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JUDUL: POLYMORPHISM OF X-RAY REPAIR CROSS-COMPLEMENTING GROUP 1 (XRCC1) IN GASTROINTESTINAL CANCER

IJAZAH: SARJANA MUDA DENGAN KEPUJIAN DALAM BIOTEKNOLOG

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POLYMORPHISMS OF X-RAY REPAIR CROSS-COMPLEMENTING GROUP 1 (XRCC1) IN GASTROINTESTINAL CANCER

JENIFFER CHIEW LEE HWA

THIS DISSERTATION IS SUMMITED TO FULFILL PARTIAL OF THE REQUIREMENT TO OBTAIN A DEGREE IN BACHELOR OF SCIENCE WITH HONOUR

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APRIL 2008

DECLARATION

I hereby declare that this dissertation is the result of my own independent work, except for the citations and quotations, in which the sources for each of them are acknowledge.

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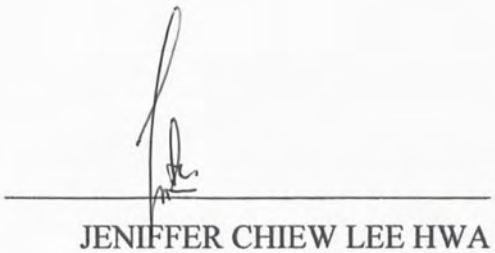

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ABSTRACT

This research is done to identify the polymorphisms of x-ray repair cross-complementing group 1 (XRCC1) gene in gastrointestinal (GI) cancer. DNA was isolated from the blood samples, which were taken from Hospital Queen Elizabeth. The quality of DNA isolated was checked by spectrophotometer and were used for polymerase chain reaction. Polymerase chain reaction was carried out using a pair of primer specifically to amplify 517bp of the XRCC1 gene that contained the Arg399Gln site. After the PCR, the amplified DNA was cut by using specific enzyme which will only cut at specific sequence of the DNA (*Bcn*I). After the restriction enzyme digestion, the result of restriction enzyme digestion was analyzed by using agarose gel electrophoresis in order to determine whether there is any polymorphism in the region of interest. From the result of the agarose gel electrophoresis, out of 10 samples, four samples with genotype frequency of 0.3481 were homozygote wild-type (Arg/Arg), three samples with genotype frequency of 0.17 were homozygote variant (Gln/Gln), and three samples with genotype frequency of 0.48 were heterozygote (Arg/Gln). However, this is just a preliminary result because we are not able to get large number of samples.

ABSTRAK

Kajian ini dijalankan untuk mengenal pasti polimorfisme gen XRCC1 (x-ray repair cross-complementary group 1) dalam kanser pencernaan. DNA dipencilkan dalam sampel darah yang didapati dari Hospital Queen Elizabeth. Kualiti DNA yang dipencilkan itu diperiksa dengan menggunakan spektrofotometer dan digunakan dalam polimerase reaksi berantai (PCR). Polimerasi reaksi berantai dilakukan dengan menggunakan sepasang primer yang spesifik, dan mengamplifikasi 517pb dalam gen XRCC1 yang mengandungi tapak Arg399Gln. Selepas polimerase reaksi berantai, DNA yang telah diamplifikasi itu akan dipotong menggunakan enzim *Bcn*I. Enzi mini akan mengenal pasti sesetengah susunan dalam DNA dan akan memotong di bahagian tersebut. Selepas dipotong menggunakan enzim, keputusan ini akan dikaji dalam agarose gel elektroforasi untuk menentukan sama ada polimorfisme berlaku dalam gen tersebut. Daripada keputusan agarose gel elektroforesis, dengan menggunakan 10 sampel, empat sampel dengan frequensi genotip 0.35 adalah homozigot dominan, tiga sampel dengan frequensi genotip 0.17 adalah homozigot varian, dan tiga sampel dengan frequensi genotip 0.48 adalah heterozigot. Walau bagaimanapun, ini cuma mewakili sebahagian daripada keputusan kerana kami tidak dapat jumlah sampel darah yang banyak.

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LIST OF SYMBOLS

%	percentage
µl	microlitre
µg	microgram
ml	mililitre
mm	millimeter
kb	kilo bases
U	unit
ºC	celcius
rpm	revolutions per minute
xg	centrifugal force
ng	nanogram
nm	nanometer
µM	micromole
mM	miliMole
s	second
min	minutes
DNA	deoxyribonuclei acids
TE buffer	Trisethylenediaminetretraacetic acid buffer
SNP	single nucleotide polymorphism
PCR	polymerase chain reaction

CHAPTER 1

INTRODUCTION

1.1 Background of study

Gastrointestinal cancer refers to malignant conditions of the gastrointestinal tract, including the esophagus, stomach, liver, biliary system, pancreas, bowels, and anus (National Cancer Institute, 2004.) Gastrointestinal cancer can be further divided into different types of diseases, which are gastrointestinal stromal tumors (GIST), esophageal cancer, stomach cancer (also called gastric cancer), liver cancer (also called hepatocellular carcinoma, HCC, and hepatoma), gallbladder cancer, pancreatic cancer, colorectal cancer – large intestine cancer (also called colon cancer, bowel cancer, and rectal cancer), small intestine cancer and anal cancer (Figure 1.1).

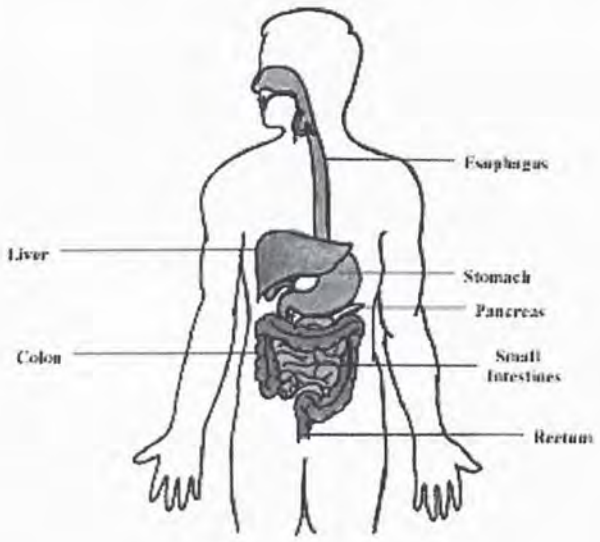

Figure 1.1: The different parts of the body that associated with Gastrointestinal cancer (National Cancer Institute, 2005).

Gastrointestinal cancers account for approximately 20% of all cancer incidences (National Cancer Institute, 2005) and are the leading cause of cancer deaths in many countries around the world (Murray & Lopez, 1997). In the United States, colorectal cancer, in particular, represents the second leading cause of cancer related mortality in males, and the third leading cause in females (Jemal *et al*, 2006). Recently, there has been an increased recognition that chronic inflammation could play a key role in the onset and progression of these cancers .Given that gastrointestinal cancers are extremely difficult to treat, detection is challenging and often occurs late in disease progression, and that surgery offers the only chance for a cure, evidence for an inflammatory basis provides a new avenue for intervention.

Gastrointestinal cancers also involve an indolent (slow-growing) cancer that forms in cells that make hormones in the lining of the gastrointestinal tract (the

stomach and intestines). It usually occurs in the appendix (a small fingerlike pouch of the large intestine), small intestine, or rectum. Having gastrointestinal carcinoid tumor increases the risk of forming other cancers of the digestive system. Gastrointestinal cancer may be diagnosed during the evaluation of symptoms or during a screening colonoscopy.

XRCC1 (x-ray cross-complementing group 1) is a DNA repair protein and it complexes with DNA ligase III. The protein encoded by this gene is involved in the efficient repair of DNA single-strand breaks formed by exposure to ionizing radiation and alkylating agents. This protein interacts with DNA ligase III, polymerase beta and poly (ADP-ribose) polymerase to participate in the base excision repair pathway. It may play a role in DNA processing during meiogenesis and recombination in germ cells. A rare microsatellite polymorphism in this gene is associated with cancer in patients of varying radiosensitivity. X-ray repair cross complementing group 1 (XRCC1) is one of the proteins involved in the repair of gaps in the restorative phase of base excision repair (BER). Other enzymes involved in BER include apurinic/apyrimidinic endonuclease (APE), polynucleotide kinase, DNA polymerase β and DNA ligase III. (Kubota *et al.*, 1996). BER, nucleotide excision repair, mismatch repair and double strand break repair are pathways of DNA repair involved in maintaining genomic integrity against damage caused by endogenous and exogenous mutagens. Because of the importance of maintaining cellular genomic integrity, the many enzymes involved in complex DNA repair processes are thought to be candidate cancer-susceptibility genes.

In this research, the polymorphisms of XRCC1 gene was identified in associated with the occurrence of gastrointestinal cancer. Also, genetic differences

such as single nucleotide polymorphisms (SNP) may contribute to GI cancer. Polymorphisms in XRCC1 gene (x-ray cross-complementing group 1) have been identified at conserved sites, including two SNPs at codons 194 (Arg to Trp) and 399 (Arg to Gln) (Cao *et al.*, 2006). Recently, several studies have shown that polymorphisms of XRCC1 gene are directly related to the formation of gastrointestinal cancers. A recently study revealed that XRCC1 codon 399 polymorphisms are risk factors of bladder cancer, which is one of the most popular gastrointestinal cancer. (Arizono *et al.*, 2008). In addition, another study revealed that single nucleotide polymorphisms (SNPs) in XRCC1 gene predicted overall survival in gastric cancer patients receiving oxaliplatin-based chemotherapy in Chinese population. (Liu *et al,* 2007).

In Malaysia, a regional population-based cancer registry survey carried out between 1988 and 1990, the incidence rates for males and females were 56.3 and 56.9 per 100 000, respectively (Kementerian Kesihatan Malaysia, 1993). Colorectal cancer is the third commonest cause of cancer deaths in Malaysia. Data from the Ministry of Health of Malaysia confirms an increase in colorectal cancer admission rates from 8.1% in 1987 to 11.9% in 1995 (Moh 1995). Genetics, experimental, and epidemiological data suggest that colorectal cancer develops from complex interactions between inherited susceptibility and environmental factors. The current hypothesis is that adenomatous polyps are the precursors of the vast majority of colorectal cancers. According to the WHO, stomach cancer and esophageal cancer accounted for about 6.3% (1,500) and 1.7% (400) out of 23,965 deaths in Malaysia for the year 2002 respectively (World Health Organization, 2007). Since there is high

number of cases that involved gastrointestinal cancer in Malaysia, therefore this research was conducted.

In this research, blood samples were obtained from Hospital Queen Elizabeth. DNA was extracted from these samples and polymorphism of XRCC1 gene at Arg399Gln site was analyzed.

1.2 Objectives

a) To isolate DNA from blood samples of gastrointestinal cancer patients.

b) To study the polymorphisms of x-ray repair cross-complementing group 1 (XRCC1) gene in gastrointestinal cancer.

c) To analyze the pattern of genetic inheritance of XRCC1 gene and gastrointestinal cancer phenotype.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Gastrointestinal Cancer

Gastrointestinal disease refers to ulcerative disorders of the upper gastrointestinal tract. Collectively, cancers of the esophagus, stomach, and small intestine are referred to as upper gastrointestinal tract (UGI) cancers. UGI cancers represent the second most common site and cause of death among the digestive system cancers (Erickson *et al.*, 2001). In addition to the UGI, gastrointestinal stromal tumors (GIST), liver cancer (also called hepatocellular carcinoma, HCC, and hepatoma), gallbladder cancer, pancreatic cancer, colorectal cancer – large intestine cancer (also called colon cancer, bowel cancer, and rectal cancer), and anal cancer also included in the gastrointestinal cancer (gastrointestinal cancer) (Evans *et al.*, 1997).

Symptoms of gastrointestinal disease are indigestion, heartburn, nausea, loss of appetite, abdominal pain that is often worse after eating, and gastrointestinal bleeding (signs of this are vomiting material that looks like coffee-grounds, or having dark stools). Some other symptoms are acid bile reflux in the throat, asthma-like symptoms, often irritable bowel syndrome, and chronic poor digestion with sharp abdominal and chest pains, hoarseness and chronic cough.

2.2 Factors contributing to gastrointestinal cancer

There are many factors that contribute to gastrointestinal cancer; there are dietary factors, environmental factors and genetic factors.

2.2.1 Dietary factors

A diet high in salt and nitrates and low in vitamins A and C increases the risk for stomach cancer (Hunt *et al.*, 2002). Other dietary risk factors include food preparation (e.g., preserving food by smoking, salt-curing, pickling, or drying) and environment (e.g., lack of refrigeration, poor drinking water).

2.2.2 Environmental factors

Others than dietary factors, environmental factors such as cigarette smoking, infection with Heliobacter pylori bacteria (a bacteria related to stomach ulcers) or Epstein-Barr virus, obesity, occupational factors (e.g., working in rubber and coal industries), personal history of gastrointestinal cancer, previous abdominal radiation, stomach surgery, etc, also lead to GI cancer (Mortele *et al.*, 2002).

2.2.3 Genetic factors

Besides dietary factors, genetic (hereditary) risk factors include hereditary nonpolyposis colon cancer (HNPCC) syndrome and Li-Fraumeni syndrome (conditions that result in a predisposition to cancer), and a family history of gastrointestinal cancer (Micames *et al.*, 2003). People with type A blood also have an increased risk for stomach cancer. Medical conditions that increase the risk for the disease include pernicious anemia (vitamin B-12 deficiency), chronic inflammation of the stomach (atrophic gastritis), and intestinal polyps (noncancerous growths).

In addition, polymorphisms of x-ray cross-complementing group 1 (XRCC1) gene also involved in gastrointestinal cancer. According to the journal reported by Luke *et al* (2003), there is a higher risk of esophageal and gastric cancer associated with the polymorphisms of XRCC1 gene (Ratnasinghe *et al,* 2004). Besides that, a report by Arizono *et al* (2008) revealed that there is a high risk of bladder cancer of people with polymorphisms of XRCC1 at codon 399 in Japanese population. In this research, we analyzed the polymorphisms of XRCC1 gene in the blood samples obtained from gastrointestinal cancer patients. Through this research, we will able to determine whether the polymorphism of XRCC1 gene contribute to the occurrence of gastrointestinal cancer in Sabah.

2.3 X-ray repair cross-complementing group 1 (XRCC1) gene

X-ray repair cross-complementing group 1 (XRCC1) is an important DNA repair protein. XRCC1 is a single-strand break DNA repair protein which is found in eukaryotic species ranging from insects to humans. Arg194Trp, Arg280His, and Arg399Gln are three polymorphisms of XRCC1 that commonly exist in human. In this context, we obtained the relevant articles through a PubMed search and examined the association of XRCC1 polymorphisms and the risk of cancer in Asian populations (Qu *et al.*, 2005). Generally, a single XRCC1 polymorphism is weakly associated with cancer in Asian populations. However, when combined with other genetic polymorphisms or such lifestyle factors as smoking, XRCC1 polymorphisms show a stronger association with the risk of cancer. The interaction of the 399Gln/Gln genotype and smoking might be associated with a three-fold increase in the risk of cancer.

As mentioned above, the XRCC1 gene exhibits polymorphic variations, including three common single nucleotide polymorphisms (SNPs) that result in amino acid substitutions in exon 7 (Arg194Trp), exon 9 (Arg280His) and exon 10 (Arg399Gln).

An exon 10 variant at codon 399 of XRCC1 leads to an Arg to Gln amino acid change. The 399Gln allele is associated with an increased risk of several types of cancers, increased DNA adducts and chromosomal changes; therefore, it appears that the 399Gln allele may alter the role of the XRCC1 protein in DNA repair. An XRCC1 knockout is embryonic-lethal in mice and cells deficient in XRCC1 have increased sensitivity to X-ray, UV, ethylmethane sulfonate and other DNA damaging agents.

2.3.1 Function of XRCC1 gene

XRCC1 is required for the efficient repair of single-strand breaks and damaged bases in DNA. XRCC1 has no known enzymatic activity, and it is thought to act as a scaffold protein for both single-strand break repair and base excision repair activities (Lindahl *et al.*, 1999). In addition, XRCC1 provides multiple functions in DNA base excision repair that include assembly of DNA repair complexes containing DNA polymerase β (β-Pol), poly-(ADP-ribose) polymerase (PARP) and DNA ligase III, and polynuclcetide kinase/phosphatase (PNK) (Thompson *et al.*, 2000). It is thought that by binding β-Pol and damaged DNA, XRCC1 may protect the template strand at the site of DNA damage, such that short patch repair can proceed efficiently (Tebbs *et al.*, 1999). XRCC1 has been shown to physically interact with DNA polymerase ß, polyadenosine diphosphate-ribose polymerases 1 and 2, APE1/APEX1, OGG1, and proliferating cell nuclear antigen. Its absence leads to a substantial reduction in the levels of its partner ligase III (Fan *et al.*, 2004). Also, the XRCC1 protein plays a

crucial role in base excision repair by acting as a scaffold for other base excision repair enzymes.

XRCC1 may also stimulate the activity of some of the repair enzymes; and XRCC1 helps protect DNA in the vicinity of the single-strand break from further damage.

2.3.2 Location of XRCC1 gene

The gene is located on chromosome 19q13.2; it consists of 17 exons and encodes a protein of 633 amino acids (Goode *et al.*, 2002). More than 60 validated single nucleotide polymorphisms in XRCC1 are listed in the Ensembl database, among which approximately 30 variants are located in exons or promoter regions. The most extensively studied single nucleotide polymorphisms are Arg194Trp on exon 6 (dbSNP no. rs1799782), Arg280His on exon 9 (dbSNP no. rs25489), and Arg399Gln on exon 10 (dbSNP no. rs25487) (Wang *et al.*, 2003).

In this research, I will concentrate on the polymorphisms of XRCC1 gene in Arg399Gln site. The 399Gln allele is located at the carboxylic acid terminal side of the polyadenosine diphosphate-ribose polymerase-interacting domain. It was shown to be associated with higher levels of aflatoxin B 1 -DNA adducts and higher bleomycin sensitivity in several studies (Wang *et al.*, 2003) (Lunn *et al.*, 1999) (Matullo *et al.*, 2001), while another study did not find such an association (Palli *et al.*, 2001).

2.4 Polymorphisms of XRCC1 gene that associated with cancer

Studies have shown significant associations between the Arg399Gln variant and various cancers (Goode *et al.*, 2002). However, in bladder cancer, the results have been inconsistent. A recent meta-analysis of 38 case-control studies concluded that the Arg194Trp variant had a protective effect on cancer risk, while individuals carrying the Arg280His variant allele had increased cancer risk compared to those with the wild type genotypes (odds ratio [95% confidence intervals], 1.19 [1.00–1.42]). (Hu *et al*, 2005).

Recently, a study discovered a novel T-77C polymorphism (rs3213245) in the XRCC1 gene which contributes to diminished promoter activity and increased risk of non-small cell lung cancer (Hao *et al,* 2004). Another report discovered that genetic variants in XRCC1 and APEX1 may alter susceptibility to biliary tract cancer and stones (Huang *et al*, 2005).

Another paper revealed that polymorphisms of XRCC1 gene at codon 399 are associated with micronucleus frequencies in human lymphocytes in vivo (Mateuca *et al.*, 2008). A study revealed that DNA repair gene XRCC1 and XPD polymorphisms and their association with coronary artery disease risks and micronucleus frequency (Guven *et al*, 2007).

Besides that, another study revealed that XRCC1 and other genes were directly related to the occurrence of bladder cancer (Angeline *et al.*, 2007). According to Baorui *et al* (2007), single nucleotide polymorphisms (SNPs) in xeroderma pigmentosum group D (XPD), X-ray repair cross complementing group 1 (XRCC1) and glutathione S-transferase P1 (GSTP1) predicted overall survival in gastric cancer

patients receiving oxaliplatin-based chemotherapy in Chinese population. Patients with XRCC1-399 Gln/Gln genotype demonstrated a significant worse survival.

2.5 Principle of polymerase chain reaction (PCR)

In this research, polymerase chain reaction (PCR) was used to amplify the sequence if DNA interest. The polymerase chain reaction (PCR) is a technique widely used in molecular biology. It derives its name from one of its key components, a DNA polymerase used to amplify a piece of DNA by in vitro enzymatic replication. As PCR progresses, the DNA thus generated is itself used as template for replication. This sets in motion a chain reaction in which the DNA template is exponentially amplified. With PCR it is possible to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating millions or more copies of the DNA piece. PCR can be performed without restrictions on the form of DNA, and it can be extensively modified to perform a wide array of genetic manipulations.

Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the bacterium *Thermus aquaticus*. This DNA polymerase enzymatically assembles a new DNA strand from DNA building blocks, the nucleotides, using single-stranded DNA as template and DNA oligonucleotides (also called DNA primers) required for initiation of DNA synthesis. The vast majority of PCR methods use thermal cycling, i.e., alternately heating and cooling the PCR sample to a defined series of temperature steps. These thermal cycling steps are necessary to physically separate the strands (at very high temperatures) in a DNA double helix (DNA melting) used as template during DNA synthesis (at lower temperatures) by the DNA polymerase to selectively amplify the target DNA. The power and selectivity of PCR are primarily due to selecting primers

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