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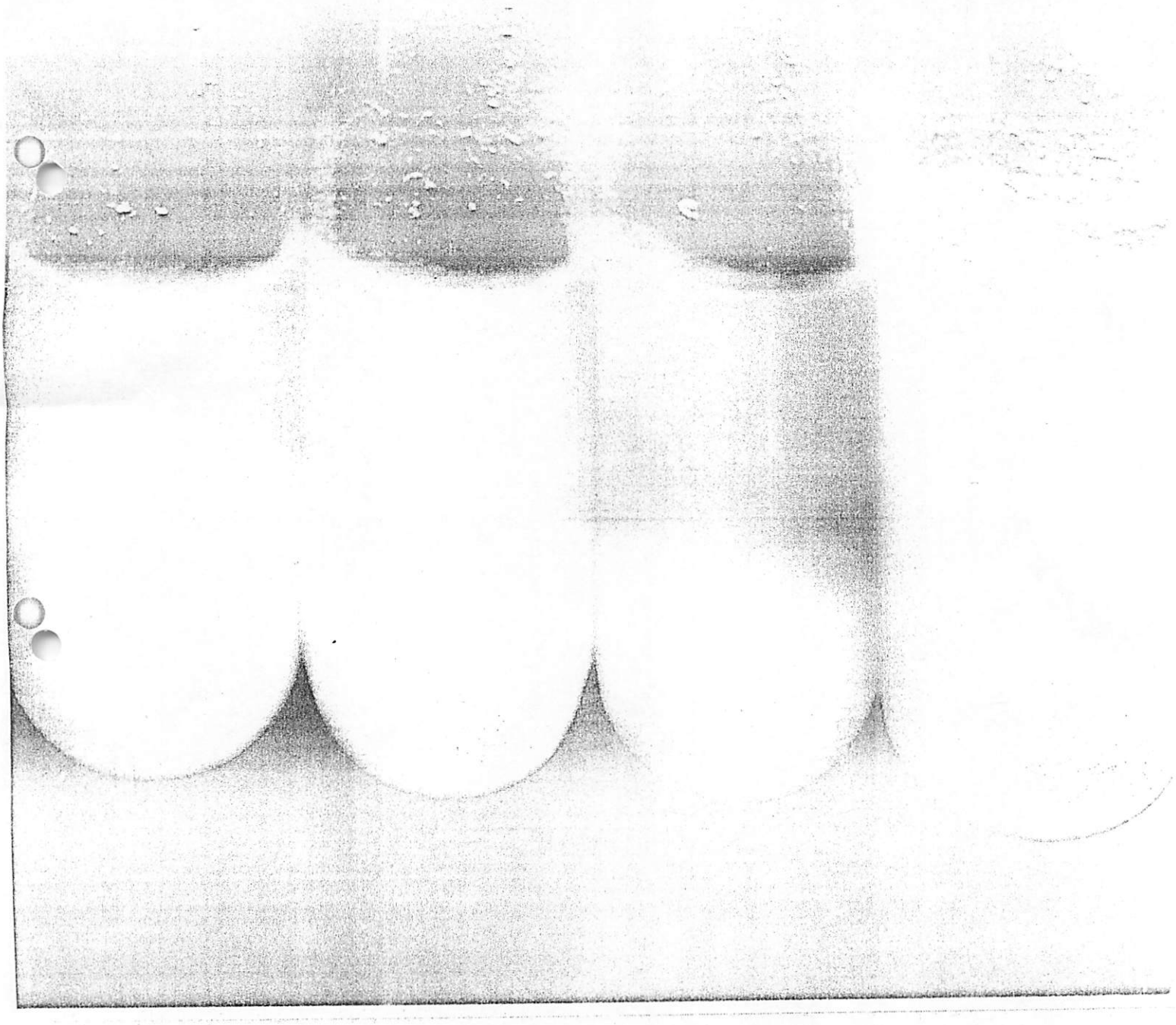

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Preliminary Study on the Single Nucleotide Polymorphism (SNP) of XRCC1 Gene Identificationto Improve the Outcomes of Radiotherapy for Cervical Cancer

Studi Awal Identifikasi Single Nucleotide Polymorphism (SNP)GenXRCC1 Untuk Memperbaiki Keluaran Radioterapi Kanker Serviks

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Abstract

Cervical cancer is the most fatal disease among Indonesian women. In recognition of the substantial variation in the intrinsic response of individuals to radiation, an effort had been done to identify the genetic markers, primarily Single Nucleotide polymorphisms (SNPs), which are associated with responsiveness of cancer cells to radiation therapy. One of these SNPs is X-ray repair cross-complementing protein 1 (XRCC1) that is one of the most important genes in deoxyribonucleic acid (DNA) repair pathways. Meta-analysis in the determination of the association of XRCC1 polymorphisms with cervical cancer revealed the potential role of XRCC1 polymorphisms in predicting cell response to radiotherapy.Our preliminary study with real-time polymerase chain reaction (RT-PCR) showed that radiotherapy affected the XRCC1 gene analyzed in blood of cervical cancer patient. Other published study found three SNPs of XRCC1 (Arg194Trp, Arg280His, and Arg399Gln) that cause amino acid substitutions. Arg194Trp is only SNPs that associated with high risk of cervical cancer but not others. Additionally, structure and function of this protein can be altered by functional SNPs, which may lead to the susceptibility of individuals to cancers. Anotherstudy found G399A polymorphisms. We concluded that SNP of this DNA repair genes have been found to be good predictors of efficacy of radiotherapy.

Abstrak

Kanker serviks adalah penyakit yang paling fatal pada perempuan di Indonesia. Untuk memahami variasi substansial respon intrinsik individual terhadap radiasi, suatu usaha telah dilakukan untuk mengidentifikasi petanda genetik, terutama Single Nucleotide polymorphism (SNP), yang berkaitan dengan responsel kanker terhadap terapi radiasi. Satu dari SNP tersebut adalah X-ray repair cross-complementing protein 1 (XRCC1) yang merupakan satu dari gen paling penting dalam lajur perbaikan asam deoksiribonukleat (DNA). Meta-analysis dalam penentuan hubungan polimorfisme XRCC1 dengan kanker serviks menemukan adanya peranan potensial polimorfisme XRCC1 dalam memprediksi respon sel terhadap radioterapi. Studi awal kami menggunakan real-time polymerase chain reaction (RT-PCR) menunjukkan bahwa radioterapi mempengaruhi gen XRCC1yang dianalisis dalam darah pasien kanker serviks. Studi yang telah dipublikasi menemukan tiga SNP dari XRCC1 (Arg194Trp, Arg280His, dan Arg399Gln) yang menyebabkan substitusi asam amino. Arg194Trp merupakan satu-satunya SNP yang berkaitan dengan tingginya risiko kanker serviks, tetapi tidak pada yang lain. Di samping itu, strukturdan fungsi protein ini dapat berubah oleh SNP fungsional, yang mengarah ke kerentanan individu untuk menderita kanker. Studi lain menemukan polimorfisme G399A. Kami menyimpulkan bahwa SNP dari gen perbaikan DNA ini merupakan prediktor yang baik dari keberhasilan radioterapi.

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INTRODUCTION

Cancer is the disease caused by an uncontrolled division of abnormal cells in a part of the body or malignant growth or tumor resulting from such a division of cells. Untreated cancers can cause serious illness and death (National Cancer Institute, 2013).It is currently estimated that there will be at least 170-190 new cancer cases annually for each 100 000 people and therefore cancer has risen to become sixth in rank among deaths after infectious diseases, cardiovascular diseases, traffic accidents, nutritional deficiency and congenital diseases (Soedarmo & Suhardi, 1992; Khanna, 1992; Wasisto, 1991; Mulyadi, 1998). However, most cancer patients (60-70%) seek medical treatment when it is already too late. The most frequent and primary cancers are cervix, breast, lymph node, skin and nasopharynx. Data collected from hospitals in several regions in Indonesia shows that cancer incidence increased by 2-8% per year during the last decade (2,4). Data which have been collected from 13 pathological laboratories during the period of 1988-1991 show that in the combined picture, cervical, breast, lymph node, skin and nasopharynx are the five major anatomical sites for cancer disease (Mangunkusumo, 1998).

Cervical cancer kills an estimated 275,000 women every year and 500,000 new cases are reported worldwide. According to official reports by World Health Organization (WHO), Indonesia is the 6-th highest in cervical cancer cases with 7493 deaths, following five countries with highest case(India, China, Brazil, Bangladesh and Nigeria) (WHO Report, 2012). Cervical cancer is the most fatal disease among Indonesian women and the second leading deadly disease in global (Tjindarbumi & Mangunkusumo, 2002).But the information on this issue has not been known because cervical cancer often causes no symptoms or complaints so that women come to the doctor in a state that it was too late.

Radiation therapy is widely used to shrink tumors or eliminate cancer cells. It works by damaging a cancer cell's deoxyribonucleic acid (DNA), making it unable to multiply. Although radiation therapy can damage nearby healthy cells, cancer cells are highly sensitive to radiation and typically die when treated. Radiotherapy is involved in many curative treatments of cancer; millions of survivors live with the consequences of treatment, and toxicity in a minority limits the radiation doses that can be safely prescribed to the majority. Around half of cancer patients receive radiotherapy (Delaney et al, 2002), with an estimated 40% cured by radiotherapy compared with 49% by surgery and 11% by chemotherapy (Bentzen et al, 2005). For some cancers of the head and neck, lung, cervix, bladder and prostate, radiotherapy can be used instead of surgery to achieve similar cure but with better functional results. Radiotherapy outcomes are improving because of technical developments allowing better radiation delivery to reduce the amount of normal tissue irradiated. Further gains will require a better understanding of molecular mechanisms and personalized treatment based on an individual patient's biology.Of these, one is the radiosensitivity that is an inherited polygenic trait, dependent on the interaction of many genes/gene products involved in multiple cell processes (Barnett et al, 2009).

As with all forms of cancer treatment, the goal of radiotherapy is to provide cancer patients with a sustainable cure for their tumor, or at least prevent disease progression, without causing substantial damage to normal tissues and organ function. Clearly, there have been great advances to conform the radiation dose to the cancer. However, even with these dosimetric improvements, some volume of normal tissue still receives a substantial radiation dose during the course of radiotherapy (Rosenstein, 2011). This radiation exposure often results in toxicity that compromises organ function and affects the quality of life for the cancer survivor. In rare instances, such radiation injuries can be fatal. Thus, a fundamental goal of radiotherapy is to minimize toxicity without a loss of treatment efficacy.

One study on the measuring radiosensitivity to predict radiotherapy outcomes that can be extended to incorporate genotyping data was done by Kerns *et al.* (Kerns et al, 2010), where four SNPs identified in their genomic wide association study were better at predicting erectile dysfunction than a combination of clinical factors (age, stage, radiation dose, hormone use, diabetes, smoking). However, the finding should be interpreted with caution, as predictive value is generally overestimated when the model is created and evaluated in the same study population, particularly if the same data were first used to select the strongest genetic predictors out of a large set of genotyped variants.

Studies have shown that among women infected with human papilloma virus (HPV), only a small proportion will develop cervical cancer during their life time. It is reasonable to conclude that other factors are also involved in the tumorigenesis of cervical cancer, such as smoking, environmental factors, and genetic factors. Recent

population-based twin and family studies have demonstrated the importance of the hereditary component of cervical cancer, associated with genetic susceptibility. Consequently, SNP markers and microsatellites should be considered genetic factors for determining what combinations of genetic factors are involved in precancerous changes to cervical cancer(Shuai et al, 2012).Investigation on human papilloma virus (HPV) prevalence and genotype distribution in cervical cancer found the association with 9 genetic SNPs including *CDKN1A (p21)* C31A, *TP53* C72G, *ATM* G1853A, *HDM2* promoter T309G, *HDM2* A110G, *LIG4* A591G, *XRCC1* G399A, *XRCC3* C241T and *TGF□1* T10C, that presumed to predispose to cancer.However this review provided information on the XRCC1 expression in supporting the goal of radiotherapy treatment to cervical cancer. How this protein is significantly associated with this cancer is described in the paper.

Single Nucleotide Polymorphisms

A SNP is a single base pair mutation at a specific locus, usually consisting of two alleles (where the rare allele frequency is >1%). SNP is also defined as variations in single base pairs that are randomly dispersedthroughout the genome (every 100 to 300 bases along the 3-billion-base human genome) and are point mutations that established in at least >1% of a given population. SNP acts as measures of genetic diversitywithin the species (i.e. 90% of human genetic variation). Many SNPs have no effect on cell function, but others could predispose people to disease or influence their response to a drug or other factors.

SNPs are found to be the etiology of many human diseases and are becoming of particular interest in pharmacogenetics. SNP genotyping is the measurement of genetic variations of SNPs between members of a species. It is a form of genotyping, the measurement of the genetic variation more generally. SNPs are one of the most common types of genetic variation. Because SNPs are conserved during evolution, they have been proposed as markers for use in quantitative trait locianalysis and in association studies in place of microsatellites. SNPs can also provide a genetic fingerprint for use in identity testing (Rapley & Harbron, 2004).The increase in interest in SNPs has been reflected by the furious development of a diverse range of SNP genotyping methods, one of them is PCR-based methods.Genetic variation in non-coding regions that called SNPs is represented in Figure 1.

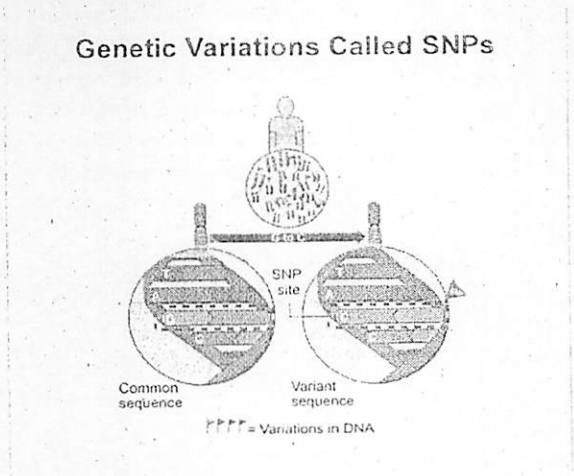

Figure 1. Genetic variation in non-coding regions that called SNPs.

Most SNPs occur in non-coding regions and do not alter genes. So that most variations in the human genome have no known effect at all because they occur in noncoding regions of the DNA. In addition, there are some changes that do occur in coding and regulatory regions, yet the effect is not entirely understood. All these are silent variations. A major SNPs that mostly studied is X-ray repair cross complementing protein 1 (XRCC1) that is one of the most important genes in DNA repair pathways. Studies have demonstrated that functional SNPs of *XRCC1* are associated with cancer risks, such as lung cancer, bladder cancer, gastric cancer and other cancers. XRCC1 belongs to the DNA base excision repair pathway and repairs single-strand breaks and XRCC1 is crucial to the integrity of chromosome. The XRCC1 protein acts as a scaffold for other DNA repair proteins, like polynucleotide kinase, human AP endonuclease (APE1), DNA polymerase □, DNA ligase III, and poly(ADP-ribose) polymerases (PARP) (Whitehouse et al, 2001). Aim of our research was to assess whether SNP of DNA repair genes can be used to be a predictorof efficacy of radiotherapy.

METHODS

All patients enrolling in this study are given radiotherapy with conventional fractionation according to tumor characteristics. Informed consent is obtained from all patients before their participation in the study.The study covering all procedures undertaken should be approved by the local ethics committee. The peripheralblood samples from all patients were taken according to standard procedure.

Genomic DNA is extracted from blood of

patient by usinga commercial Kit. For the identification or determination of SNP in cervical cancer it was selected the candidate genes and polymorphisms that are located in genes related to DNA repair mechanisms (Arg399Gln (rs25487) and Arg194Trp (rs1799782) of *XRCC1*). Polymerase chainreactions (PCRs) are then conducted in a total volume of 30 µL containing 100 ng of genomic DNA, that are performed using 0.4 mM of eachcouple of primers. After 35 cycles of PCR amplification (denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds), amplification products are electrophoresed in 2% agarose gel and visualized after staining with ethidium bromide (Terrazino et al, 2011).

The PCR products harboring the SNPs (5 µL) are then analyzed with Restriction Fragment Length Polymorphisms(RFLP) by digestingfor 3 hours in a total volume of 20 µL by 2 to 10 U of specific restriction enzymes at recommended temperaturesthat recognized and cut either the wild-type or variant sequence site. The digested products are then electrophoresed in 2.5% agarose gel and visualized after staining with ethidium bromide. All PCR reactions were set up in a dedicated PCR area with dedicated PCR pipettes and reagents. For quality control purposes, each PCR and restriction enzyme digestion included both negative and positive controls (Terrazino et al, 2011).

RESULTS AND DISCUSSION

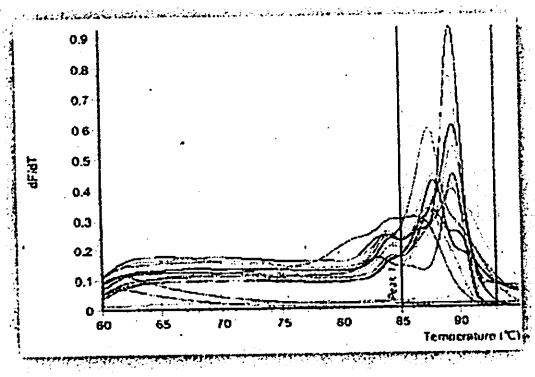

Figure 2. The curve of melting temperature of DNA analysed for SNP study in BATAN with real-time PCR.

Our preliminary study on the SNP with Real-time PCR that have been done on 15 cervical cancer samples of patient underwent radiotherapy showed that 1 sample had a mutation in *XRCC1* gene examined. Radiotherapy affecting the *XRCC1* gene analyzed in blood of cancer patient. Based on the fact that every sample has its own melting peak, the alteration in cancer cells occured due to radiotherapy treatment can be seen fromthe alteration of melting peaks and melting curve pre- and post-radiotherapy (Figure 2) (Kurnia et al, 2014).

SNP array technology was also developed in 1998 for genotyping. Since then, the technique has been improved dramatically and has become one of the most powerful genomic analysis tools (Mao et al, 2007).Research by Shuai et al (2012) demonstrated from a total of 13 studies, they found that the Arg194Trp polymorphism (Trp vs. Arg, OR=1.342, 95% CI: 1.176) was associated with increased risk of cervical cancer, while no significant association was found with Arg280His (His vs. Arg, OR=1.059, 95% CI: 0.863, 1.299) or Arg399Gln (Gln vs. Arg, OR=1.144, 95% CI: 0.938, 1.394). As for response to platinum-based chemotherapy, the variant *XRCC1* 399Gln allele (Gln vs. Arg, OR=0.345, 95% CI: 0.163, 0.729) was linked with a poor response; however, the Arg194Trp polymorphism (TrpArg vs. ArgArg, OR=6.421, 95% CI: 1.573, 26.205) predicted a good response. Thus three SNPs (Arg194Trp, Arg280His, and Arg399Gln) cause amino acid substitutions. Arg194Trp and Arg280His polymorphisms locate at the linker region connecting the domains that interact with PARP and DNA polymerase β, while Arg399Gln resides in PARP-binding domain. Additionally, proteins' structure and function can be altered by functional SNPs, which may lead to the susceptibility of individuals to cancers (Tudek, 2007). Thus, it is reasonable to conclude that the functional SNPs of XRCC1 are associated with cervical cancer risks.They conclude that the Arg194Trp polymorphism of *XRCC1* increases risk of cervical cancer; the variant 399Gln allele predicts poor response to platinum-based chemotherapy, while the Arg194Trp polymorphism indicates a good response.

Other study had been done by Alsbeih et al. (Alsbeih et al,2013) using 100 cervical cancer patients (90 squamous cell carcinomas and 10 adenocarcinomas). SNPs were genotyped by direct sequencing. They found only *XRCC1* SNP was significantly associated with cervical cancer. However, nested analysis revealed a preponderance of HPV-positivity in patients harboring the presumed risk allele *TP53* G (P=0.06). Both *XRCC1* and *TP53* SNPs tended to deviate from Hardy-Weinberg equilibrium (HWE; P=0.03-0.07). From this, they concluded that *XRCC1* G399A was significantly associated with cervical cancer, *TP53* G72C showed borderline associati-

on only in HPV-positive patients. SNPs could be more relevant biomarkers of susceptibility to cervical cancer when associated with HPV infection.

Two investigators independently searched the Medline, Embase, CNKI, and Chinese Biomedicine Databases. Their analysis reported that the variant genotypes of Arg194Trp were associated with a significantly increased cervical cancer risk (Trp/Trp vs Arg/Arg, OR = 2.21, 95% CI = 1.60–3.06; Arg/Trp vs Arg/Arg, OR = 1.23, 95% CI = 1.02–1.49; dominant model, OR = 1.36, 95% CI = 1.14–1.63; recessive model, OR = 2.06, 95% CI = 1.51–2.82). For Arg280His polymorphism, no obvious associations were found for all genetic models. For Arg399Gln polymorphism, also no obvious associations were found for all genetic models. In the subgroup analyses by ethnicity/country, a significantly increased risk was observed among Asian, especially among Chinese. To get more precise evidences, adjusted ORs (95%CI) by potential confounders (such as age, ethnicity or smoking, etc) were also calculated for *XRCC1* Arg399Gln and Arg194Trp, however, the estimated pooled adjusted OR still did not change at all.This meta-analysis suggests that Arg194Trp polymorphism may be associated with CC risk, Arg399Gln polymorphism might be a low-penetrent risk factor for cervical cancer only in Asians, and there may be no association between Arg280His polymorphism and cervical cancer risk (Li. Et al, 2012).Quantitative analyses for the relationships between *XRCC1*polymorphisms and cervical cancer risk is summarized in Table 1.

Barbisan. G et al. (2011) from Institute of Veterinary Genetics, Faculty of Veterinary Science, National University of La Plata, Buenos Aires, Argentina conducted a case-control study comprising 217 cervical samples, including 103 cervical carcinomas and 114 normal tissue samples. Cervical samples were genotyped for two *XRCC1* SNPs (Arg194Trp and Arg399Gln) by PCR-RFLP. Subjects carrying heterozygous Arg399Gln or the combined Gln399Gln + Arg399Gln variant genotypes had a significantly reduced risk for cervical cancer development. In addition, the 194Arg-399Gln haplotype was also found to be associated with a decreased risk for cervical carcinoma. Their findings suggest that XRCC1 genotypes and haplotypes contribute in reducing the risk for cervical cancer development. Furthermore, genetic susceptibility conferred by Arg399Gln polymorphism operates independently of human papillomavirus infection of cervical tissue.Number of SNPs in XRCC1R399Q and XRCC1R194W in control and patient groups and its p value is presented in Table 2.

Cheng et al. (2009)studied on SNPs of *XRCC1* (at codon 194 and 399) and XRCC1 protein expression in seventy patients with locally advanced cervical carcinoma who underwent neo-adjuvant chemotherapy (NAC). The association of *XRCC1* gene SNPs and protein expression with NAC response were also analyzed. Results showed the response to NAC was not statistically significant in three genotypes, Arg/Arg, Arg/Trp, Trp/Trp of *XRCC1* at codon 194(X^2 = 1.243, P = 0.07), while responses were significantly dif-

Table 1. Quantitative analyses for the relationships between XRCC1 polymorphisms and cervical cancer risk based on adjusted OR (95% CI) [modified from 20].

Genetic Polymorphisms	Comparisons	Sample Size (N)	AdjustedOR 95%CI	$P_{x\text{-test}}$	$P_{heterogeneity}$	Model
XRCC1 Arg399Gln (Total)	Gln/Gln vs. Arg/Arg	6	1.23(0.94,1.61)	0.127	0.012	Random
	Arg/Gln vs. Arg/Arg	6	1.04(0.74,1.45)	0.843	0.001	Random
	Gln/Gln+Arg/Gln vs. Arg/Arg	6	1.06(0.76,1.48	0.736	0.000	Random
XRCC1 Arg399Gln (Asian)	Gln/Gln vs. Arg/Arg	5	1.34(1.01,1.78)	0.046	0.017	Random
	Arg/Gln vs. Arg/Arg	5	1.29(1.11,1.50)	0.001	0.276	Fixed
	Gln/Gln+Arg/Gln vs. Arg/Arg	5	1.25(0.98,1.59)	0.078	0.054	Random
XRCC1 Arg194Trp (Total)	Trp/Trp vs. Arg/Arg	3	2.15(1.53,3.04)	0.000	0.372	Fixed
	Arg/Trp vs. Arg/Arg	3	1.19(0.97,1.46)	0.106	0.338	Fixed
	Trp/Trp+Arg/Trp vs. Arg/Arg	3	1.33(1.09,1.61)	0.004	0.606	Fixed

Table 2. Number of SNPs in *XRCC1* in control and patient groups and its p value [21].

Genotype	Control group (n = 10)	Patient group (n = 35)	p value
XRCC1R194W			
Arg/Arg	9	29	0.579
Arg/Trp	1	6	-
Arg allele frequency	18 (90%)	64 (91%)	0.843
Trp allele frequency	2 (60%)	6 (9%)	-
XRCC1R399Q			
Arg/Arg	1	19	-
Arg/Gln	6	13	0.029*
Gln/Gln	3	3	-
Arg allele frequency	8 (40%)	50 (71%)	0.01*
Gln allele frequency	12 (60%)	20 (29%)	-

ferent in genotypes Arg/Arg, Arg/Gln, Gln/Gln of *XRCC1* at codon 399 ($X^2 = 2.283$, P = 0.020). The risk of failure to chemotherapy in the patients with a Gln allele(Arg/Gln+Gln/Gln) was significantly greater than that with Arg/Arg(OR = 3.254, 95%CI 1.708 ~ 14.951). The expression level of XRCC1 protein was significantly associated with response to NAC. Moreover, the genotype with the Gln allele(Arg/Gln+Gln/Gln) at codon 399, but not codon at 194, presented a significantly higher level of XRCC1 protein expression than that with Arg/Arg genotype (F = 2.699, p = 0.009).They concluded thatSNP of *XRCC1* gene at codon 399 influences the response of cervical carcinoma to platinum-based NAC. This is probably due to changes in expression of XRCC1 protein, affecting response to chemotherapy.

Furthermore, many different polymorphisms of *XRCC1* are found in several types of cancer. Polymorphisms of *XRCC1* Arg399Gln are associated with colorectal cancer.*XRCC1* Arg194Trp polymorphism was associated with non-cardia gastric cancer.In HNSCC, mutated homozygous *XRCC1* AA was found less frequently observed genotype in analyzed groups (YA = 4.7%, YH = 17.1%, and OP = 10.8%). In the YA group, genotype AA occurred significantly less frequently in comparison to YH (p = 0.0116).*XRCC1* Arg399Gln, PARP1 Val762Ala and APE1 Asp148Glu SNPs in the base excision repair pathway may influence the prognosis of advanced non-small cell lung cancer patients following platinum-based chemotherapy. Meta-analysis also suggested that the *XRCC1* Arg399Gln polymorphism is moderately associated with increased risk of gliomas in Asians, while Arg194Trp and Arg280His polymorphisms demonstrated no significant influence.*XRCC1* 194Trp/Trp genotype was strongly significantly associated with an increased risk of hepatocellular carcinoma when compared with the wild-type genotype.*XRCC1* Arg194Trp and *XRCC1*Arg399Gln SNPs were related to the metabolism of platinum-based chemotherapy, and could be used as predictors of clinical outcome of non-small cell lung cancer (NCBI,). One study also found that *XRCC1* codon 399Gln polymorphism is associated with radiotherapy-induced acute dermatitis and mucositis in nasopharyngeal carcinoma (Li et al, 2013). Significant relationships are found between SNPs in *XRCC1* and outcome in esophagus cancer receiving cisplatin based concurrent chemoradiotherapy (XingMing, 2009).

Although cancer treatment with radiation can produce high cure rates, adverse effects often result from this radiotherapy.The identification of molecular variables that predict either sensitivity or resistance of cells to radiotherapy is of major interest in selecting the first-line treatment most likely to be effective. Many investigations have identified a SNP which confers better outcome in patients with cervical cancer.So far, numerous studies have been performed, but the results obtained are heterogeneous and often conflicting. There has been increasing evidence that decreased DNA repair capacity resulting from genetic polymorphisms of various DNA repair genes such as *XRCC1* is associated with improved survival of cancer patients treated with radiotherapy (Jung et al, 2001). Because *XRCC1* is one of the most important DNA repair genes, it is important todetermine whether the *XRCC1* genetic polymorphisms could predict clinical response of patients with cervical carcinoma to radiotherapy. Some studies have assessed the association between *XRCC1* gene polymorphisms and radiotherapy response in various carcinomas, but the re-

sults are inconsistent.Findings above suggest that *XRCC1* polymorphisms including genotypes and haplotypes contribute to susceptibility to the development of cervical cancer, and the increased susceptibility is probably not through increasing susceptibility to human papillomavirus infection.

There is great interest in establishing methods which can be used to predict the individual risk of normal tissue effects after radiotherapy. If these risks were known prior to the onset of therapy, the total dose applied could be reduced in the small proportion of highly sensitive patients and, conversely, radiation dose and possibly the chance to cure could be increased for normal and resistant patients.The results obtained in the study conducted indicate that the endpoints of acute and late tissue toxicity are determined by different molecular and cellular pathways, respectively. Therefore, analyzing the association of SNPs with both acute and late effects will help us not only to identify the SNPs which might be used as markers of the respective risks, but also to unravel the underlying biological mechanisms (Ryu et al, 2004).Although positive associations have been reported between certain SNPs and several normal tissue toxicities, no specific SNP has been definitively linked with radiation sensitivity.

CONCLUSION

We concluded that decreased DNA repair capacity resulting from genetic polymorphisms of *XRCC1*as DNA repair genes is closely associated with improved survival of cancer patients treated with radiotherapy.Even thought the results obtained are heterogeneous and often conflicting, we concluded that SNP of this DNA repair genes have been found to be good predictors of efficacy of radiotherapy.

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