RESEARCH COMMUNICATION

Genetic Risk of DNA Repair Gene Polymorphisms (*XRCC1* and *XRCC3*) for High Risk Human Papillomavirus Negative Cervical Cancer in Northeast Thailand

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Abstract

To identify risk factors other than high risk human papillomavirus infection for the development of cervical cancer, functional polymorphisms of DNA repair genes, *XRCC1* Arg399Gln and Arg194Trp and *XRCC3* Thr241Met, were studied among Northeastern Thai women. Cases (n=111) were defined as squamous cell cervical cancer and controls (n=118) were recruited from healthy women without cervical abnormalities. The *XRCC1* 194Trp/Trp genotype significantly increased the risk for cervical cancer (OR=5.52; 95%CI=1.14-26.64; p=0.03). Among the HPV infection negative group, significantly higher risks for cervical cancer were visualized for *XRCC1* 399Arg/Gln (adjusted OR=3.69; 95%CI=1.04-13.06; p=0.04) and *XRCC1* 194Arg/Trp (adjusted OR=4.13; 95%CI=1.13-15.12; p=0.03). This study indicates that variant types of DNA repair genes play partial roles in modifying individual susceptibility to cervical cancer. Since cervical cancer is a multi-factorial disease, the contribution of DNA repair enzymes to the development of cervical cancer, if it exists may be concealed by HPV infection.

Keywords: Genetic risk - DNA repair genes - cervical cancer - non HPV - Northeast Thailand

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Introduction

Cervical cancer has been a serious national health problem in Thailand, especially in North and Northeast Thailand (Khuhaprema et al., 2010). To reduce new cases of this cancer, risks for the cancer should be identified and regulated. Human papillomavirus (HPV) infection is a principal cause for cervical cancer and more than 80 % of the cases were associated with high risk HPV (hrHPV) infection in this region (Settheetham-Ishida et al., 2005). Although prophylactic vaccines against HPV have been developed, they are effective only in preventing new infections of certain types of HPV (HPV16 and HPV18), and the current hrHPV carriers and new carriers with hrHPV other than type 16 and 18 remain facing the risk. Since only a small part of hrHPV carriers develop cervical cancer, it is indicated that the presence of other factor(s) other than hrHPV infection is responsible for the development of cervical cancer. To prevent and/or reduce cervical cancer cases, it is thus important to unveil cryptic risks for cervical cancer.

Our previous study indicated that number of sexual partners, age at the first intercourse, number of parities and smoking are the risks for cervical cancer (Settheetham-Ishida et al., 2004); among them, parameters other than smoking are directly related with the risk of HPV infection. It is widely accepted that tobacco smoking increases risks for many types of cancer (Stern et al., 2001; Cote et al., 2009). Carcinogens in tobacco smoke, such as nicotine, cotinine and tobacco-specific nitrosamines that can induce DNA damages (Pryor and Stone, 1993) have been detected in the cervical mucus of smokers (Schiffman et al., 1987; McCan et al., 1992). On the other hand, DNA-repair systems are essential for the maintenance of integrity of the genetic material and dysfunction of the systems thus plays critical roles in cancer development (Mathonet et al., 2003). Among the DNA-repair systems, base-excision repair (BER) pathway and double-strand break (DSB) repair pathway constitute a primary defense against lesions generated by ionizing radiation and strong alkylating agents as well as lesions formed by DNA-damaging agents such as smoke (Pryor and Stone, 1993).

X-ray repair cross complementing group 1 (*XRCC1*) is a major DNA repair gene involved in BER, whereas *XRCC3* is in DSB repair (Mathonet et al., 2003). Mutation and polymorphism in DNA repair genes associated with repair efficiency against DNA damage may predispose an individual's cancer susceptibility. Functional genetic polymorphisms of the *XRCC1* are Arg399Gln in the exon 10 and Arg194Trp in the exon 6 and that of the *XRCC3* is

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Table 1. PCR-RFLP Assays for Detecting Polymorphisms in *XRCC1* and *XRCC3*

Polymorphism primer sequence (5'–3')	PCR products	RE	Genotype	Fragment size(s) (bp)
XRCC1 codon 399				
Upper: caagtacagccaggtcctag	248	*Nci* I	Arg/Arg (G/G): 159/89	
Lower: ccttccctcatctggagtac			Arg/Gln (G/A): 248/159/89	
			Gln/Gln (A/A): 248	
XRCC1 codon194				
Upper: gccagggcccctccttcaa	485	*Pvu* II	Arg/Arg (C/C): 485	
Lower: taccctcagacccacgagt			Arg/Trp (C/T): 485, 396, 89	
			Trp/Trp (T/T): 396, 89	
XRCC3 codon 241				
Upper: ggtcgagtgacagtccaaac	455	*Nla* III	Thr/Thr (C/C): 315/140	
Lower: tgcaacggctgagggtctt			Thr/Met (C/T): 315/210/140/105	
			Met/Met (T/T): 210/140/105	

RE, Restriction enzyme

Thr241Met in the exon 7. There has been considerable interest in understanding genetic variability in DNA repair genes and their influence on modifying an individual's susceptibility to cancer (Spitz et al., 2003); however, the former observations were not consistent in terms of their roles in cancer susceptibility (Au et al., 2004; Goode et al., 2002) and influence of the polymorphisms of the *XRCC1* and *XRCC3* on DNA repair capacity is still unclear. In this study, we have selected BER related *XRCC1* and DBS related *XRCC3* to evaluate their roles in the development of squamous cell cervical carcinoma (SCCA).

Materials and Methods

Subjects

Women aged 27-74 years were recruited at Srinagarind Hospital, Khon Kaen University, Thailand, between 2002 and 2003. Cases (n=111) were defined as squamous cell cervical cancer (SCCA) by cytological, colposcopical and histological diagnosis. Controls (n=118) were recruited from healthy women without cervical abnormalities that were diagnosed by the cytological examination. The subjects were informed of the purpose and experimental procedures of this study and written informed consent was obtained. This study was approved by the Ethics Committee of Khon Kaen University.

Genotyping assays

DNA was extracted from peripheral blood cells and used as template in the following PCR assays. Genotyping of DNA repair genes was performed by PCR-restriction fragment length polymorphism (RFLP) methods with appropriate primer sets (Table 1). The primers were designed to amplify the regions of DNA that contain polymorphic sites of interest: *XRCC1* Arg399Gln in the exon 10 (G>A), *XRCC1* Arg194Trp in the exon 6 (C>T) and *XRCC3* Thr241Met in the exon 7 (C>T). The PCR condition consisted basically of as follows; i) activation of Taq polymerase (AmpliTaq Gold, Applied Biosystems) at 95 ˚C for 9 min, ii) 40 cycles of denaturation at 94˚C for 1 min; annealing at 58 ˚C for 1 min; elongation at 72 ˚C for 1 min, and iii) extension at 72 ˚C for 5 min. The PCR products were electrophoresed on 2.5% agarose gel and visualized by ethidium bromide staining to confirm DNA amplification. Each PCR product was digested with an appropriate restriction enzyme (Table 1) for genotyping.

Detection of hrHPV DNA

In this study hrHPV infection in the subjects was evaluated by the presence of the viral genome in the scraped cervical cells by the specific PCR-RFLP tests (Settheetham-Ishida et al., 2005); briefly, DNA was extracted from cells in the Pap test specimens and used as templates in the PCR to amplify a fragment of E6 and E7 of malignant types of HPV including HPV-16, -18, -31, -33, -35, -52b and -58. The PCR products were then digested with appropriate restriction enzymes for strain identification.

Statistical analyses

The chi-square test was used to compare genotype frequency in between the cervical carcinoma patients and the controls. Associations between the *XRCC* genotypes and risk of SCCA with or without smoking history or hrHPV infection were studied using odds ratios and 95% confidence interval (OR and 95% CI). Multivariate logistic regression analysis with 800-STATA-PC was also employed to calculate adjusted ORs. P-values less than 0.05 were considered significantly different.

Table 2. Risk of *XRCC* genotypes for SCCA

Genotype and frequency of variant allele		Number of cases	Number of controls	OR [95% CI]	*p*-value	Adjusted OR* [95% CI]	*p*-value
XRCC1 codon 399	Arg/Arg	66	69	1		1	
	Arg/Gln	41	44	0.97 [0.56-1.67]	0.95	1.45 [0.66-3.18]	0.34
	Gln/Gln	4	5	0.84 [0.21-3.25]	0.79	2.41 [0.36-16.1]	0.36
	Arg/Gln+Gln/Gln	45	49	0.96 [0.57-1.63]	0.88	1.47 [0.69-3.37]	0.31
	freq. allele Gln	0.221	0.229 (p>0.05)				
XRCC1 codon 194	Arg/Arg	53	65	1		1	
	Arg/Trp	49	51	1.18 [0.69-2.01]	0.54	1.21 [0.57-2.59]	0.61
	Trp/Trp	9	2	5.52 [1.14-26.6]	0.03	6.73 [0.92-48.8]	0.06
	Arg/Trp+Trp/Trp	58	53	1.34 [0.79-2.25]	0.27	1.38 [0.67-2.87]	0.38
	freq. allele Trp	0.302	0.233 (p>0.05)				
XRCC3 codon 241	Thr/Thr	101	106	1		1	
	Thr/Met	10	12	0.87 [0.36-2.11]	0.76	2.13 [0.61-7.43]	0.23
	freq. allele Met	0.045	0.051 (p>0.05)				

OR [95% CI]: Odds ratios [95% confidence interval] *adjusted with multiple logistic regression for age, HPV status and smoking

Table 3. *XRCC* genotype, HPV status and risk for SCCA

Genotype		Number of cases	Number of controls	OR [95% CI]	*p*-value	Adjusted OR* [95% CI]	*p*-value
HPV negative group							
XRCC1 codon 399	Arg/Arg	5	56	1		1	
	Arg/Gln	9	35	2.88 [0.89-9.29]	0.08	3.69 [1.04-13.1]	0.04
	Gln/Gln	1	5	2.24 [0.22-23.1]	0.49	4.53 [0.34-59.7]	0.25
	Arg/Gln+Gln/Gln	10	40	2.80 [0.89-8.82]	0.08	2.83[0.89- 9.04]	0.08
XRCC1 codon 194	Arg/Arg	5	56	1		1	
	Arg/Trp	9	38	2.65 [0.82-8.53]	0.10	4.13 [1.13-15.1]	0.03
	Trp/Trp	1	2	5.60 [0.43-73.1]	0.19	7.23 [0.50- 103]	0.14
	Arg/Trp+Trp/Trp	10	40	2.80 [0.89-8.82]	0.08	3.06 [0.95- 9.83]	0.06
XRCC3 codon 241	Thr/Thr	13	84	1		1	
	Thr/Met	2	12	1.07 [0.21-5.37]	0.93	1.55 [0.27- 8.74]	0.62
HPV positive group							
XRCC1 codon 399	Arg/Arg	61	13	1		1	
	Arg/Gln	32	9	0.76 [0.29- 1.96]	0.57	0.71 [0.26- 1.93]	0.51
	Arg/Gln+Gln/Gln	35	9	0.83 [0.32- 2.13]	0.70	0.82 [0.32- 2.14]	0.70
XRCC1 codon 194	Arg/Arg	48	9	1		1	
	Arg/Trp	40	13	0.57 [0.22- 1.48]	0.25	0.57 [0.21- 1.55]	0.27
	Arg/Trp+Trp/Trp	48	13	0.69 [0.27- 1.77]	0.44	0.69 [0.27- 1.78]	0.45

OR [95% CI]: Odds ratios [95% confidence interval]; *adjusted with multiple logistic regression for age and smoking

Results

Allele frequencies and distribution of genotypes of the *XRCC1* codon 399 and 194, and *XRCC3* codon 241 are shown in Table 2. No significant deviation from Hardy-Weinberg equilibrium in the genotype distribution for the three loci was confirmed in the controls. Prevalence of the *XRCC1* 194Trp allele was not significantly different in the cases and controls (p>0.05) but *XRCC1* 194Trp/Trp genotype significantly increased the risk for SCCA (OR=5.52; 95%CI=1.14-26.64; p=0.03), whereas heterozygous genotype did not (OR=1.18; 95%CI=0.69-2.01; p=0.54). The genotype and allele distribution of *XRCC1* 399 and *XRCC3* 241 polymorphisms did not alter the risk for SCCA (Table 2). When ORs were calculated for combined genotypes of *XRCC1* 399 and 194, there was a trend to increase the risk for SCCA in Arg/Arg-Trp/Trp genotype (OR=4.31; 95%CI=0.82-22.53; *p*=0.08) . When genotypes were combined for three loci, a trend of increased risk was still observed in the presence of *XRCC1* 194Trp/Trp genotype (OR=4.08; 95%CI=0.77-21.54; *p*=0.09).

Interaction between the *XRCC* genotypes and the risk for SCCA by the status of hrHPV infection was analyzed (Table 3). The type of hrHPV included HPV 16, 18, 31, 33, 52b and 58. Among the hrHPV infection negative group, significantly higher risks for SCCA were visualized for *XRCC1* 399Arg/Gln (adjusted OR=3.69; 95%CI=1.04-13.06; *p*=0.04) and *XRCC1* 194Arg/Trp (adjusted OR=4.13; 95%CI=1.13-15.12; *p*=0.03).

When risk of the *XRCC* polymorphisms for SCCA was evaluated by the smoking status, none of the genotypes showed deviation in the risk for the cancer susceptibility.

Discussion

Among the genotypes defined by the three missense variants, only *XRCC1* 194 Trp/Trp showed an increased risk for SCCA (5.5-fold). A similar finding for the *XRCC1* polymorphism in SCCA development was observed in Chinese (Huang et al., 2007); this report also observed the *XRCC1* 399 Gln showed higher risks for the cervical cancer. On the contrary, Niwa et al. (2005) reported that *XRCC1* 399 Gln was not the risk for SCCA, but a risk for cervical adenocarcimona/adenosquamous cell carcinoma in Japanese. Controversial results in epidemiological studies have often been found in between Occidental and Oriental populations and they may be attributable to the difference in the allele frequencies; however, these three Asian populations showed similar allele frequencies. This suggests that the *XRCC* variant alleles may not be a critical risk for SCCA, and that their contribution may be cryptic but visible in the presence of other risk factor(s).

Among SCCA cases, 13.5 % were found to be negative for hrHPV DNA in our study (Settheetham-Ishida et al., 2005). When subjects were sorted by the status of hrHPV infection, the following trend was visualized (Table 3); contribution of *XRCC1* variant alleles to the risk for SCCA was identified in the hrHPV negative group, whereas in the hrHPV infected group, there was a trend that rather low risks for SCCA were found in the presence of the *XRCC1* variant alleles. Among hrHPV negative individuals, increased risk for SCCA was found in both heterozygous genotypes, *XRCC1* 399 Arg/Gln and 194 Arg/Trp, with the OR of 3.7 and 4.1-fold, respectively. Contribution of *XRCC1* 399 Gln was thus unveiled by excluding influence of the principal risk factor, hrHPV infection. This is somehow comparable situation observed in the Japanese cervical cancers (Niwa et al., 2005) where the risk of *XRCC1* 399Gln was shown in cervical acenocarcinoma/adeno squamous cell carcinoma of which HPV infection rate was rather low. Our study indicates that among hrHPV carriers, a strong driving force of hrHPV infection leads to the development of SCCA irrespective of *XRCC* genotypes.

Our previous study showed that smoking, including

passive smoking, is one of the critical risks for the development of SCCA (Settheetham-Ishida et al., 2004). This means that some of the pro-carcinogens/carcinogens in tobacco smoke may be responsible for the development of SCCA; however, the null genotype of phase I detoxification enzymes, GSTM1 and GSTT1, did not increase the risk for SCCA in the smokers (Settheetham-Ishida et al., 2009) and moreover, in this study, the variant allele for DNA repair genes, *XRCC1* and *XRCC3*, did not increase the risk as in the Japanese (Niwa et al., 2005) and Chinese (He et al., 2008) cervical carcinoma. It is implicated that modification or activation of pro-carcinogens/carcinogens by phase II metabolizing enzymes may play direct roles in the development of SCCA. Studying the role of phase II enzymes, such as CYP1 and CYP2 families, in the development of cervical cancer is strongly recommended.

This study indicates that variant types of DNA repair genes play partial roles in modifying individual susceptibility to cervical cancer. Cervical cancer is a multi-factorial disease, and thus the contribution of repair enzymes if it ever exists to the development of the cervical cancer may be concealed by the major risk factor, hrHPV infection, otherwise the increased risk should be found not only among hrHPV negative individuals but also hrHPV positive individuals.

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