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Polymorphism of XRCC1 Codon 399 and Prognosis of Non-Small Cell Lung Cancer Patients After Radiotherapy

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Abstract

To assess that the XRCC1 399Gln variant contributes to sensitivity to ionizing radiation treatment and is associated with progression-free and overall survival, one hundred and ninety-five lung cancer patients were recruited at the Asan Medical Center from 2000 to 2003. We determined the genotypes of the *XRCC1* genes by PCR-RFLP. Kaplan-Meier survival curves and the log-rank test were used to analyze the effects of genotypes on survival. Hazard ratios, adjusted for age, sex, and other potential confounders, were calculated using the Cox-proportional hazard model. Patients carrying the 399Gln variant allele under radiotherapy only had a shorter progression-free and overall survival than those with the 399Arg homozygote. However, when we analyzed for the effect of the XRCC1 Arg399Gln polymorphism in the combined treatment of surgical resection and radiotherapy, we found that patients with the 399Gln variant allele had a longer progression-free and overall survival. This study shows different associations between the XRCC1 Arg399Gln polymorphism and progression-free or overall survival depending on treatment protocol in patients with NSCLC.

Keywords: XRCC1, Polymorphism, Lung cancer, Radiotherapy, Prognosis, Survival analysis

Lung cancer is the leading cause of cancer mortality in South Korea as in the Western countries[1-3]. Despite efforts to improve survival in patients with lung cancer, there has been no significant advance in the prognosis. The overall 5-year survival of patients with this disease across all stages is only about 10-15%[1]. Because lung tumors with the same histopathology have been known to show widely varying sensitivities to ionizing radiation, genetically determined susceptible patients may show enhanced sensitivity to radiotherapy[4-6]. Therefore, we need to find an optimized cancer treatment by adjusting the administered dose on the basis of an individual sensitivity to ionizing radiation[4]. In addition, since NSCLC responds poorly to radiotherapy, biological markers predicting individual radiosensitivity are greatly needed[1].

The cytotoxic effect of radiation treatment is principally attributable to the formation of single-strand breaks, and base excision repair is a major pathway for removal of these lesions from genomic DNA[7]. Therefore, the DNA repair mechanism may play a central role in tumor development and in the response of cancer cells to radiotherapy[6].

The human XRCC1 gene, one of the DNA repair genes, was identified because of its ability to restore DNA repair activity in a Chinese hamster ovary mutant cell line EM9[8]. The XRCC1 protein is involved in base-excision repair, and interacts with DNA ligase III, DNA polymerase β, poly (ADP-ribose) polymerase (PARP), polynucleotide kinase and AP endonuclease I[9-12]. The base-excision repair pathway is designed to remove non-bulky base adducts, which are produced by oxidation due to ionizing radiation or oxidative damage[13,14].

One polymorphism of the XRCC1 gene at codon 399 results in an amino acid substitution from arginine to glutamine in the BRCT I domain of the gene[15], indicating that the XRCC1 variant may have functional significance. Previous studies have shown that the XRCC1 399Gln allele is associated with higher levels of DNA adduct and an increased lung cancer risk[16,17]. Moreover, it contributes to ionizing radiation susceptibility as measured by prolonged G2 cell cycle delay[18]. Since XRCC1 399Gln variants are relatively common and base excision repair is particularly important for repairing oxidative base damage due to ionizing radiation exposure[18-20], these findings may have clinical significance in the manage-

ment and prognosis of lung cancer patients. If oxidative DNA damage is potentially lethal, then the inhibition of its repair should lead to increased tumor cell death after radiotherapy. Therefore, reduced base-excision repair capacity would prolong the lifetime of oxidative DNA damage, making an eventual DNA breakage more likely in tumor cells[21].

In the present study, we hypothesized that the XRCC1 399Gln variant contributes to sensitivity to ionizing radiation treatment, and that it is inversely associated with progression-free and overall survival, because the genetic susceptibility conferred by a reduced base-excision repair capacity probably modifies the effect of radiotherapy at the individual level.

In this study, 69 patients (53.9%) had stage I-IIIa disease and 59 (46.1%) stage IIIb or IV disease. At present, 56 patients are alive without evidence of lung cancer and 54 are alive with the disease. Thir-teen patients have died from the disease and three patients have died from other causes. Two patients were lost during follow-up. Sixty-six patients receiv-ed radiotherapy after surgical resection of the primary tumor, and 62 underwent radiotherapy after chemotherapy.

The number of patients with the XRCC1 399Arg homozygote was 67 and of those with XRCC1 399Gln allele was 61 (Table 1). Chi-square tests for heterogeneity showed that age, sex, ECOG performance status, weight loss, tumor stage, and cell types were not significantly dependent upon the XRCC1 Arg399Gln polymorphism ($P > 0.05$). In addition, no significant association was observed between the XRCC1 polymorphism and the treatment protocol ($P = 0.385$).

Univariate analysis was performed upon progression-free and overall survival of lung cancer patients after radiotherapy (Table 2). Significant progression-free and overall survival advantage was observed for tumor stage I-IIIa versus tumor stages IIIb and IV ($P < 0.01$). However, other variables were not found to be independent prognostic markers of either progression-free or overall survival. In the subsequent analyses, the effects on survival were evaluated separately according to the tumor stage (I-IIIa vs. IIIb and IV).

Figures 1 and 2 show the effect of the XRCC1 Arg-399Gln polymorphism on progression-free survival. Patients in tumor stage I-IIIa carrying the 399Gln variant allele had a significantly longer progression-free survival than those with the 399Arg homozygote [Mean progression-free survival 68.2 months (SE 4.2) compared with 21.5 months (SE 1.7); $P = 0.003$]. However, patients carrying the 399Arg homozygote showed longer progression-free survival in the tumor stage IIIb and IV, even though the XRCC1 Arg-399Gln polymorphism was not a statistically significant predictor of survival ($P = 0.105$). When we controlled for other potential predictors, such as age, sex, ECOG performance status, weight loss, cell types, and treatment protocol in the Cox-proportional hazards model, the XRCC1 codon 399 polymorphism remained significantly correlated with survival in tumor stage I-IIIa ($P = 0.018$), whereas it was not a significant predictor in tumor stages IIIb and IV ($P = 0.291$) (Table 3).

When we analyzed the effect of the XRCC1 Arg-399Gln polymorphism on overall survival, it did not reach statistical significance according to the Cox-proportional hazards model after controlling for other potential predictors, or by the log-rank test for univariate analysis for both stage I-IIIa and IIIb-IV ($P > 0.05$).

Survival analysis in lung cancer patients was used

Table 1. Characteristics of patients receiving radiotherapy after surgical resection or radiotherapy alone for lung cancer, and the relationship with XRCC1 Arg399Gln polymorphism.

	XRCC1 Codon 399		
	Arg/Arg	Arg/Gln, Gln/Gln	P-value*
	Number of patients (%)		
Sex			
Male	95 (82.6)	72 (90.0)	
Female	20 (17.4)	8 (10.0)	0.148
Age			
<= 61	53 (46.1)	43 (53.7)	
> 61	62 (53.9)	37 (46.3)	0.29
ECOG			
0	38 (33.0)	20 (25.0)	
1	74 (64.4)	53 (66.3)	
2	3 (2.6)	7 (8.8)	0.104
Wt loss			
<=5%	97 (84.4)	65 (81.3)	
5-10%	10 (8.7)	10 (12.5)	
>10%	8 (7.0)	5 (6.2)	0.685
Stage			
I	8 (6.9)	6 (7.5)	
II	34 (29.6)	23 (28.8)	
III	73 (63.5)	51 (63.7)	0.985
Histopathology			
Squamous cell carcinoma	76 (66.7)	47 (58.8)	
Adenocarcinoma	38 (33.3)	33 (41.2)	0.260
Treatment protocol			
Radiation only	55 (47.8)	39 (48.8)	
Surgery+Radiation	60 (52.2)	41 (51.2)	0.899

*P-values obtained after Chi-square tests

Table 2. Univariate analysis of the progression-free and overall survival of lung cancer patients using Kaplan-Meier survival analysis

Variables	No. of patients	Progression-free survival (%)		P-value	Overall survival (%)		P-value
		1-year	3-year		1-year	3-year	
Gender							
Male	167	74.8	56.5		83.2	64.2	
Female	28	77.9	58.4	0.867	85.0	-	0.767
Age							
<= 61	96	70.7	59.0		81.2	68.9	
>61	99	80.4	52.4	0.915	85.8	58.5	0.511
ECOG							
0	58	76.0	58.8		82.3	68.8	
1	127	73.6	53.6		83.8	62.3	
2	10	-	-	0.241	-	-	0.746
Wt loss							
<= 5%	162	74.9	55.0		84.6	65.4	
5-10%	20	71.0	-		71.6	-	
>10%	13	91.7	-	0.754	87.5	-	0.805
Stage							
I	14	80.8	-		92.3	-	
II	57	93.8	82.9		96.2	83.7	
III	124	66.2	45.4	0.001	76.6	57.9	0.017
Histopathology							
Squamous carcinoma	123	80.0	65.4		84.6	74.9	
Adenocarcinoma	71	67.7	41.4	0.007	81.5	47.9	0.070
Treatment protocol							
Radiation only	94	57.2	-		75.8	49.2	
Surgery+Radiation	101	92.1	74.6	<0.001	90.2	78.0	<0.001

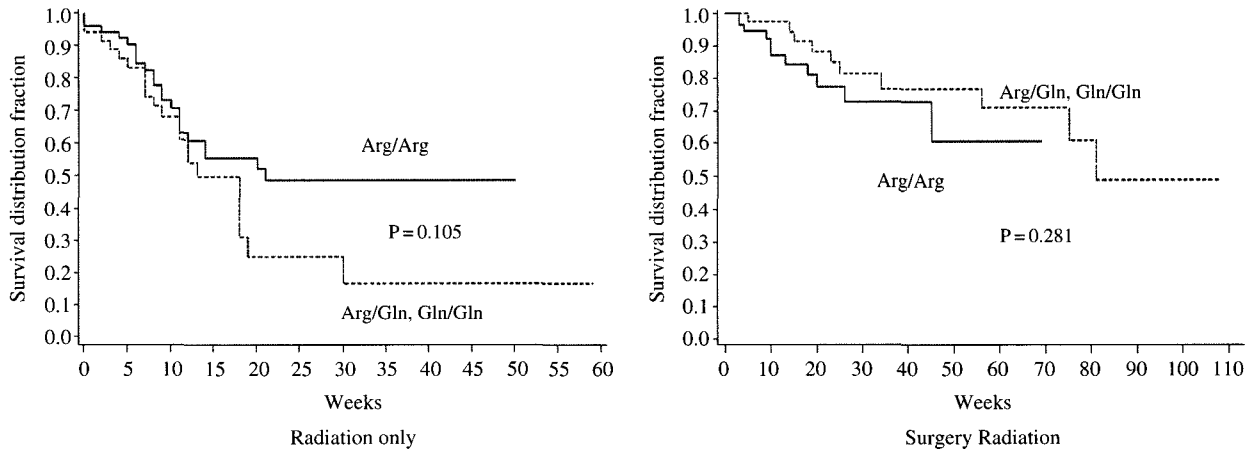

Fig. 1. Progression-free survival of patients with respect to the XRCC1 Arg399Gln polymorphism according to the treatment protocol

to investigate whether the XRCC1 Arg399Gln polymorphism could be used as a predictor of prognosis in lung cancer patients. Two different outcomes, progression-free and overall survival, were used as indicators to evaluate its effects on survival time.

This study found an association between XRCC1 Arg399Gln polymorphism and progression-free survival after radiotherapy for patients with NSCLC tumor stage I-IIIa, whereas no significant relationship was found between the two in tumor stages IIIb and IV. Since multivariate analyses showed the strength of effect of the XRCC1 polymorphism on time to

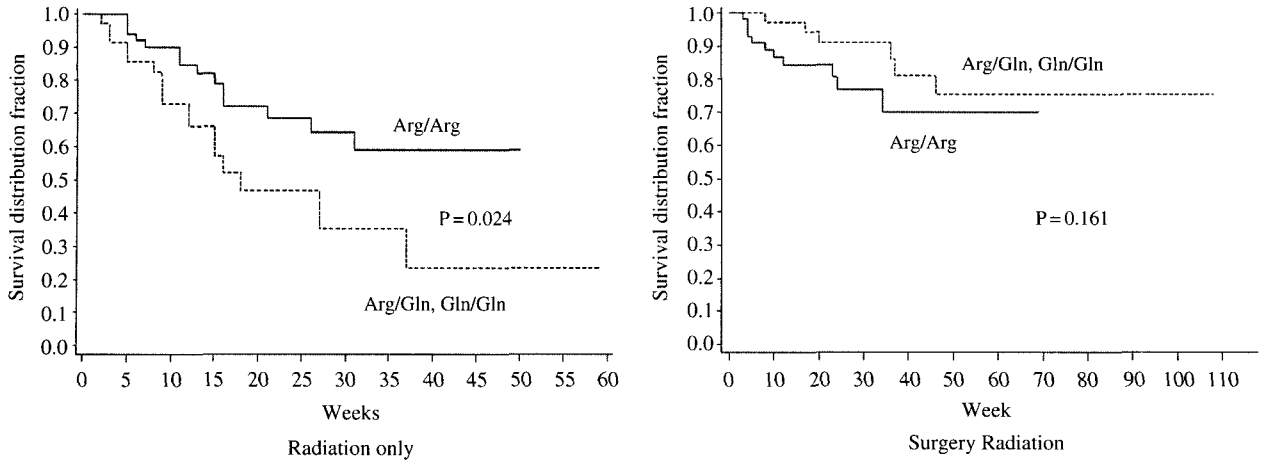

Fig. 2. Overall survival of patients with respect to the XRCC1 Arg399Gln polymorphism according to the treatment protocol

Table 3. Multivariate analysis of the progression-free survival of lung cancer patients after radiotherapy according to tumor stage using Cox-proportional hazards regression

Predictors	Radiation only		Surgery+Radiation	
	RR (95% CI)	P-value	RR (95% CI)	P-value
XRCC1 Arg399Gln	1.46 (0.78, 2.71)	0.236	0.42 (0.16, 1.12)	0.082
Sex	1.36 (0.50, 3.67)	0.547	0.16 (0.04, 0.67)	0.012
Age	1.00 (0.96, 1.04)	0.926	0.95 (0.90, 1.01)	0.101
Wt loss	0.70 (0.40, 1.23)	0.219	0.58 (0.08, 4.18)	0.588
ECOG	0.70 (0.38, 1.27)	0.234	1.85 (0.66, 5.14)	0.241
Stage	1.26 (0.68, 2.30)	0.463	2.95 (1.11, 7.87)	0.030
Histology	1.99 (1.04, 3.80)	0.037	6.40 (2.16, 18.98)	0.001

Table 4. Multivariate analysis of the overall survival of lung cancer patients after radiotherapy according to tumor stage using Cox-proportional hazards regression

Predictors	Radiation only		Surgery+Radiation	
	RR (95% CI)	P-value	RR (95% CI)	P-value
XRCC1 Arg399Gln	2.11 (1.01, 4.37)	0.046	0.42 (0.15, 1.19)	0.503
Sex	2.15 (0.59, 7.80)	0.247	0.29 (0.06, 1.35)	0.114
Age	1.02 (0.97, 1.07)	0.433	1.02 (0.96, 1.10)	0.503
Wt loss	0.73 (0.39, 1.38)	0.336	1.06 (0.23, 4.82)	0.944
ECOG	0.66 (0.33, 1.31)	0.238	2.16 (0.69, 6.71)	0.183
Stage	1.17 (0.61, 2.24)	0.639	2.69 (0.92, 7.89)	0.071
Histology	1.68 (0.79, 3.56)	0.181	4.92 (1.57, 15.40)	0.006

progression in NSCLC patients, particularly in those with stage I-IIIa tumors, the use of molecular predictors of survival may provide important guidelines for the prognosis of patients under radiotherapy. Our study results are supported by the finding that the XRCC1 399Gln allele is associated with prolonged ionizing radiation-induced cell cycle delay[18,22]. The 399Gln allele of the XRCC1 gene has also been reported to be associated with higher levels of aflatoxin B1-DNA adducts and glycophorin NN mutations in placental DNA, suggesting that the Arg399Gln polymorphism may result in deficient DNA repair[16].

However, we observed no favorable effect of variant types of the XRCC1 Arg399Gln polymorphism on overall survival time. The reason for this absence of a significant difference on overall survival may be that the number of deaths was too small to allow statistical analysis.

Although cancer radiotherapy depends on cell death due to DNA damage inflicted upon rapidly dividing tumor cells, it also causes severe damage to normal cells, thus leading to enhanced susceptibility to tumor recurrence or metastasis, particularly in the patients with a reduced DNA repair capacity[6]. However, it would be difficult to determine exactly those with a favorable prognosis or those at risk of progression. We suspect that tumor mass burden as well as DNA repair genes may play key roles in radiosensitivity and tumor progression. Therefore, our observation of a lack of an effect or of a reverse effect of reduced DNA repair capacity by XRCC1 399Gln allele in NSCLC patients of tumor stage IIIb or IV, is not surprising because the advanced tumor stage of these patients may restrict the potential benefit of reduced DNA repair capacity during radiotherapy, or the reduced DNA repair capacity could render tumor recurrence or metastasis more likely.

The XRCC1 Arg399Gln polymorphism has been reported to be linked to an increased risk of colorectal, gastric, breast, and lung cancers[17,23-25]. Therefore, the application of DNA repair gene polymorphisms has been considered primarily as a target for cancer prevention, because the cancer cases attributable to them may be large due to the high frequency for variant genotypes in the general population[22]. However, genetically determined DNA repair capacity may also modulate treatment response and lung cancer susceptibility[26]. Our present data demonstrate an extension of the idea of primary prevention based on gene-environmental interaction or the effect of environmental risk factors on cancer treatment, to a secondary prevention based on genetically tailored treatment strategies. Therefore, the identification of radiosensitive lung cancer patients before therapy may allow the individual tailoring of treatment, the minimization of normal tissue damage, and the enhancement of the effectiveness of ionizing radiation[6,27]. This approach can thus potentially increase response rates and survival outcomes while reducing unnecessary treatment.

Several genetic markers have been shown to be related with chemotherapy resistance[26]. Cancer patients with a lower expression level of ERCC1 mRNA have enhanced response and survival on cisplatin-based chemotherapy[28,29]. However, most of these reports have focused on the prognosis of cancer patients after chemotherapy. Therefore, the role of DNA repair genetic markers in the outcome of lung cancer after radiotherapy has not been evaluated. In the present study, we hypothesized that lung cancer patients with the XRCC1 399Gln variant allele might have a more favorable prognosis than those with XRCC1 399Arg wild homozygotes. Our results provide some evidence that genetic markers for DNA repair may affect sensitivity to radiation therapy, thus showing that lung cancer patients with stage I-IIIa harboring the XRCC1 399Gln variant allele are sensitized to ionizing radiation treatment.

Methods

Patients and Samples

128 lung cancer patients were recruited at the Asan Medical Center from 2000 to 2003. Patients were eligible if their tumor stages were I to IV NSCLC, and they had an ECOG performance status of 2 or less. Patients receiving radiotherapy after surgical resection or chemotherapy were eligible, but patients having received previous radiation before surgical resection were excluded. All patients entered in the study received radiation therapy using high-energy linear accelerators. Radiation was delivered to the tumor in fractions of 1.8 Gy daily, 5 days per week, and a total of 50-60 Gy was administered to each patient.

Subjects completed a self-administered questionnaire for demographic information. Information about clinical condition was obtained from the subject's medical records. All patients were required to give written informed consent, and the study protocol was approved by the medical center's Institutional Review Board. Blood samples were collected from all study subjects at the time of enrollment. Patients were followed at least every 3 months from time of study entry.

Genotyping Assays

Genomic DNA was extracted from peripheral blood lymphocytes using a QIAamp DNA Blood Mini Kit. We determined the genotypes of the XRCC1 genes by PCR-RFLP. All of the PCR reactions were performed in a total reaction volume of 20 μL containing 50 ng of DNA, 1 U *Taq* polymerase in 1 X PCR buffer, 1.5 mM $MgCl_2$, 250 μM dNTPs and 1 μM of each primer. Thermal cycling conditions consisted of an initial denaturation step at 94°C for 5 min, then 30 cycles of 94°C for 1 min, annealing at 62°C for 1 min and at 72°C for 1 min, followed by a final extension step at 72°C for 7 min. The PCR products were digested with *Msp*I and analyzed in a 3% Metaphor gel (BMA, USA).

Statistical Analysis

The Chi-square test for heterogeneity was used to test for significant associations between the XRCC1

polymorphism (homozygous 399Arg vs. homozygous and heterozygous 399Gln) and categorical variables, i.e., sex, age above and below the median age, ECOG, weight loss, tumor stage, and histopathology.

Kaplan-Meier survival curves and the log-rank test were used to analyze for the genotype effect on progression-free and overall survival. Progression-free survival was defined as time from study entry to tumor recurrence or the occurrence of metastasis. Overall survival was defined as the time from study entry to death from any cause. Hazard ratios, adjusted for age, sex, and other potential confounders, were calculated by Cox-proportional hazards regression. A probability level of 0.05 was used as the criterion of statistical significance. All analyses were performed using SAS (ver 6.12) statistical software.

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