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XRCC1 POLYMORPHISM IN GASTROINTESTINAL CANCER AMONG SABAH POPULATION

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Abstract

Gastrointestinal cancer (esophagus, pancreas, gastric, colon and rectum cancer) is a common cancer causing high mortality in the world. Studies of gastrointestinal cancer have revealed that factors that cause this cancer includes individual age, dietary and genetic factors. DNA repair capacity is one of the genetic factors that contribute to variation in susceptibility to cancer. This study aims to identify polymorphism of XRCC1 gene, which is a DNA repair gene and explores the risk to gastrointestinal cancer. Peripheral blood was obtained from healthy and cancer patients with consent. DNA was extracted from the blood and analysed for Arg194Trp polymorphism of the XRCC1 gene using PCR-RFLP approach. In this study, Pvu II restriction enzyme was used to detect wild-type (Arg/Arg) and variant forms (Trp/Trp) of XRCC1 gene. We found that the risk of developing gastrointestinal cancer is two times higher in individuals with Arg/Trp genotype (RR=2.3, 95% CI: 0.5-1.2) and 10 times higher in individuals with Trp/Trp genotype (RR=10.6, 95% CI: 2-2.8) compared to those with the Arg/Arg genotype. Our findings suggest that XRCC1 polymorphism is a determinant of gastrointestinal cancer risk.

INTRODUCTION

Cancer is a disease of major impact in world health. Gastrointestinal cancer (esophagus, pancreas, gastric, colon and rectum cancer) is a common cancer causing high mortality. Studies of gastrointestinal cancer have revealed that factors that cause this cancer includes individual age, dietary and genetic factors. Genetic makeup is one of the factors that influences whether and when the individual will develop cancer (Huilie et al., 2009). In the human body, there are two types of control systems which are, the growth –promoting systems that promote cell growth or cell proliferation and safeguard systems that protect against abnormal cell growth. When one of the growth-promoting systems malfunctions, a cell may begin to proliferate inappropriately. Malfunction of growth-promoting systems occur because of gene that specifies proteins in these systems is mutated. So, to protect against malfunctions in the control systems that promote cell proliferation, our body has equipped with safeguard systems. Safeguard systems involved in repairing mutated DNA. However, if one of the safeguard systems are also disrupted by mutation, the mutation rate in a cell can soar, making it much more likely that the cell will accrue the multiple mutations required to turn it into a cancer cell (Van et al., 2001).
Recent studies have focused on identifying the changes at the molecular level that lead to cancer for new diagnostics and treatment modalities. DNA repair capacity is one of the genetic factors that contribute to variation in susceptibility to cancer. Studies of cancer predisposition have focused on DNA repair genes that influenced capacity of DNA repair in individual. Mutations of these genes have a major impact on cancer formation and/or progression. Therefore in this study, the relationship between polymorphism of \textit{XRCC1} genes, which is one of the DNA repair gene with gastrointestinal cancer risk had been analyzed. X-ray repair complementing group 1 (\textit{XRCC1}) is one of DNA repair gene that is important in base excision repair. \textit{XRCC1} gene has been mapped to human chromosome 19q13.2-13.3 (Mohrenweiser \textit{et al}., 1989) and consists of 17 exons and encodes a protein of 633 amino acids (Parisa and Mostafa, 2008). This \textit{XRCC1} protein functions in the repair of single-strand breaks (SSB) and in base excision repair (BER) of damaged bases caused by endogenous and exogenous oxidants (Camilla \textit{et al}., 2006). In addition, \textit{XRCC1} also acts as a scaffold protein that is able to coordinate and facilitate the steps of various DNA repair pathways. Proteins that \textit{XRCC1} associated to facilitate the base-excision repair (BER) and single-strand break-repair processes are polymerase beta, DNA ligase III and poly (ADP-ribose) polymerase (PARP) (Caldecott \textit{et al}., 1996 and Julie \textit{et al}., 2008).

The \textit{XRCC1} gene exhibits polymorphic variations including three common single nucleotide polymorphisms that result in amino acid substitutions in exon 6 (Arg194Trp), exon 9 (Arg280His) and exon 10 (Arg399Gln) (Mariana \textit{et al}., 2001 and Hongbing \textit{et al}., 2000). In this study, a case-control study of gastrointestinal cancer in Sabah population was performed to examine one polymorphic site (Arg194Trp) in exon 6 of the \textit{XRCC1} gene to the risk of gastrointestinal cancer.

**METHODOLOGY**

**Sampling and DNA Extraction**

Peripheral blood from 81 gastrointestinal cancer patients and 121 healthy controls were obtained with consent. DNA was extracted using the FlexiGene blood and tissue kit (Qiagen) as recommended by the manufacturer.

**\textit{XRCC1} genotyping**

\textit{XRCC1} genotypes at the Arg94Trp site was determined by PCR-based restriction fragment length polymorphism. The primers used for amplifications were 5’GCC AGG GCC CCT CCT TCA A 3’ and 5’ TAC CCT CAG ACC CAC GAG T 3’. PCR was performed in a 25\mu l reaction mixture containing 100ng DNA, 0.5\mu M each primer, 0.2mM each dNTP, 2.0mM MgCl2, 1.0U Taq DNA polymerase (Promega) with 1X reaction buffer with conditions: 95°C for 2 min, followed by 35 cycles of 94°C for 30 s, 65°C for 30 s and 72°C for 45 s and a final elongation of 7 min at 72°C. PCR products were analyzed by using agarose gel electrophoresis and stained with ethidium bromide. For RFLP analysis, restriction enzyme \textit{Pvu} II (New England Biolabs) was used to distinguish the Arg194Trp polymorphism. PCR products were digested at 37°C overnight with 2U of the enzyme. The restriction products were analyzed by 2% agarose gel electrophoresis.
Statistical analysis

The association between \textit{XRCC1} polymorphisms and risk of gastrointestinal cancer was tested using relative risk (RR) at 95\% confidence intervals (CI). SPSS version 12 was used for statistical analysis.

RESULTS AND DISCUSSION

A total of 81 case subjects and 121 control subjects regardless of ethnic groups were included in the analyses. In the PCR-RFLP analysis, the PCR product of 485 bp was obtained. This is the expected size using the designed primers. The PCR products were subsequently digested with \textit{Pvu} II which resulted in different band patterns (Fig. 1).

![Figure 1](image)

Statistical analysis showed that among the control subjects, the frequencies of \textit{XRCC1} 194Arg and 194Trp polymorphisms were 85.5\% and 14.5\%, respectively. In cases subjects, the frequency were 72.2\% and 27.8\%, respectively. Allele frequency of the mutated 194Trp was higher in cases (27.8\%) compared to controls (14.5\%). Table 1 indicated that homozygous for Trp variant has 10.6-fold increased risk of developing gastrointestinal cancer (RR=10.6, 95\% CI: 1.9-2.8) compared to those with homozygous for Arg allele. Those with heterozygous allele were at 2.3-fold higher risk of developing gastrointestinal cancer (RR=2.3, 95\% CI: 0.5-1.2) compared to those with homozygous for Arg allele.

Table 1. Frequency distribution among cases and controls and relative risk associated with genotypic variants of \textit{XRCC1} detected by restriction enzyme \textit{Pvu} II
Results from this study suggest an association between \textit{XRCC1} genetic polymorphism at exon 6 and risk of gastrointestinal cancer. The significant increase in the risk of gastrointestinal cancer indicates that \textit{XRCC1} gene polymorphism play an important role in variation of cancer susceptibility among individuals in Sabah. Association between polymorphism of \textit{XRCC1} at codon 194 with cancer risk was also reported by study in South Korea and Chinese population where it increased the risk of colorectal, gastric and pancreatic cancer (Yun \textit{et al.}, 2005, Hongbing \textit{et al.}, 2000 and Jiao \textit{et al.}, 2006). However, Ratnasinghe \textit{et al.} (2004) reported that polymorphism of \textit{XRCC1} at codon 194 do not confer significant risk to both upper and lower gastrointestinal cancer in North Central China population.

In addition to gastrointestinal cancer, \textit{XRCC1} polymorphism at codon 194 was reported to increase risk to other type of cancer such as breast and lung cancers (Priya \textit{et al.}, 2005 and Pachouri \textit{et al.}, 2007). Among Indian women (Priya \textit{et al.}, 2005) the risk was increased to two fold higher but not Cyprus women (Maria \textit{et al.}, 2008). Polymorphism of \textit{XRCC1} at codon 194 was also reported having positive association with oral cancer risk among Indian population where individuals carrying at least one variant Trp allele have three fold increased risk in developing oral cancer compared to individuals carrying wild type allele (Ramachandran \textit{et al.}, 2006).

**CONCLUSIONS**

Our study suggests that \textit{XRCC1} Trp194Trp variant genotype is associated with an increased risk of developing gastrointestinal cancer among Sabah population where the risk is 10-fold higher when both alleles of \textit{XRCC1} are mutated. Nevertheless, we are now analyzing a larger sample size for further verification.

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