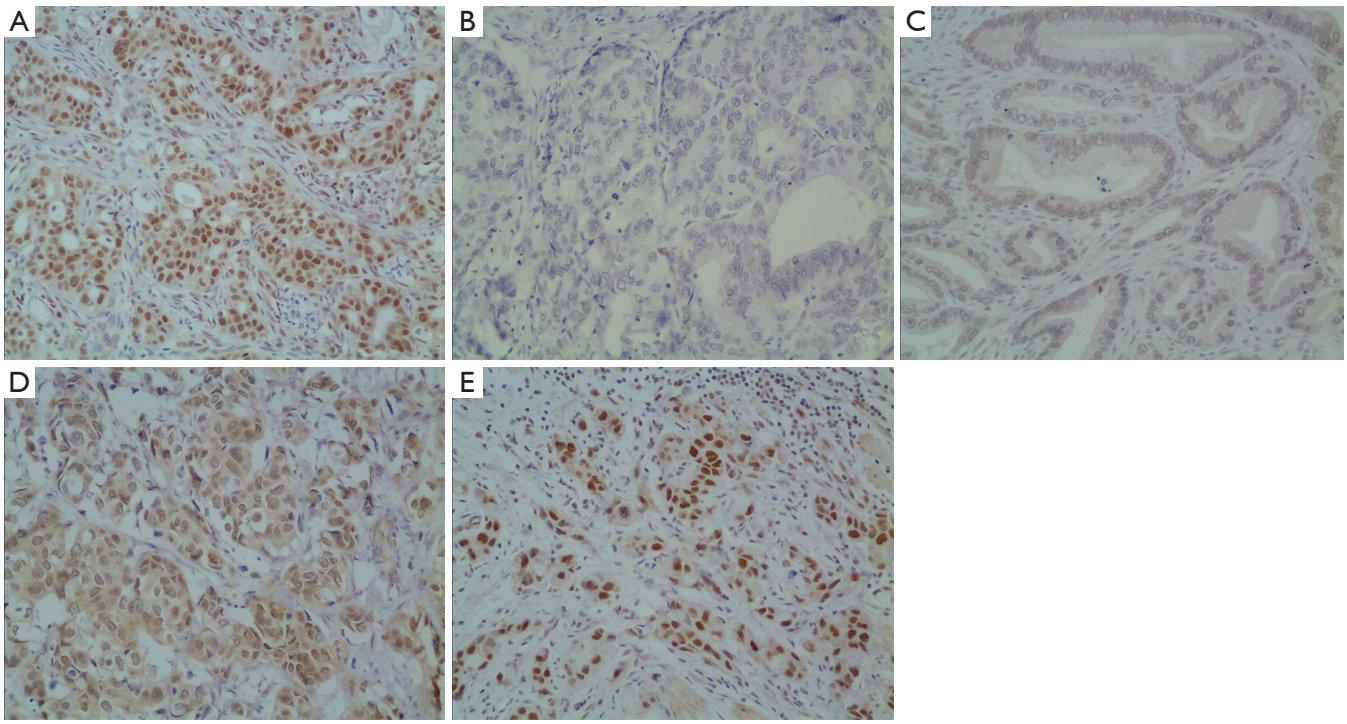

Figure 1 Immunohistochemical detection of *XRCC1* protein in gastric cancer. (A) In gastric cancer, *XRCC1* expression was located in the nucleus, *XRCC1* expression located in the nucleus was varied; (B) no staining; (C) weak staining; (D) moderate staining; (E) strong staining (×200).

alive. There is gastric cancer recurrence rate of 49.8% [305] from our study. The median OS and DFS were 37.357 months with 95% CI: 31.049–43.218 months and 18.072 months with 95% CI: 16.807–21.059 months, respectively.

Neither OS (P=0.206, *Figure 2A*) nor DFS (P=0.973) was significantly prolonged among the patients with positive *XRCC1* expression when compared with patients with negative *XRCC1* expression. The patients received combination of surgery and platinum-based adjuvant chemotherapy were associated with better OS compared with patients received surgery alone (P=0.031, *Figure 2B*). There was no statistically significant difference of OS between tumors with and without *XRCC1* expression in the patients received platinum-based adjuvant chemotherapy (P=0.326, *Figure 2C*), as well as the patients that only received surgery (P=0.414, *Figure 2D*). However, after stratification by *XRCC1* expression, this survival benefit was only found among the patients without *XRCC1* expression (P=0.049, *Figure 2E*), and it was not found among the patients with positive *XRCC1* expression (P=0.327, *Figure 2F*).

There was no significant association between the expression of *XRCC1* and age, gender, tumor size, differentiation, T category, N category, Vascular or nerves invasion, stage and adjuvant chemotherapy. Tumor size, T category, N category, vascular or nerves invasion, and adjuvant chemotherapy were significant predictors for OS according to multivariate analysis (*Table 2*). Age, gender, tumor differentiation and *XRCC1* expression were not significant prognostic predictors in this study.

Discussion

One of the most challenging problems in oncology is how to select the right candidates for treatment with good response. Even in patients with similar clinical or pathological features, their survival outcomes were quite different. Thus discovery of new biomarkers predicting better response and avoiding unnecessary toxicity in adjuvant chemotherapy is urgently needed. Two previous studies have suggested that gastric cancer patients with low expression of either *BRCA1* (9) or *ERCC1* (10) could benefit more from platinum-based chemotherapy, whereas the expression of *ERCC1* or *BRCA1* contributed to a significantly prolonged OS, respectively. Since *XRCC1*,

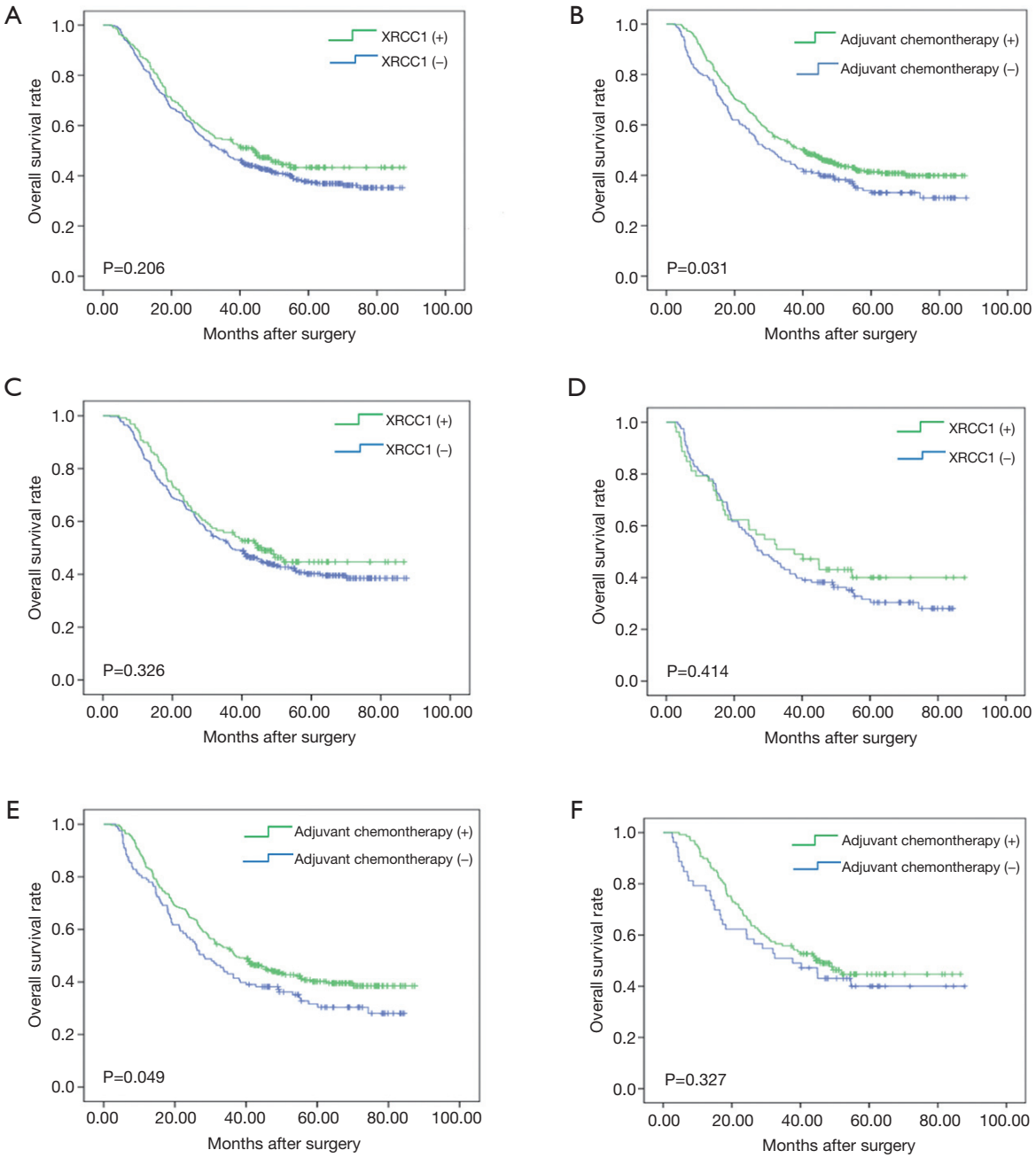

Figure 2 Overall cumulative survival rates of patients according to *XRCC1* expression. (A) OS between tumors with and without *XRCC1* expression (P=0.206); (B) OS between patients with and without platinum-based adjuvant chemotherapy (P=0.031); (C) OS between tumors with and without *XRCC1* expression in the patients received platinum-based adjuvant chemotherapy (P=0.326); (D) OS between tumors with and without *XRCC1* expression in the patients that did not received platinum-based adjuvant chemotherapy (P=0.414); (E) OS of patients with negative *XRCC1* expression received platinum-based adjuvant chemotherapy or not (P=0.049); (F) OS of patients with positive *XRCC1* expression received platinum-based adjuvant chemotherapy or not (P=0.327).

Table 2 Predictors from Cox proportional hazard model

Factors	RR	95% CI	P
Age	1.059	0.859–1.307	0.590
Gender	0.946	0.748–1.196	0.641
Tumor size	1.381	1.116–1.709	0.003
Differentiation	0.909	0.679–1.218	0.523
T category	2.847	1.755–4.620	0.000
N category	1.602	1.447–1.773	0.000
Vascular or nerve invasion	1.366	1.087–1.718	0.008
Adjuvant chemotherapy	0.620	0.492–0.781	0.000
XRCC1	0.862	0.683–1.088	0.347

XRCC1, X-ray cross-complementing gene 1.

BRCA1 and *ERCC1* are all DNA repair genes, we considered that the expression of *XRCC1* could reflect the cell's internal ability to repair DNA damage to some extent and *XRCC1* might have similar prognostic significance in gastric cancer (11). In a recent study, Wang *et al*. (12) showed that *XRCC1* protein levels were significantly down-regulated in gastric cancer lesions compared with normal tissues, and low expression of *XRCC1* was significantly associated with unfavorable clinical and pathological parameters and decreased OS. Similar phenomenon was also found in pancreatic cancer (13). However, in our study, there was no significant difference between patients with *XRCC1*-positive expression and patients with *XRCC1*-negative expression in OS (P=0.206). And *XRCC1* might not be a good prognostic predictor according to the Cox Proportional hazard model. In our view, this consequence may partly attribute to the polymorphism of *XRCC1*, since an altered DNA repair activity has been suggested to be associated with the *XRCC1* polymorphism. On the other hand, human cells have evolved a set of complex DNA repair systems and the multiple effects may cause the function of *XRCC1* to be less obvious. Besides, the prognostic power of *XRCC1* might be hampered by the sample size and retrospective nature of this study.

Molecular epidemiologic studies indicate that single nucleotide polymorphisms (SNP) of *XRCC1* were associated with the risk of various cancers including gastric cancer as well as being predictive for chemotherapy outcomes (14-16). The current studies of *XRCC1* mainly focused on the relationship between gene polymorphisms and cancer susceptibility. Several studies have reported the association of *XRCC1*-399 with the risk in non-small-cell lung cancer (NSCLC) (17), colorectal cancer (18), gastric cancer (19) and prostate cancer (20). Some previous studies also showed that the polymorphism of XRCC1 could influence the effect of the platinum agents by altering the DNA repair capacity. However, the relationship between the expression of *XRCC1* and the sensitivity to platinum-based chemotherapy in gastric cancer was rarely reported. In our analysis, patients in *XRCC1* IHC-negative could benefit from platinum-based adjuvant chemotherapy in a certain degree compared with which in XRCC1 IHC-positive subgroup (P=0.049). This outcome supports the notion that *XRCC1* negative expression sensitized cancer to platinum-based adjuvant chemotherapy. Some recent reports also showed that *XRCC1* plays an important part in repairing cisplatin adducts through DNA BER pathway (21), and *XRCC1* negative expression would sensitize cancer to cisplatin or oxaliplatin as a result of the reduced BER capacity (22).

In conclusion, our study suggested that the patients with *XRCC1*-negative expression benefited more from platinum-based adjuvant chemotherapy. Detecting the expression of *XRCC1* in gastric cancer tissues may provide clinical guidance in choosing the right candidate for adjuvant chemotherapy. However, further large-scale studies are called to find out the exact mechanisms.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
2. GASTRIC (Global Advanced/Adjuvant Stomach Tumor Research International Collaboration) Group, Paoletti X, Oba K, et al. Benefit of adjuvant chemotherapy for resectable gastric cancer: a meta-analysis. JAMA 2010;303:1729-37.
3. Van Cutsem E, Moiseyenko VM, Tjulandin S, et al. Phase

III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. J Clin Oncol 2006;24:4991-7.
4. Borst P, Rottenberg S, Jonkers J. How do real tumors become resistant to cisplatin? Cell Cycle 2008;7:1353-9.
5. Au WW, Salama SA, Sierra-Torres CH. Functional characterization of polymorphisms in DNA repair genes using cytogenetic challenge assays. Environ Health Perspect 2003;111:1843-50.
6. Whitehouse CJ, Taylor RM, Thistlethwaite A, et al. XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. Cell 2001;104:107-17.
7. Xu J, Ma J, Zong HT, et al. Pharmacogenetic role of XRCC1 polymorphisms on the clinical outcome of gastric cancer patients with platinum-based chemotherapy: a systematic review and meta-analysis. Genet Mol Res 2014;13:1438-46.
8. Vilmar A, Garcia-Foncillas J, Huarriz M, et al. RT-PCR versus immunohistochemistry for correlation and quantification of ERCC1, BRCA1, TUBB3 and RRM1 in NSCLC. Lung Cancer 2012;75:306-12.
9. Chen W, Wang J, Li X, et al. Prognostic significance of BRCA1 expression in gastric cancer. Med Oncol 2013;30:423.
10. Ozkan M, Akbudak IH, Deniz K, et al. Prognostic value of excision repair cross-complementing gene 1 expression for cisplatin-based chemotherapy in advanced gastric cancer. Asian Pac J Cancer Prev 2010;11:181-5.
11. Lu Z, Luo T, Nie M, et al. Genetic polymorphisms of XRCC1 gene and susceptibility to gastric cancer in Chinese Han population. Biomarkers 2013;18:542-6.
12. Wang S, Wu X, Chen Y, et al. Prognostic and predictive role of JWA and XRCC1 expressions in gastric cancer. Clin Cancer Res 2012;18:2987-96.
13. Crnogorac-Jurcevic T, Efthimiou E, Nielsen T, et al. Expression profiling of microdissected pancreatic adenocarcinomas. Oncogene 2002;21:4587-94.
14. Ju H, Lim B, Kim M, et al. A regulatory polymorphism at position -309 in PTPRCAP is associated with susceptibility to diffuse-type gastric cancer and gene expression. Neoplasia 2009;11:1340-7.
15. Ding WJ, Fang JY, Chen XY, et al. The expression and clinical significance of DNA methyltransferase proteins in human gastric cancer. Dig Dis Sci 2008;53:2083-9.
16. Motoya S, Kitamura K, Matsuda A, et al. Interaction between CD45-AP and protein-tyrosine kinases involved in T cell receptor signaling. J Biol Chem 1999;274:1407-14.
17. Kiyohara C, Takayama K, Nakanishi Y. Association of genetic polymorphisms in the base excision repair pathway with lung cancer risk: a meta-analysis. Lung Cancer 2006;54:267-83.
18. Stern MC, Siegmund KD, Conti DV, et al. XRCC1, XRCC3, and XPD polymorphisms as modifiers of the effect of smoking and alcohol on colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev 2006;15:2384-90.
19. Huang WY, Chow WH, Rothman N, et al. Selected DNA repair polymorphisms and gastric cancer in Poland. Carcinogenesis 2005;26:1354-9.
20. Hirata H, Hinoda Y, Tanaka Y, et al. Polymorphisms of DNA repair genes are risk factors for prostate cancer. Eur J Cancer 2007;43:231-7.
21. Weaver DA, Crawford EL, Warner KA, et al. ABCC5, ERCC2, XPA and XRCC1 transcript abundance levels correlate with cisplatin chemoresistance in non-small cell lung cancer cell lines. Mol Cancer 2005;4:18.
22. Zhang R, Niu Y, Zhou Y. Increase the cisplatin cytotoxicity and cisplatin-induced DNA damage in HepG2 cells by XRCC1 abrogation related mechanisms. Toxicol Lett 2010;192:108-14.

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