RESEARCH ARTICLE

Implication of Polymorphisms in DNA Repair Genes in Prognosis of Hepatocellular Carcinoma

Ai-Min Yue*, Zhen-Bin Xie, Shu-Ping Guo, Qi-Dong Wei, Xiao-Wei Yang

Abstract

XRCC1 genetic polymorphisms could be associated with increased risk of various cancer, including hepatocellular carcinoma (HCC), the fifth most common cancer. We here conducted a study to explore the role of selective SNPs of the XRCC1 and XPD genes in the prognosis of HCC. A total of 231 cases were collected, and genotyping of XRCC1 Arg194Trp, XRCC1 Arg399Gln, XPD Lys751Gln and XPD Asp312Asn was performed by duplex polymerase-chain-reaction with the confronting-two-pair primer method. Our findings indicated XRCC1 399Gln/Gln genotype was associated with a significant difference in the median survival time compared with patients carrying Arg/Trp and Arg/Arg genotypes, and individuals with XPD 751 Gln/ Gln genotype had a significantly greater survival time than patients carrying Lys/Lys and Lys/Gln genotypes. The Cox's regression analysis showed individuals carrying XRCC1 399Trp/Trp genotype had 0.55 fold risk of death from HCC than Arg/Arg genotype. Similarly, XPD 751Gln/Gln had a strong decreasein comparison to XPD Lys/Lys carriers with an HR of 0.34. These results suggest that polymorphisms in XRCC1 and XPD may have functional significance in the prognosis of HCC.

Keywords: XRCC1 - XPD - polymorphism - hepatocellular carcinoma - prognosis

Asian Pacific J Cancer Prev, **14 (1)**, 355-358

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common primary cancer in men (523 000 cases, 7.9% of the total) and the seventh in women (226 000 cases, 6.5% of the total), and most of the burden is in developing countries (IARC, 2008). It is well known that chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) are the main risk factors for the pathogenesis of HCC. It is estimated 30% to 50% of the HBV related deaths are attributable to HCC, however, only less than 10% of HBV and HCV infected individuals developed HCC in their later life (Lavanchy, 2004; Bowen et al., 2005). It could be hypothesis that other factors might play a role in the development of HCC, such as environment and genetic factors. It is reported that individuals with HCC presented with DNA damaged by hepatitis virus, and this is a major underlying risk factors of HCC (Bowen et al., 2005).

It is reported that majority of HCC cases result from DNA damage caused by hepatitis viruses, which is the main potential risk factors to the development of HCC (McKillop et al., 2006). Damage due to endogenuous or exogenous exposure may be repaired by enzymes encoded by the DNA repair pathways, such as base excision repair, nucleotide excision repair, mismatch repair, homologous recombination repair and non-homologous end joining. The nucleotide excision repair (NER) is the main DNA repair system, and constitutes the main defense against lesions generated by ionizing radiation and strong alkylating agents as well as lesions formed by endogenous DNA-damaging agents like viruses (Smith et al., 2003). The NER is reported to association with the development of several cancers (Han et al., 2012; Mandal et al., 2012). Defective DNA repair can lead to the accumulation of mutations and microsatellite instability in the genome, increasing the chance of malignant transformation (Hussain et al., 2007). Genetic variation may alter the function of DNA repair proteins, influence the development and clinical outcome of HCC. The role of XRCC1 and XPD polymorphisms has been reported to be associated with function of chemotherapy of several cancers, and the chemotherapy could induce the DNA damage of cancer cells, while the low activity of XRCC1 and XPD polymorphisms might influence the therapy outcome. There is still no study on the role of these two gene polymorphisms on the HCC prognosis in Chinese population. Therefore, we conducted a study to explore the role of selective SNPs of the two gene polymorphisms in the prognosis of HCC.

Materials and Methods

Subjects

A total of 242 cases with HCC were histological confirmed in Xinxiang Central Hospital between January 2008 and Dec. 2011. Finally, 231 patients finished the

*Department of Surgical Oncology, Xinxiang Central Hospital, Xinxiang, China *For correspondence: hezhonglin_9h@163.com*

Table 1. PCR Primers of Selected SNPs

Single nucleotide polymorphism	Primer	Sequence
XRCC1 Arg194Trp (rs1799782)	1st-primer	5'-GCC AGG GCC CCT CCT TCA A-3
	2nd-primer	5'-TAC CCT CAG ACC CAC GAG T-3'
XRCC1 Arg399Gln (rs25487)	1st-primer	5'-TTG TGC TTT CTC TGT GTC CA-3'
	2nd-primer	5'-TCC TCC AGC CTT TTC TGA TA-3'
XPD Lys751Gln (rs13181)	1st-primer	5'-TTG TGC TTT CTC TGT GTC CA-3'
	2nd-primer	5'-CTA TCA TCT CCT GGC CCC C-3'
XPD Asp312Asn (rs1799793)	1st-primer	5'–CCCACCTGGCCAACCCCGTGCTGCCC-3'
	2nd-primer	5'–ACGAAGTGCTGCAGGGTGAGCCCCG-3'

follow-up in our study, with the participation rate of 95.5%. Case with secondary or recurrent tumors was excluded. All the patients were followed up until the end of August 2012.

The diagnosis of HCC was made by liver biopsy or the combination of increased alpha-fetoprotein (AFP of ≥200 ng/ml) and the typical vascular pattern on angiography or dynamic imaging. The clinical information, such as tumor differentiation, tumor size, child-pugh class and surgery types were collected from medical records with patients' consent. All the patients were followed up until the end of April. 2012. Tumor progression was defined as postoperative tumor recurrence and distant metastasis. The overall survival time was defined as the data of tumor section or the first local treatment to the date of death. The ethics committee of the Xinxiang Central Hospital reviewed and approved the study.

DNA collection and genotyping

A 2 ml sample of blood was collected from each subject. DNA was extracted from frozen white blood cells using the buffy-coat fractions with TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). Primers for polymerase chain reaction (PCR) amplification and single base extension (SBE) assays were designed by Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA) according to the manufacturer's instructions (Table1).

PCR was carried out in a reaction volume of 20 μl containing 50ng of genomic DNA, 200μM dNTP, 2.5 units of Taq DNA polymerase (Promega Corporation, WI, USA), and 200μM primers. The parameters of PCR reaction used was as follows: 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, annealing temperature reduced to 64°C for 30 seconds, and 72°C for 1 minute seconds. PCR product was electrophoresis on 1.0% agrose gel to testify PCR products. For quality control, genotyping was performed without knowledge of the case/control status of the subjects, and a random sample of 5% of cases and controls was genotyped again by different researchers. The reproducibility was 100%. For quality control, genotyping was performed without knowledge of the case/control status of the subjects, and a random sample of 5% of cases and controls was genotyped again by different researchers. The reproducibility was 100%.

Statistical analysis

The SPSS software package version 16.0 (SPSS Inc. Chicago, USA) was used for statistical analyses. Mean±standard deviation (SD) was used for continuous variables, and frequencies and percentages were used for categorical variables. The homozygous genotypes for these SNPs were used as reference. The Kaplan-Meier method was adopted to estimate survival curves, and the log-rank test was used to compare patients' survival time between genotype groups. Cox's proportional hazard model was used to assess the associations between these two gene polymorphisms and survival. Primary death from HCC was defined as the failure event, and the time of survival was the time between diagnosis and death. The cause of death was determined by specialists based on clinical documents from hospital or reports by patients' family members. If a patient died of causes other than HCC, he was censored at the date of death. P value was considered statistically significant which was less than or equal to 0.05.

Table 2. Comparison of the Selected Characteristics of HCC Cases

Characteristics		Case (N=231)	%
Age (mean±SD), years		50.9±9.6	
Gender	Male	154	66.5
	Female	77	33.5
HBsAg	+	86	37.4
	-	145	62.6
Anti-HCV	+	11	4.6
	-	220	95.4
Tumor differentiation	Well	84	36.5
	Moderate	76	32.8
	Poor	56	24.4
	Unknown	15	6.3
Child-Pugh class	A	142	61.3
	B	66	28.6
	C	23	10.1
Surgery	Yes	101	43.8
	No	130	56.2

Results

All the patients were followed up until the end of Dec. 2012. Among 242 patients, 11 patients were lost to follow-up due to migration, while the remaining 231 patients completed the study. The median follow-up time was 25.3 months (range: 2 to 53 months). A total of 195 patients (42.8%) died of HCC during the follow-up period. The demographic characteristics of subjects included and clinical features of HCC patients were shown in Table 2. The average age was 50.9±9.6 years in HCC cases, and was 51.2±9.1 years in controls. Most of the HCC patients had well differentiation. Almost 60% of the HCC patients were grade A of Child-Pugh class, and most of the HCC

Table 3. The Survival of HCC by Polymorphisms in XRCC1 and XPD Genes

Genotype	Case N=231	%	Death N=99	%	Mean survival time (months)	HR(95% CI)[1]
XRCC1 Arg194Trp (rs1799782)						
Arg/Arg	158	68.5	71	71.3	21.3±6.7	1.0(Ref.)
Arg/Trp	59	25.6	24	24.2	23.4±7.9	0.82(0.54-1.27)
Trp/Trp	14	5.9	4	4.5	26.3±5.5	0.63(0.32-1.58)
XRCC1 Arg399Gln (rs25487)						
Arg/Arg	118	50.9	56	56.6	20.5±7.1	1.0(Ref.)
Arg/Gln	80	34.5	33	33.1	23.6±7.5	0.83(0.54-1.21)
Gln/Gln	34	14.6	10	10.3	29.2±6.1	0.55(0.30-0.96)
XPD Lys751Gln (rs13181)						
Lys/Lys	112	48.5	54	54.7	21.4±6.5	1.0(Ref.)
Lys/Gln	100	43.2	42	42	21.8±8.0	0.83(0.57-1.18)
Gln/Gln	19	8.3	3	3.3	28.9±7.4	0.34(0.12-0.85)
XPD Asp312Asn (rs1799793)						
Asp/Asp	149	64.3	67	67.6	26.7±7.3	1.0(Ref.)
Asp/Asn	59	25.5	24	23.9	29.8±8.2	0.86(0.55-1.30)
Asn/ Asn	24	10.2	8	8.5	35.4±6.9	0.73(0.38-1.36)

[1]Adjusted for age, sex, tumor differentiation and Child-Pugh class

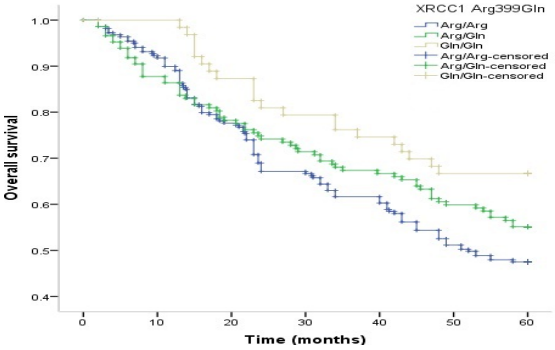

Figure 1. Kaplan-Meier Estimates of Overall Survival with XRCC1 Arg399Gln Polymorphism

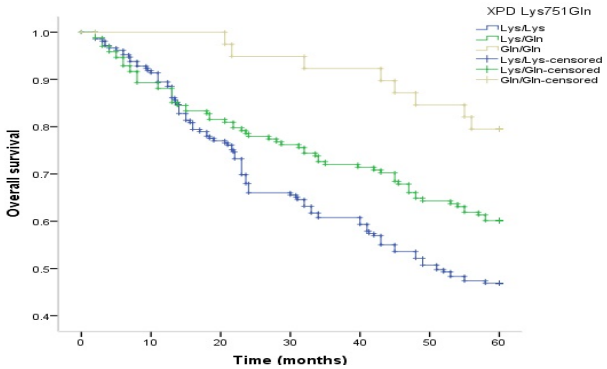

Figure 2. Kaplan-Meier Estimates of Overall Survival with XPD Lys751Gln Polymorphism

patients received surgery treatment.

XRCC1 399Gln/Gln genotype had a significant difference in the median survival time compared with patients carrying Arg/Trp and Arg/Arg genotypes (29.2±6.1 months vs 23.6±7.5 and 20.5±7.1 months) (Table 3, Figure 1). Individuals with XPD 751 Gln/Gln genotype had significant difference survival time than patients carrying Lys/Lys and Lys/Gln genotypes (28.9±7.4 months vs 21.8±8.0 and 20.5±7.1 months, figure 2). The Cox's regression analysis showed individuals carrying XRCC1 399Trp/Trp genotype had 0.55 fold risk of death from HCC than Arg/Arg genotype. Meanwhile, XPD 751Gln/Gln had a strong decreased risk of death from HCC in comparison to XPD Lys/Lys carriers (HR=0.34, 95% CI=0.12-0.85). However, XRCC1 Arg194Trp and XPD Asp312Asn did not show significant association with survival of HCC.

Discussion

The present study evaluated the effect of polymorphisms in XRCC1 Arg399Gln, and XPD Lys751Gln on the prognosis of HCC risk in Chinese population. To the best of our knowledge, this is the first study to describe the association of XRCC1 Arg399Gln and XPD Lys751Gln polymorphisms with the clinical outcomes of HCC. We demonstrated that XRCC1 399Gln/Gln and XPD 751Gln/Gln genotypes were associated with a reduction of death from HCC (adjusted HR: 0.55 for XRCC1 399Gln/Gln; 0.34 for XPD 751Gln/Gln). These results suggest that polymorphisms in XRCC1 and XPD may have functional significance in the overall survival of HCC patients.

Various DNA damage may be caused during the endogenous metabolic processes or by environmental carcinogens. If not repaired for these DNA damages, genetic instability, mutagenesis, cell death and genome integrity as well as induce carcinogenesis might be occurred. NER is the main DNA damage repair pathway for the small base lesions from oxidation and alkylation's damage. XRCC1 protein is an important protein in the multistep NER pathway, and consists of three functional domains that interact with different enzymes to initiate DNA repair of different stages and types (16). HBV is known to be the primary risk factor for the development of HCC by inducing either chromosomal instability or insertional mutations (Pang et al., 2006). Any alteration in the activity of XRCC1 may be associated with polymorphisms may lead to modification of the clinical behavior of HBsAg positive patients.

A previous study conducted in South Korea indicated that A allele of XRCC1 Gln399Arg might be associated with an unfavorable prognosis in patients with HCC by multivariate Cox regression analysis, which suggested the SNP of XRCC1 rs25487 was significantly associated with reduced overall survival of HCC patients (Jung et al., 2012). Another study conducted in Taiwan indicated the A allele of XRCC1 Gln399Arg was associated with a dose-dependent increased risk of early-onset HCC patients less than 50 years old (Yu et al., 2003), and the SNP of XRCC1 Gln399Arg was significantly associated with a lower risk of distant metastasis and microsatellite nodule. In our study, Gln allele of XRCC1 Arg399Gln was more likely to reduce the overall survival of HCC, which was in line of previous studies (Jung et al., 2012; Yu et al., 2003).

XPD protein, encoded by XPD gene, plays a role in NER pathway. During the NER, XPD participates in the opening of the DNA helix to allow the excision of the DNA fragment containing the damaged base (Benhamous and Sarasin, 2002; Manuguerra et al., 2006). 312 (Asp to Asn) and 751 (Lys to Gln) were the main two polymorphisms that induce amino acid changes in the proteins (Shen et al., 1998). The associations of the Lys751Gln polymorphism in XPD gene with the prognosis of various types of cancer have been widely reported in cancer epidemiologic

studies, such as colorectal cancer, advanced lung cancer, oral cancer and pancreatic cancer (Giovannetti et al., 2011; Biason et al., 2012; Chen et al., 2012; Mahimkar et al., 2012; Slyskova et al., 2012). In our study, we have found polymorphism in XPD Lys751Gln is related to the prognosis of HCC, which has provided evidence that the XPD protein influences the overall survival of HCC through NER pathway.

Up to now, there are limited reports on the role of polymorphisms in XRCC1 and XPD on the survival of HCC. We have found modern decreased survival in XRCC1 399Gln/Gln or XPD 751Gln/Gln carriers, which is obvious because XRCC1 399Gln/Gln or XPD 751Gln/Gln have reduced the enzyme activity and thus may have decrease DNA repair captivities. The chemotherapy for HCC is to induce the DNA damage of cancer cells, the low activity of XRCC1 and XPD polymorphisms would strengthen the efficacy of therapy. The role of XRCC1 and XPD polymorphisms has been reported to be associated with increased function of chemotherapy of several cancers, including non-small cell lung cancer, breast cancer, head and neck squamous cell cancer as well as pancreatic cancer (Giovannetti et al., 2011; Zhang et al., 2011; Azad et al., 2012). Our study was in line with previous studies, which provided solid evidence for the above hypothesis. XRCC1 399Gln/Gln or XPD 751Gln/Gln carriers had significant prognostic role on the survival of HCC patients in Chinese population. Moreover, the genetic information on the polymorphisms and gene expression could play an important role in creating successful pharmacogenetic-guided chemotherapy. The use of rapid and sensitive PCR assays for diagnostic screening, coupled with ready accessibility to peripherad blood from patients with HCC, will help facilitate application of our study.

In conclusion, our present data provide evidences to suggest that polymorphisms in XRCC1 Arg399Gln, and XPD Lys751Gln were related to HCC risk in Chinese population. Moreover, the genotype of XRCC1 399Gln/Gln and XPD 751Gln/Gln are associated with a reduction of death from HCC. It is suggested that the XRCC1 Arg399Gln and XPD Lys751Gln polymorphisms should be routine detected to HCC patients who are more likely benefit from radiotherapy and chemotherapy. Our finding are based on relative small numbers and limited by small number subjects. Larger sample studies from Chinese population are still needed.

Acknowledgements

We thanks for the help from staff of Xinxiang Central Hospital.

References

Azad AK, Bairati I, Samson E, et al (2012). Validation of genetic sequence variants as prognostic factors in early-stage head and neck squamous cell cancer survival. *Clin Cancer Res*, **18**, 196-206.

Benhamou S, Sarasin A (2002). ERCC2/XPD gene polymorphisms and cancer risk. *Mutagenesis*, **17**, 463-9.

Biason P, Hattinger CM, Innocenti F, et al (2012). Nucleotide excision repair gene variants and association with survival in osteosarcoma patients treated with neoadjuvant chemotherapy. *Pharmacogenomics J*, **12**, 476-83.

Bowen DG, Walker CM (2005). Adaptive immune responses in acute and chronic hepatitis C virus infection. *Nature*, **436**, 946-52.

Chen X, Sun H, Ren S, et al (2012). Association of XRCC3 and XPD751 SNP with efficacy of platinum-based chemotherapy in advanced NSCLC patients. *Clin Transl Oncol*, **14**, 207-13.

Giovannetti E, Pacetti P, Reni M, et al (2011). Association between DNA-repair polymorphisms and survival in pancreatic cancer patients treated with combination chemotherapy. *Pharmacogenomics*, **12**, 1641-52.

Han W, Kim KY, Yang SJ, et al (2012). SNP-SNP interactions between DNA repair genes were associated with breast cancer risk in a Korean population. *Cancer*, **118**, 594-602.

Hussain SP, Schwank J, Staib F, et al (2007). TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. *Oncogene*, **26**, 2166-76.

International Agency for Research on Cancer (2011). Liver cancer incidence and mortality worldwide in 2008; http://globocan.iarc.fr/factsheets/cancers/liver.asp. 2011.

Jung SW, Park NH, Shin JW, et al (2012). Polymorphisms of DNA repair genes in Korean hepatocellular carcinoma patients with chronic hepatitis B: possible implications on survival. *J Hepatol*, **57**, 621-7.

Lavanchy D (2004). Hepatitis B virus epidemiology, disease burden, treatment and current and emerging prevention and control measures. *J Viral Hepat*, **11**, 97-107.

Mahimkar MB, Samant TA, Kannan S, et al (2012). Polymorphisms in GSTM1 and XPD genes predict clinical outcome in advanced oral cancer patients treated with postoperative radiotherapy. *Mol Carcinog*, **51**, E94-103.

Mandal RK, Gangwar R, Kapoor R, et al (2012). Polymorphisms in base-excision & nucleotide-excision repair genes & prostate cancer risk in north Indian population. *Indian J Med Res*, **135**, 64-71.

Manuguerra M, Saletta F, Karagas MR, et al (2006). XRCC3 and XPD/ERCC2 single nucleotide polymorphisms and the risk of cancer: a HuGE review. *Am J Epidemiol*, **164**, 297-302.

Pang R, Tse E, Poon RT (2006). Molecular pathways in hepatocellular carcinoma. *Cancer Lett*, **240**, 157-69.

Ramachandran S, Ramadas K, Hariharan R (2006). Single nucleotide polymorphisms of DNA repair genes XRCC1 and XPD and its molecular mapping in Indian oral cancer. *Oral Oncol*, **42**, 350-62.

Rybárová S, Vecanová J, Hodorová I, et al (2011). Association between polymorphisms of XRCC1, p53 and MDR1 genes, the expression of their protein products and prognostic significance in human breast cancer. *Med Sci Monit*, **17**, BR354-63.

Shen MR, Jones IM, Mohrenweiser H (1998). Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. *Cancer research*, **58**, 604-8.

Slyskova J, Naccarati A, Pardini B, et al (2012). Differences in nucleotide excision repair capacity between newly diagnosed colorectal cancer patients and healthy controls. *Mutagenesis*, **27**, 225-32.

Smith TR, Miller MS, Lohman K, et al (2003). Polymorphisms of XRCC1 and XRCC3 genes and susceptibility to breast cancer. *Cancer Lett*, **190**, 183-90.

Xu C, Wang X, Zhang Y, et al (2011). Effect of the XRCC1 and XRCC3 genetic polymorphisms on the efficacy of platinum-based chemotherapy in patients with advanced non-small cell lung cancer. *Zhongguo Fei Ai Za Zhi*, **14**, 912-7.

Yu MW, Yang SY, Pan IJ, et al (2003). Polymorphisms in XRCC1 and glutathione S-transferase genes and hepatitis B-related hepatocellular carcinoma. *J Natl Cancer Inst*, **95**, 1485-8.

Zhang H, Li W, Franklin MJ, et al (2011). Polymorphisms in DNA repair gene XRCC1 and skin cancer risk: a meta-analysis. *Anticancer Res*, **31**, 3945-52.