

Original Article

Genetic variability of DNA repair mechanisms influences chemotherapy outcome of gastric cancer

HaiYu^{1,2}, Xinghua Wu³, Yonggang Zhang², Zhihong Jin⁴, Guoxin Li¹, Haiping Zhao⁴

¹Department of General Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, China; ²Department of General Surgery, Affiliated Renmin Hospital of Inner Mongolia Medical University, Huhhot, China; ³Department of Feature Section, The Second Affiliated Hospital of Inner Mongolia Medical University, Huhhot, China; ⁴Department of Feature section, The Second Affiliated Hospital of Inner Mongolia Medical University, Huhhot, China

Received January 26, 2015; Accepted March 23, 2015; Epub April 1, 2015; Published April 15, 2015

Abstract: Genetic variability of DNA repair mechanisms influences chemotherapy treatment outcome of gastric cancer. We conducted a cohort study to investigate the role of ERCC1-ERCC2 gene polymorphisms in the chemotherapy response and clinic outcome of gastric cancer. Between March 2011 and March 2013, 228 gastric patients who were newly diagnosed with histopathology were enrolled in our study. Genotypes of ERCC1 rs11615, rs3212986, rs2298881 and ERCC2 rs3212986 were conducted by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. We found that individuals carrying TT genotype of ERCC1 rs11615 and CC genotype of ERCC1 rs2298881 were associated with better response to chemotherapy and longer survival time of gastric cancer. Moreover, individuals with AA genotype of ERCC2 rs1799793 were correlated with shorter survival of gastric cancer. In conclusion, ERCC1 rs11615, rs2298881 and ERCC2 rs1799793 polymorphism play an important role in the treatment outcome of gastric cancer.

Keywords: ERCC1, ERCC2, polymorphism, response to chemotherapy, overall survival, gastric cancer

Introduction

Gastric cancer is one of the common types of malignancies, ranking the fourth common malignancy and second leading cause of cancer deaths worldwide, and it is estimated that 989,600 new cases and 738,000 deaths occurred every year [1]. In China, it is approximately 221,478 people killed by gastric cancer in 2012 [2].

Postoperative neoadjuvant chemotherapy shows its effectiveness in improving survival time of patients with advanced gastric cancer. The 5-fluorouracil (5-FU), Platinum-based chemotherapy and the folinic acid/5-FU/oxaliplatin (FOLFOX) combination has been regarded as main treatment agent for gastric cancer. However, patients showed different response to chemotherapies, hence showed different clinic outcomes. Previous studies found that genetic variations play roles in response to chemotherapy and development of gastric cancer [3, 4].

DNA repair system could repair the damaged DNA induce by endogenous and/or exogenous factors, including therapeutic agents. Therefore, DNA repair mechanisms influence the development of gastric cancer and treatment effect [5, 6]. Nucleotide excision repair (NER) pathway is one of DNA repair mechanism pathways which maintains genomic integrity through removing DNA lesions or correcting the abnormal DNA structures, such as replication errors, inter-strand adducts, oxidative DNA damage, and so on [7, 8]. The excision repair cross-complimentary group 1 (ERCC1) and excision repair cross-complimentary group 2 (ERCC2) are two important endonucleases in the NER pathway, and both were located at chromosome 19. Previous studies have reported that ERCC1 and ERCC2 genetic variation can be a predictive marker for prognostic and response to chemotherapy for patients with non-small cell lung cancer (NSCLC) [9], colorectal cancer [10] and osteosarcoma [11]. However, only few studies have reported the effect of ERCC1 and ERCC2 expression on

Table 1. Demographic and clinical characteristics of included patients

Characteristics	Cases (N)	Percent (%)
Gender		
Male	137	60.09
Female	91	39.91
Age		
<60	106	46.49
≥60	122	53.51
TMN stage		
I or II	81	35.53
III or IV	147	64.47
Histological type		
Intestinal	104	45.61
Diffuse	88	38.60
Other	36	15.79
Histological grade		
moderate	77	33.77
Poor	139	60.96
Unknown	12	5.26
Response to chemotherapy		
Good	139	60.96
Poor	89	39.04

clinical outcome of patients with gastric cancer treated with FOLFOX chemotherapy [12]. Therefore, we conducted a cohort study to investigate the association between ERCC1-ERCC2 gene polymorphisms and chemotherapy response, as well as clinic outcome of gastric cancer.

Patients and methods

Patients

Between March 2011 and March 2013, 228 gastric patients who were newly diagnosed with histopathology were enrolled in our study from the Affiliated Renmin Hospital of Inner Mongolia Medical University. Those patients who had recurrent tumors, pregnant, breast feeding, had organ failure and been treated elsewhere before were excluded. This study was approved by the ethic committee of our hospital. All the patients included were followed up until December 2014, with follow-up time ranged from 11 to 40 months (median: 28.6 months). All patients were followed up by telephone or attending clinics every month until death or the end of study.

Assessment of treatment outcome

All the patients received FOLFOX chemotherapy until unacceptable toxicity or progressive disease presented. Demographic and clinical information of included subjects were obtained from the medical records. Tumor response was evaluated by World Health Organization (WHO) criteria [13], patients with complete response (CR) or partial remission (PR) were regarded as good responder, and patients with stable disease (SD) and progressive disease (PD) were considered as poor responder. The overall survival (OS) was calculated from the date of the first time of treatment to the date of death or last contact follow-up.

Blood samples and genotyping

Each patient agreed to provide 5 ml peripheral blood after enrolling into our study. The blood samples were kept in -70°C before use. The potential gene SNPs were selected from the National Center for Biotechnology Information (NCBI) dbSNP database. Genomic DNA was isolated from blood sample using the QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Genotypes of ERCC1 rs11615, rs3212986, rs2298881, and ERCC2 rs3212986 and rs1799793 were conducted by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. Probes and primers for ERCC1 rs11615, rs3212986, rs2298881, and ERCC2 rs13181 and rs1799793 were designed by Sequenom Assay Design 3.1 software (Sequenom®) according to the manufacturer's instructions. The PCR cycling was done at 50°C for 2 min, 95°C for 10 min, 94°C for 20 s, and then 62°C for 60 s for 35 cycles. 5% of blood samples were randomly selected to maintain genotyping quality, and the results of repeated samples showed 100% concordant.

Statistical analysis

Continuous variables were shown as the mean ± standard deviation (SD) and analyzed by student t test. Categorical variables were expressed as number (N) and percentage (%) and analyzed by χ^2 -test. Conditional logistic regression analysis were taken to calculate the association between ERCC1 rs11615, rs3212986, rs2298881, ERCC2 rs13181 and rs1799793 polymorphisms and response to chemothera-

DNA repair genes and gastric cancer

Table 2. Association between DNA repaired gene polymorphisms and response to chemotherapy in gastric cancer patients

Genotypes	Total frequencies N=228	Percent (%)	Good response N=139	Percent (%)	Poor response N=89	Percent (%)	Adjusted OR (95% CI) ¹	P value
ERCC1 rs11615								
CC	102	44.74	55	39.57	47	52.81	1.00 (ref)	--
CT	88	38.60	57	41.01	31	34.83	1.58 (0.90-2.84)	0.11
TT	38	16.67	27	19.42	11	12.36	2.11 (1.03-4.72)	0.04
ERCC1 rs3212986								
CC	116	50.88	67	48.20	49	55.06	1.00 (ref)	--
CA	79	34.65	49	35.25	30	33.71	1.15 (0.62-2.01)	0.52
AA	33	14.47	23	16.55	10	11.24	1.67 (0.71-3.80)	0.19
ERCC1 rs2298881								
AA	139	60.96	78	56.12	61	68.54	1.00 (ref)	--
AC	57	25.00	37	26.62	20	22.47	1.42 (0.74-2.71)	0.21
CC	32	14.04	24	17.27	8	8.99	2.35 (1.11-5.60)	0.03
ERCC2 rs13181								
TT	136	59.65	86	61.87	50	56.18	1.00 (ref)	--
GT	78	34.21	46	33.09	32	35.96	0.85 (0.48-1.49)	0.53
GG	14	6.14	7	5.04	7	7.87	0.57 (0.18-1.73)	0.32
ERCC2 rs1799793								
GG	121	53.07	79	56.83	42	47.19	1.00 (ref)	--
AG	92	40.35	54	38.85	38	42.70	0.77 (0.43-1.34)	0.32
AA	15	6.58	6	4.32	9	10.11	0.37 (0.12-1.03)	0.06

¹Adjusted for age, gender, tumor histology and TMN stage.

py, and the results were expressed by Odds ratio (ORs) and 95% confidence interval (CI). The Cox proportional hazards model was taken to assess the association between four gene polymorphisms and prognostic by describing hazard ratio (HR) and 95% CI. The Kaplan-Meier method was used to estimate survival probabilities. All analyses were conducted using the SPSS version 17.0 statistical software. Two-tailed *P* values set at <0.05 used and regarded as statistically difference.

Results

Patients and clinical characteristics

The distributions of selected characteristics of included subjects were presented in **Table 1**. Of 228 gastric cancer patients, 137 (60.09%) were male and 91 (39.91%) were female, and the mean age of subjects was 55.7±13.8 years old (ranging from 41 to 79 years old). 81 (35.35%) patients presented TMN stage I or II and 147 (64.47%) with stage III or IV. The distributions of histological type were 104 (46.61%)

patients with intestinal carcinoma, 88 (38.60%) with diffuse carcinoma and 36 (15.79%) with other types. The distribution of histological types showed that 77 (33.77%) patients were moderate cases, 139 (60.96%) were poor cases and 12 (5.26%) were unknown. For response to chemotherapy, 139 (60.96%) patients showed good response to chemotherapy and 89 (39.04%) showed poor response to chemotherapy.

Association between DNA repaired gene polymorphisms and response to chemotherapy

139 gastric cancer patients showed good response to FOLFOX chemotherapy, with a response rate of 60.69%. In conditional logistic regression model, after adjusting for potential confounding factors, patients carrying TT genotype of ERCC1 rs11615 showed a significant higher respond rate than CC genotype, and the adjusted OR (95% CI) was 2.11 (1.03-4.72) (**Table 2**). Subjects carrying CC genotype of ERCC1 rs2298881 showed a higher respond rate than AA genotype, the adjusted OR (95%

DNA repair genes and gastric cancer

Table 3. Association between gene polymorphisms and overall survival in gastric cancer patients

Genotypes	Total frequencies N=228	Percent (%)	Death N=75	Percent (%)	Median survival time (months)	Log-rank P value	Adjusted HR (95% CI) ¹	P value
ERCC1 rs11615								
CC	102	44.74	40	53.33	24.3	0.003	0.69 (0.38-1.27)	0.16
CT	88	38.60	27	36.00	28.9			
TT	38	16.67	8	10.67	31.4			
ERCC1 rs3212986								
CC	116	50.88	44	58.67	26.3	0.224	0.69 (0.38-1.30)	0.23
CA	79	34.65	23	30.67	28.1			
AA	33	14.47	8	10.67	29.2			
ERCC1 rs2298881								
AA	139	60.96	53	70.67	23.2	0.045	0.70 (0.37-1.41)	0.25
AC	57	25.00	17	22.67	27.6			
CC	32	14.04	5	6.67	32.7			
ERCC2 rs13181								
TT	136	59.65	41	54.67	29.7	0.315	1.31 (0.73-2.36)	0.39
GT	78	34.21	28	37.33	28.5			
GG	14	6.14	6	8.00	27.8			
ERCC2 Rs1799793								
GG	121	53.07	33	44.00	33.4	0.016	1.52 (0.75-2.86)	0.20
AG	92	40.35	33	44.00	28.1			
AA	15	6.58	9	12.00	25.5			

¹Adjusted for age, gender, tumor histology and TMN stage.

CI) was 2.35 (1.11-5.60). However, the ERCC1 rs3212986, ERCC2 rs13181 and ERCC2 rs1799793 polymorphisms were not correlated with response to FOLFOX chemotherapy (**Table 2**).

Association between DNA repaired gene polymorphisms and overall survival

During the follow-up time, 77 patients died from gastric cancer, and the survival rate is 66.23%. By the Cox proportional hazards analysis, after adjusting for potential confounding factors, we found that individuals carrying TT genotype of ERCC1 rs11615 showed a better prognosis when compare with CC genotype, and the HR (95% CI) was 0.40 (0.16-0.89) (**Table 3**). Subjects carrying CC genotype of ERCC1 rs2298881 were associated with decreased risk of death from gastric cancer (HR: 0.33, 95% CI: 0.12-0.80), when compared with AA genotype. Moreover, we found a significant increased risk of death from gastric cancer among patients with ERCC2 rs1799793 AA genotype compared to GG genotype, and HR (95% CI) was 3.86 (1.36-12.23). By Kaplan-Meier method, individuals with CC genotype of ERCC1 rs11615, CC genotype of ERCC1 rs22-

98881 and GG genotype of ERCC2 rs1799793 had a longer overall survival time of gastric cancer (**Figures 1** and **2**). However, we found no association between ERCC1 rs3212986 and ERCC2 rs13181 polymorphisms and overall survival in gastric patients (**Table 3**).

Discussion

In the present study, we investigated the association between polymorphisms of ERCC1 and ERCC2 in NER pathways and treatment response or overall survival in gastric cancer patients treated with FOLFOX chemotherapy. We observed that TT genotype of ERCC1 rs11615 and CC genotype of ERCC1 rs2298881 were associated with better response to chemotherapy when compared with TT genotype and AA genotype respectively, and these genotypes could also influence the OS of gastric cancer patients. Moreover, patients with AA genotype of ERCC2 rs1799793 would suffer high risk of death when compared with GG genotype.

It is well known that the DNA repair machinery plays an important role in determining the efficiency of DNA crosslinking agents [14], the NER

DNA repair genes and gastric cancer

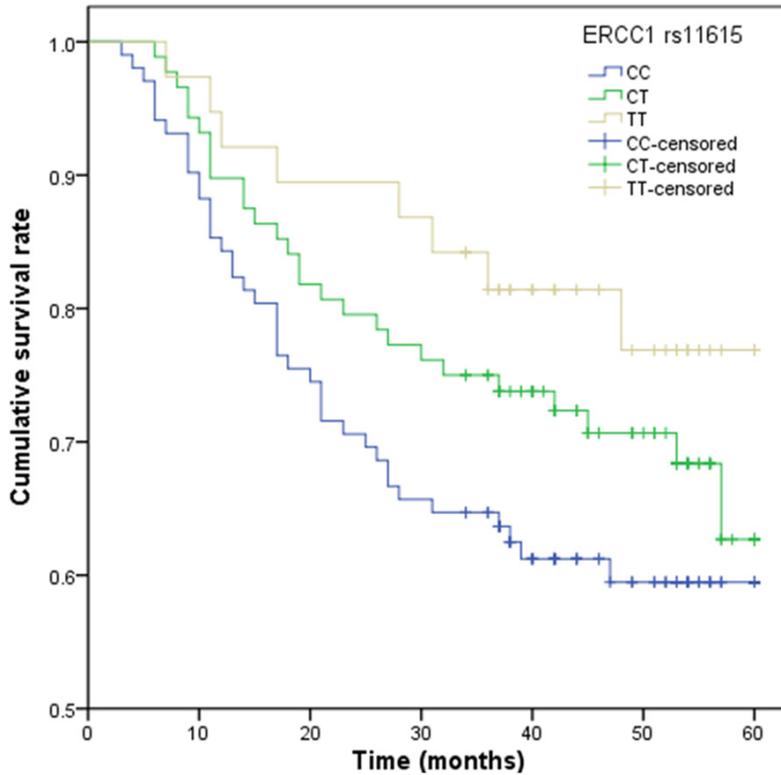


Figure 1. Kaplan-Meier estimates of overall survival of gastric cancer with ERCC1 rs11615.

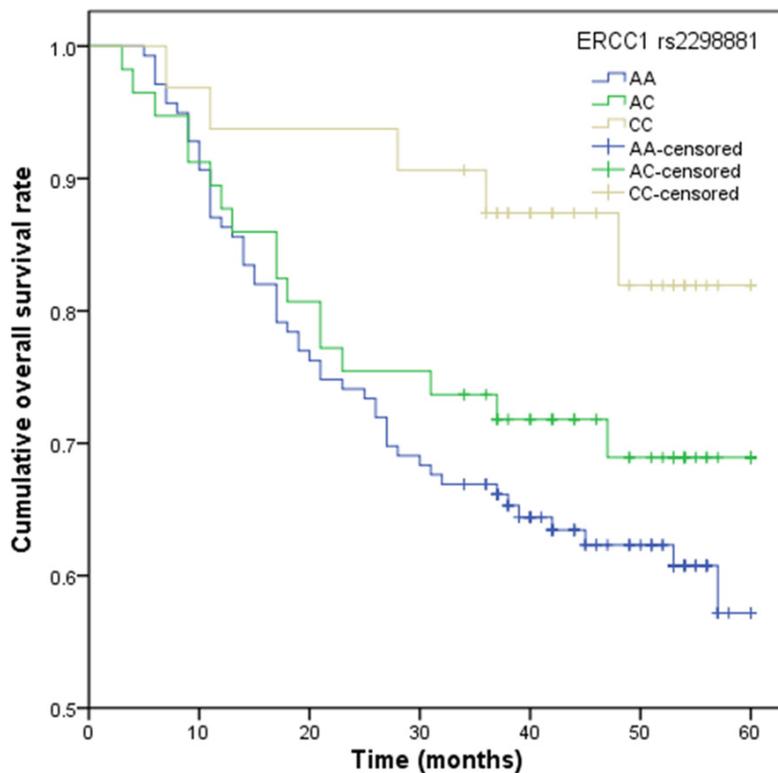


Figure 2. Kaplan-Meier estimates of overall survival of gastric cancer with ERCC1 rs2298881.

process is one of critical DNA repair mechanism which could maintain genomic integrity and repair many forms of DNA damage both in tumor and normal cells, including platinum DNA adducts [15], thus may influence susceptibility of tumor, response to chemotherapy and clinic outcomes. ERCC1 and ERCC2 gene polymorphisms are two important rate-limiting enzymes which been reported as potential predictor for clinical outcomes of various cancers, such as non-small cell lung cancer [16], breast cancer [17], ovarian cancer [18] and gastric cancer [19].

ERCC1 have an important role in DNA damage recognition. Previous studies on the association between ERCC1 rs11615, ERCC1 rs2298881 and response to chemotherapy or OS were controversial. Lu et al. reported that ERCC1 rs11615 and ERCC1 rs2298881 are associated with response to chemotherapy and prognosis of gastric cancer patients, but in that study, carriers of the ERCC1 rs11615 TT and T allele had a marginally significantly higher response rate to chemotherapy. However, several previous studies indicated that ERCC1 rs11615 CC had a higher response rate to chemotherapy not only in gastric cancer patients but also in non-small cell cancer, ovarian cancer and colorectal cancer patients [4, 20]. Besides, a few studies founded that ERCC1 rs11615 and rs2298881 polymorphism was not associated with OS of gastric cancer

[4, 17]. Our study also indicated that there was significant association between the ERCC1 rs11615, rs2298881 polymorphism and response to chemotherapy and prognosis of gastric cancer. The differences of the results may be caused by the sample size, ethnic variation, and source of cases.

ERCC2 is also called xeroderma pigmentosum group D (XPD) gene, which was one of human transcriptional initiation factor (TFIIH) with ATP-dependent helicase activity. The association between ERCC2 rs13181 and rs1799793 and clinic outcome have also been investigated in previous studies. Rzeszowska-Wolny J, et al. founded that ERCC2 rs13181 G allele and rs1799793 A allele were associated with lower NER capacity when compare with common genotypes [21]. Li et al. conducted a study with 360 gastric cancer patients, and found that ERCC2 rs1799793 AA genotype was associated with poor OS in gastric cancer patients [17], which is consistent with our findings.

There were several limitations should be considered in our study. First, selection bias may exist in our study, since cases were selected from one hospital. Second, although common functional SNPs were included in our analysis, but some other genetic variability of DNA repair mechanisms could also influence the response to chemotherapeutics and prognosis, which may have been missed, and need to be included in further study. Third, the sample size of our study is relatively small, which may reduce the statistical power and lead to statistical bias. Therefore, further multicenter, large sample and multi ethnic studies are greatly needed to verify our results.

In conclusion, this study suggest that ERCC1 rs11615, rs2298881 and ERCC2 rs1799793 polymorphism in the DNA repair pathways can be used as a predictor of response to FOLFOX chemotherapy and clinical outcome of gastric cancer. In the future, these SNPs could contribute to identification of patients, less likely to achieve better response to FOLFOX chemotherapy or better prognosis. Further multicenter studies involving various populations are essential to confirm our findings.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Guoxin Li, Department of General Surgery, Nanfang Hospital, Southern Medical University, Nanfang Hospital, Southern Medical University, NO. 1838, North of Guangzhou Avenue, Guangzhou, China. Tel: +86-13664785587; Fax: +86-13664785587; E-mail: zhangnan_nan61@163.com

References

- [1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; 61: 69-90.
- [2] International Agency for Research on Cancer. *Globocan 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012*. IARC; 2012.
- [3] Li Y, Liu Z, Liu H, Wang LE, Tan D, Ajani JA, Wei QY. ERCC1 and ERCC2 variants predict survival in gastric cancer patients. *PLoS One* 2013; 8: e71994.
- [4] Lu ZM, Luo TH, Nie MM, Fang GE, Ma LY, Xue XC, Wei G, Ke CW, Bi JW. Influence of ERCC1 and ERCC4 polymorphisms on response to prognosis in gastric cancer treated with FOLFOX-based chemotherapy. *Tumour Biol* 2014; 35: 2941-8.
- [5] Wang LE, Yin M, Dong Q, Stewart DJ, Merriman KW, Amos CI, Spitz MR, Wei Q. DNA repair capacity in peripheral lymphocytes predicts survival of patients with non-small-cell lung cancer treated with first-line platinum-based chemotherapy. *J Clin Oncol* 2011; 29: 4121-8.
- [6] Jagdis A, Phan T, Klimowicz AC, Laskin JJ, Lau HY, Petrillo SK, Siever JE, Thomson TA, Magliocco AM, Hao D. Assessment of ERCC1 and XPF protein expression using quantitative immunohistochemistry in nasopharyngeal carcinoma patients undergoing curative intent treatment. *Int J Radiat Oncol Biol Phys* 2013; 85: 1340-5.
- [7] Wood RD, Mitchell M, Sgouros J, Lindahl T. Human DNA repair genes. *Science* 2001; 229: 1284-9.
- [8] McNeil EM and Melton DW. DNA repair endonuclease ERCC1-XPF as a novel therapeutic target to overcome chemoresistance in cancer therapy. *Nucleic Acids Res* 2012; 40: 9990-10004.
- [9] Vilmar A and Sørensen JB. Excision repair cross-complementation group 1 (ERCC1) in platinum-based treatment of non-small cell lung cancer with special emphasis on carboplatin: a review of current literature. *Lung Cancer* 2009; 64: 131-9.
- [10] Gan Y, Li XR, Chen DJ, Wu JH. Association between polymorphisms of XRCC1 Arg399Gln and XPD Lys751Gln genes and prognosis of colorectal cancer in a Chinese population. *Asian Pac J Cancer Prev* 2012; 13: 5721-4.

DNA repair genes and gastric cancer

- [11] Biason P, Hattinger CM, Innocenti F, Talamini R, Alberghini M, Scotlandi K, Zanusso C, Serra M, Toffoli G. Nucleotide excision repair gene variants and association with survival in osteosarcoma patients treated with neoadjuvant chemotherapy. *Pharmacogenomics J* 2012; 12: 476-83.
- [12] Yin M, Yan J, Martinez-Balibrea E, Graziano F, Lenz HJ, Kim HJ, Robert J, Im SA, Wang WS, Etienne-Grimaldi MC, Wei Q. ERCC1 and ERCC2 polymorphisms predict clinical outcomes of oxaliplatin-based chemotherapies in gastric and colorectal cancer: a systemic review and meta-analysis. *Clin Cancer Res* 2011; 17: 1632-40.
- [13] Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981; 47: 207-14.
- [14] Mohseni-Meybodi A, Mozdarani H, Mozdarani S. DNA damage and repair of leukocytes from Fanconi anaemia patients, carriers and healthy individuals as measured by the alkaline comet assay. *Mutagenesis* 2009; 24: 67-73.
- [15] Liu T, Ghosal G, Yuan J, Chen J, Huang J. FAN1 acts with FANCD2 to promote DNA inter-strand cross-link repair. *Science* 2010; 329: 693-6.
- [16] Xu TP, Shen H, Liu LX, Shu YQ. Association of ERCC1-C118T and C8092A polymorphisms with lung cancer risk and survival of advanced-stage non-small cell lung cancer patients receiving platinum-based chemotherapy: a pooled analysis based on 39 reports. *Gene* 2013; 526: 265-74.
- [17] Chu H, Gu D, Xu M, Xu Z, Gong Y, Gong W, Tang Y, Zhou J, Tong N, Zhang Z, Chen J, Wang M. A genetic variant in ERCC2 is associated with gastric cancer prognosis in a Chinese population. *Mutagenesis* 2013; 28: 441-6.
- [18] Gerhard R, Carvalho A, Carneiro V, Bento RS, Uemura G, Gomes M, Albergaria A, Schmitt F. Clinicopathological significance of ERCC1 expression in breast cancer. *Pathol Res Pract* 2013; 209: 331-6.
- [19] Qiao Y, Spitz MR, Shen H, Guo Z, Shete S, Hedayati M, Grossman L, Mohrenweiser H, Wei Q. Modulation of repair of ultraviolet damage in the host-cell reactivation assay by polymorphic XPC and XPD/ERCC2 genotypes. *Carcinogenesis* 2002; 23: 295-9.
- [20] Rumiato E, Cavallin F, Boldrin E, Cagol M, Alfieri R, Basso D, Castoro C, Ancona E, Amadori A, Ruol A, Saggioro D. ERCC1 C8092A (rs321-2986) polymorphism as a predictive marker in esophageal cancer patients treated with cisplatin/5-FU-based neoadjuvant therapy. *Pharmacogenet Genom* 2013; 23: 597-604.
- [21] Rzeszowska-Wolny J, Polanska J, Pietrowska M, Palyvoda O, Jaworska J, Butkiewicz D, Hancock R. Influence of polymorphisms in DNA repair genes XPD, XRCC1 and MGMT on DNA damage induced by gamma radiation and its repair in lymphocytes in vitro. *Radiat Res* 2005; 164: 132-40.