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The association between four SNPs of X-ray repair cross complementing protein 1 and the sensitivity to radiotherapy in patients with esophageal squamous cell carcinoma

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Abstract. Early stage diagnosis and therapeutic outcomes of esophageal squamous cell carcinoma remain poor. In order to evaluate the association between 4 single nucleotide polymorphisms (SNPs) of X-ray repair cross complementing protein 1 (XRCC1) and the sensitivity to radiotherapy in patients with esophageal squamous cell carcinoma (ESCC), the present study identified 4 SNPs of XRCC1 and evaluated the distribution of these genotypes among patients with ESCC. Venous blood samples from 175 patients with ESCC were collected and DNA was extracted. The 4 SNPs of the XRCC1 gene fragment were amplified using three primer pairs, which were sequenced. The mismatches were analyzed and identified using Basic Local Alignment Search Tool software. The sensitivity to radiotherapy was graded as effective and non-effective, according to the treatment results of the patients. The present study successfully amplified and sequenced 4 SNPs of XRCC1 in 112 out of the 175 patients with ESCC. The effective response rate of radiotherapy was 84.8% among the 112 patients. The effective response rate of patients with no mutation in the SNPs was 74.3%, and the rate increased to 89.6% in patients that had ≥1 mutation out of the 4 SNPs (χ^2=4.389; P=0.036). For G28152A and G28152A mutations the effective response rate of patients was 91.2% (χ^2=4.014; P=0.045) and 91.5% (χ^2=4.451; P=0.035), respectively, which was significantly different compared to patients with no mutation (P=0.045 and P=0.035, respectively). The present results suggest that the 4 SNPs of XRCC1 are associated with the effective response rate of radiotherapy in patients with ESCC. The mutation of SNP G28152A was particularly important and may be a potential genomic predictor for radiotherapy sensitivity in patients with ESCC.

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Key words: esophageal squamous cell carcinoma, X-ray repair cross complementing protein 1, single nucleotide polymorphisms, radiotherapy, radio-sensitivity

Introduction

Worldwide, esophageal cancer is the eighth most common type of cancer, and in 2012 there were 456,000 novel cases and 400,000 mortalities (1). Esophageal squamous cell carcinoma (ESCC) is the most common type of esophageal cancer (2); however, early stage diagnosis and therapeutic outcomes of patients with ESCC remain poor (3-5). Only 15-25% of patients with ESCC survive for >5 years following diagnosis (3).

Esophagography, endoscopic ultrasonography, endoscopy, computed tomography (CT) and 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) are commonly used in the diagnosis and staging of ESCC. Endoscopy is the most sensitive method for the detection and diagnosis of esophageal cancer. Endoscopic screening has decreased the ESCC-associated mortality, and esophagography, CT and FDG-PET are used to assess the invasion and length of tumors, direct invasion to adjacent organs and lymph node and distant metastasis (6).

There are numerous approaches for the treatment of patients with ESCC, including surgery, endoscopic therapy, chemotherapy and radiotherapy. Neoadjuvant chemotherapy and radiotherapy is performed as a standard treatment for locally advanced ESCC (7). Radiotherapy is one of the most common therapeutic methods for the treatment of patients with ESCC, and leads to the apoptosis of tumor cells by directly or indirectly destroying the cell DNA (8). A previous study demonstrated that the radiosensitivity of cells depends on the extent of damage the radiation exerts on the DNA and the efficiency of the host in repairing the DNA (9). As DNA damage accumulates, it may lead to uncontrolled cell proliferation and differentiation, which may result in tumorigenesis. DNA damage may be prevented by DNA repair genes; X-ray repair cross complementing protein 1 (XRCC1) is an important gene for DNA repair (8). It is significant in maintaining chromosomal stability. An elevated level of sister chromatid

exchange, widely used as an indicator of genetic damage, is characteristic of XRCC1 functional deficiency, and single nucleotide polymorphisms (SNPs) of XRCC1 has been associated with a higher risk of cancer (10). SNP is a variation in a single nucleotide that may occur at a specific position in the genome, where each variation is present to a certain degree within a population. The repairing ability of XRCC1 is negatively associated with the radiosensitivity of cells (11).

The present study used polymerase chain reaction to detect SNPs in patients with ESCC. The present study compared the curative effect of radiotherapy in these patients with various genotypes and investigated the association between 4 SNPs of XRCC1 and the sensitivity of radiotherapy in patients with ESCC.

Materials and methods

Patients. The present study was approved by the Internal Review Board of Chaozhou People's Hospital (Chaozhou, China). In addition, written informed consent was obtained from the patients involved in the present study. The present study recruited 175 patients with ESCC that were pathologically diagnosed with ESCC and received radiotherapy as an initial treatment at Chaozhou People's Hospital between December 2011 and December 2013. Among these patients, DNA was successfully amplified and 4 SNPs of XRCC1 were sequenced and analyzed in the blood samples of 112 patients, which consisted of 81 men and 31 women (average age, 60.6 years; median age, 60.0 years). The location of tumors in the 112 patients was cervical and upper thoracic in 10 patients, middle thoracic in 71 patients and lower thoracic in 31 patients. According to the 2010 Clinical Non-Operative Treatment of Esophageal Cancer Staging Standard (12), there were 28 patients with stage II and 84 patients with stage III ESCC. All the patients were free from distant metastasis and major organ dysfunction at diagnosis. The average Karnofsky score of the patients was ≥70 (13). Written informed consent was obtained from all patients. The present study was approved by the Internal Review Board of Chaozhou People's Hospital.

Radiation treatment. All the patients received radiation treatment, which was either three-dimensional conformal radiation therapy (3DCRT; 67 patients) or intensity-modulated radiation therapy (IMRT; 45 patients). The radiation treatments were performed on patients in the supine position with the head extended and using a fixed body thermoplastic film. The patients underwent enhanced chest CT (SOMATOM Definition AS; Siemens AG, Munich, Germany) (3DCRT thickness, 5 mm; IMRT thickness, 3 mm) in order to visualize the target region for radiation. Following CT, the images were transferred to the radiotherapy treatment planning system (Eclipse™ version 8.6; Varian Medical Systems, Palo Alto, CA, USA). The clinical target volume (CTV) comprised the gross tumor volume visible on the CT scan (GTV), neck metastatic lymph nodes (GTVnd) and lymphatic drainage region, which required an 0.8 cm extension in the sagittal and coronal direction and a 5 cm extension upward and downward from the standard irradiated region of GTV and GTVnd. Appropriate adjustments were adopted to suit individual patients. The present study administered a planning target volume (PTV) extension of 0.5 cm according to the CTV region. The exposure dose was GTV 60-64 Gy and PTV 50-54 Gy. All the patients were administered with nedaplatin (20 mg/m^2, weekly for 2-4 cycles) as synchronous chemotherapy.

Evaluation. At the end of the radiation treatment and 1 month following, the 112 patients were administered with a barium swallow (Qingdao Dongfeng Chemical Co., Ltd., Qingdao, China) and subsequently underwent enhanced chest CT. According to the Response Evaluation Criteria In Solid Tumors for curative effect (14), the patients were divided into the following 4 groups: Complete response (CR), the tumor was not observed for ≥4 weeks; partial response (PR), the maximal tumor diameter was reduced by ≥30% for ≥4 weeks; stable disease (SD), the maximal tumor diameter altered between PR and progressive disease (PD); and PD, the maximum diameter of the tumor increased ≥20%. CR and PR were considered as the valid (effective) group and SD and PD were the null (non-effective) group.

Primer design and DNA amplification. A Rapid DNA Extraction kit (Yaneng Biotechnology Shenzhen Co., Ltd., Shenzhen, China) was used to extract genomic DNA from peripheral blood samples from the patients prior to radiotherapy. The present study designed 3 pairs of specific primers using reference strains identified from the GenBank® gene sequence library (National Institutes of Health, Bethesda, MA, USA), and optimized them using Primer-BLAST version 5.0 software (National Institutes of Health; available from http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Table I details the primers used in the present study, which were synthesized by Shanghai Invitrogen Biotechnology Co., Ltd. (Shanghai, China). Polymerase chain reaction (PCR) was performed using 3 pairs of specific forward and reverse primers (DNA polymerase used in the PCR reaction and the kit used for the master mix of the PCR reaction were provided by Aidlab Biotechnologies Co., Ltd., Beijing, China) in a total volume of 50 μl using a PCR machine (Life Express Thermal Cycler TC-96/G/H(b); Hangzhou Bioer Technology Co., Ltd, Zhejiang, China). The PCR products (7 μl) were run on a 2% agarose gel (Biowest, Hong Kong, China) using electrophoresis at 210 volts for 15 minutes (ethidium bromide, Aidlab Biotechnologies Co., Ltd.). A DNA Molecular Weight Marker II (200 bp; Aidlab Biotechnologies Co., Ltd.) was used as a reference molecular weight ladder. Target gene fragment amplification bands were observed under UV lamps.

Genotype analysis. Following bidirectional sequencing of the PCR products, the present study analyzed the sequences using the following software: BioEdit version 3.0 (Ibis BioSciences, Carlsbad, CA, USA; available from http://www.mbio.ncsu.edu/bioedit/bioedit.html), Basic Local Alignment Search Tool (National Institutes of Health; available from http://blast.ncbi.nlm.nih.gov/Blast.cgi) and DNASTAR® Primer Premier version 5.0 (DNASTAR, Inc., Madison, WI, USA). Subsequently, the present study compared the coding region of XRCC1 with the GenBank® gene library reference sequence and performed an analysis on the polymorphisms of the variations observed in the sequences. In the present study, the 112 collected identical sequences were merged and divided into 8 genotypes as specified in Table II.

Table I. Primers used for restriction fragment length polymorphism-PCR for 4 SNPs of the X-ray repair cross complementing protein 1 gene.

SNP	Primer, 5'-3'	PCR, bp	Temperature, ˚C
rs3213245,c.-77C>T	F, CACTTTAGCCAGCGCAGGG R, GGAAGTTCACCTATGGGCTCT	392	60
c26304t,Arg194Trp	F, TTTGGCTTGAGTTTTGTACGGTT R, CGCTGGCTGTGACTATGAAGGGA	595	62
G27466A,Arg280His and G28152A,Arg399Gln	F, CTCTTTGTCTTCTCCAGTGCCA R, CACAGGATAAGGAGCAGGGTT	752	65

PCR, polymerase chain reaction; SNP, single nucleotide polymorphisms; bp, base pairs; F, forward; R, reverse.

Table II. Association between genotypes and the curative effect of radiotherapy in patients with esophageal squamous cell carcinoma.

			Curative effect, n	
Genotypes	SNP mutation	Total, n	Null group	Valid group
Total		112	17	95
T+Arg+Arg+Arg	None	35	9	26
C+Arg+Arg+Arg	1st	11	2	9
T+Trp+Arg+Arg	2nd	10	1	9
T+Arg+His+Arg	3rd	8	1	7
T+Arg+Arg+Gln	4th	37	3	34
C+Arg+Arg+Gln	1st and 4th	7	1	6
T+Trp+His+Arg	2nd and 3rd	1	0	1
T+Arg+His+Gln	3rd and 4th	3	0	3

SNP, single nucleotide polymorphisms; T, threonine; Arg, arginine; C, cysteine; His, histidine; Gln, glutamine; Trp, tryptophan.

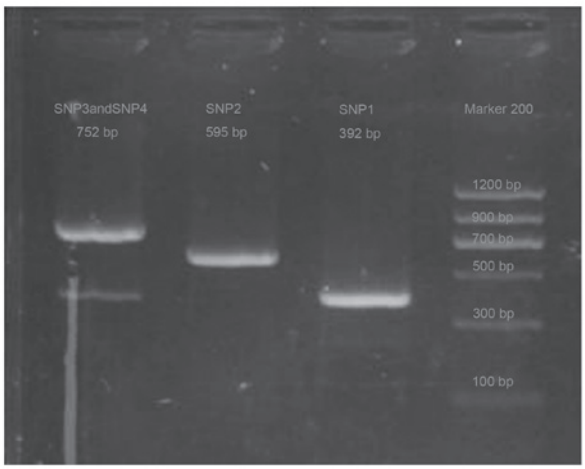

Figure 1. Polymerase chain reaction products for 4 single nucleotide polymorphisms of X-ray repair cross complementing protein 1 in the blood samples of patients with esophageal squamous cell carcinoma.

Statistics approach. SPSS version 16.0 software (SPSS, Inc., Chicago, IL, USA) was used for the analysis of data. Differences in sensitivity to radiotherapy between genotype groups were analyzed using the χ^2 test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

PCR products of 4 SNPs of XRCC1 . Following DNA amplification and electrophoresis analysis, the 112 blood samples of the patients with ESCC exhibited specific DNA bands at 392, 595 and 752 bp, which corresponded to the 4 SNPs identified in XRCC1 (Fig. 1).

Mutations in 4 SNPs of XRCC1. An analysis of the base alterations identified in the 4 SNPS demonstrated that the non-coding base alterations in the 1st SNP locus mutation (rs3213245, C.-77 C>T) indicated the position of the 77th base ahead of the initiation codon (ATG). In addition, mutations in the 2nd, 3rd and 4th SNPs resulted in alterations of the following amino acids: SNP locus mutation replaced arginine with tryptophan (C26304T, Arg194Trp); 3rd SNP locus mutation replaced arginine with histidine (G27466A, Arg280His); 4th SNP locus mutation replaced arginine with glutamine (G28152A, Arg399Gln).

According to the gene polymorphisms of the samples, the present study compared the XRCC1 gene sequence with the coding reference sequence. There were certain identical sequences of XRCC1 in the 112 patient blood samples, which were divided by the present study into 8 groups. The present

Table III. Association between the characteristics of patients with esophageal squamous cell carcinoma and the efficacy rate of radiotherapy.

Characteristic	Curative effect, n		χ^2	P-value
	Null group	Valid group		
Total	17	95		
Gender				
Male	12	69	0.030	0.862
Female	5	26		
Age, years				
≥60	10	48	0.398	0.528
<60	7	47		
Tumor location				
Cervical/upper thoracic	3	7	1.963	0.375
Middle thoracic	10	61		
Lower thoracic	4	27		
Tumor stage				
II	0	28	6.681	0.010
III	17	67		
Radiotherapy				
3DCRT	9	58	0.395	0.530
IMRT	8	37		

3DCRT, three-dimensional conformal radiation therapy; IMRT, intensity-modulated radiation therapy.

study identified that the group with the majority of patients, in which 35 gene sequences shared the same sequence in the reference strain and its 4 SNPs, had no mutations. This group was defined as Chaozhou Local Strains. There were 11 sequences that possessed the 1st SNP locus mutation (rs3213245.C.-77 C>T); 10 sequences possessed the 2nd SNP locus mutation (C26304T, Arg194Trp); 8 gene sequences possessed the 3rd SNP locus mutation (G27466A, Arg280His); and 37 sequences possessed the 4th SNP locus mutation (G28152A, Arg399Gln). There were 7 gene sequences that possessed the 1st and 4th SNP locus, 1 gene sequence possessed the 2nd and 3rd SNP locus, and 3 gene sequences possessed the 3rd and 4th SNP locus mutations (Table II).

Follow-up of patients. By the end of February 2014, the follow-up time of the patients ranged between 2.0 and 26.4 months (median, 16.2 months). The follow-up rate was 95.5%. Survival time was defined as the time between the beginning of radiotherapy and the last follow-up or patient mortality. In total, 31 patients succumbed to ESCC during follow-up, which consisted of 13 patients with local tumor recurrence, 8 patients with distant metastasis and 10 patients with tumor recurrence and metastasis.

Efficacy of radiotherapy. The effective response rate of radiotherapy in the 112 patients was 84.8%. Table III summarizes the association between the efficacy of radiotherapy and patient characteristics. The efficacy rate of radiotherapy in patients with stage II ESCC was increased compared with patients with stage III ESCC (100 and 79.8%, respectively; χ^2=6.681; P=0.01). The efficacy rate of radiotherapy had no significant association with the gender and age of the patients, tumor location and mode of radiotherapy.

Association between SNP mutations and ESCC sensitivity to radiotherapy. The efficacy rate of radiotherapy in patients with no SNP mutations was 74.3%, compared to 89.6% in patients with ≥1 SNP locus mutation (χ^2=4.389; P=0.036; Table IV). The efficacy rate of radiotherapy in patients containing only the 4th SNP locus mutation (G28152 A, Arg399Gln) and for all patients containing mutations in the 4th and other SNP locus was 91.9 and 91.5%, respectively (Table II). Compared with the non-mutation group, the difference was statistically significant (χ^2=4.014 and χ^2=4.451, respectively; P=0.045 and P=0.036, respectively; Tables V and VI). The difference in the efficacy of radiotherapy in patients with Chaozhou Local Strains and those with only 1 SNP locus mutation (1st, 2nd or 3rd) was not significantly different (P>0.05).

The stratification analysis demonstrated that the effective rate of radiotherapy in stage III patients with no SNP mutations was 74.1% and those with ≥1 mutation was 91.2% (χ^2=4.403; P=0.036). The effective rate of radiotherapy in patients with mutations in the 4th SNP locus and those containing mutations in the 4th and other SNP locus was 92.3% and 93.5%, respectively, which was not significant compared to the no mutation group (χ^2=3.333 and χ^2=3.312, respectively; P=0.068 and P=0.073, respectively). Table VII summarizes the association between the SNP

Table IV. Association between SNP mutations and the radiotherapy sensitivity of patients with esophageal squamous cell carcinoma.

SNP	Curative effect, n		χ^2	P-value
	Null group	Valid group		
Total	17	95		
None	9	26	4.389	0.036
≥1	8	69		

SNP, single nucleotide polymorphisms.

genotypes and the sensitivity to radiotherapy in patients with stage III ESCC.

Discussion

As a radical treatment, radiotherapy is important in the comprehensive management of esophageal cancer. Radiotherapy induces the apoptosis of tumor cells by damaging cell DNA directly or indirectly. However, the 5-year survival rate of patients with ESCC that are treated solely with radiotherapy is <10% and the recurrence rate is 60-80%. (12) It has been reported that an increase in the radiotherapeutic dose between 50 and 70 Gy does not achieve a corresponding improvement in efficacy, which suggests that dose is not a key factor for the efficacy of radiotherapy (15). Clinically, patients with the same tumor stage often exhibit a varied response to radiotherapy (15). This difference greatly depends on the balance between the damage to the DNA and the DNA repair ability. DNA damage and repair is a complex process that is comprised of several enzymes and proteins. If a repair gene is mutated, the DNA repairing capacity of the entire genome decreases, which may lead to adverse effects, including tumorigenesis (16). The difference in the ability to repair DNA damage also varies among individuals (17). The balance between DNA damage and repair ultimately affects the sensitivity of cells to radiotherapy. Currently, studies that focus on the genes that alter the sensitivity of tumors to radiotherapy are increasing.

XRCC1 is the first gene that was revealed to affect cell sensitivity to ionizing radiation (18). It is involved in repairing base excision and single-strand breaks, which are caused by ionizing radiation and oxidative damage (18). The repairing ability of XRCC1 and radiosensitivity is negatively associated (10). The human genome mainly manifests itself in multi-nucleotide polymorphisms. In the process of DNA repair, SNP mutations in XRCC1 alter the encoded amino acid; therefore altering the structure and activity of the repair enzymes encoded by XRCC1 (19-21). This may be the key factor that causes the differences in DNA repairing ability observed in individuals, and may be responsible for the various outcomes of radiotherapy.

The identification of SNPs of XRCC1 is valuable to predict the radiosensitivity of cells. At present, XRCC1 has 3 major SNP locus, which are located in exons 6, 9 and 10. These SNPs cause alterations at C26304T to Arg194Trp, G27466A to Arg280His and G28152A to Arg399Gln (22).

Table V. Efficacy rate of radiation in patients with only the 4th SNP locus mutation.

SNP	Curative effect, n		χ^2	P-value
	Null group	Valid group		
None	9	26	4.014	0.045
Only 4th	3	34		

SNP, single nucleotide polymorphisms.

Table VI. Efficacy rate of radiation in all patients with the 4th SNP locus mutation.

SNP	Curative effect, n		χ^2	P-value
	Null group	Valid group		
None	9	26	4.451	0.035
All 4th	4	43		

SNP, single nucleotide polymorphisms.

Table VII. Association between genotypes and radiotherapy sensitivity of patients with stage III esophageal squamous cell carcinoma.

SNP	Curative effect, n	
	Null group	Valid group
None	7	20
≥1	5	52
Only 4th	2	25
All 4th	3	31

SNP, single nucleotide polymorphisms.

The present study identified that 4 SNP locus of XRCC1, including a locus in the non coding region (rs3213245, C.-77 C>T) that has not been previously reported in radiotherapy, to the best of our knowledge. This locus is hypothesized to affect the transcription and mediation of the XRCC1 gene. Hao *et al* (23) demonstrated that polymorphisms of rs3213245, C.-77 C>T increased the binding of the XRCC1 promoter to the transcription inhibitory factor, which lead to a decrease in the promoter activity and protein expression. The present study identified that the 1st SNP locus mutation does not have a significant effect on radiosensitivity of patients compared with the non-mutation group; however, this may be due to a limited number of samples. The efficacy of radiotherapy was 83.33% (15/18) in the mutation group and 74.29% (26/35) in the non-mutation group (P=0.355).

Previous studies concerning XRCC1 focus on cancer susceptibility or risk. Sreeja *et al* (24) identified that

399 homozygous dominant XRCC1 carriers were more likely to have lung cancer compared with the heterozygous genotype carriers. Previous studies suggest that polymorphisms of XRCC1 at Arg399Gln are associated with the incidence of several types of cancer, including lung, stomach, esophagal and bladder cancer and nasopharyngeal carcinoma (25-27). Yang *et al* (25) demonstrated that the XRCC1 Gln/Gln genotype was increased in individuals with ESCC in a population in Taiwan. Therefore, XRCC1 is an important genetic risk factor for tumor development. A meta-analysis of the XRCC1 gene Arg194Trp by Huang *et al* (26) indicates that polymorphisms in XRCC1 are a risk factor for cancer in China. Wu *et al* (27) investigated the polymorphisms of XRCC1 Arg194Trp and Arg399Gln, and the roles these polymorphisms play in the development of esophageal cancer. The authors demonstrated that the risk of developing esophageal cancer is associated with polymorphisms of XRCC1 gene Arg194Trp, and indicate that homozygous 194Trp/Trp carriers with restriction endonuclease Pvu II have the highest risk of esophageal cancer.

There have been numerous contradictory studies concerning the association between XRCC1 and the sensitivity of patients to radiotherapy. Liu *et al* (28) revealed an association between the expression of XRCC1 and excision repair cross-complementation group 1 (ERCC1) and the efficacy of radiotherapy and the prognosis of patients with ESCC. The authors indicated that the expression of XRCC1 and ERCC1 may play a role in esophageal carcinogenesis. However, the study did not reveal the association between XRCC1 and radiosensitivity in esophageal carcinoma. Warnecke-Eberz *et al* (29) investigated the predictive value of ERCC1 and XRCC1 polymorphisms on the effect of chemotherapy (cisplatin and 5-fluorouracil) in esophageal cancer. The results demonstrated that ERCC1 may be a predictor for chemotherapy, and XRCC1 Arg194Trp was not a suitable marker. However, the authors concluded that SNPs of XRCC1 and ERCC1 may be applied to treatment strategies for patients in the future. Cornetta *et al* (30) used a 2 Gy X-ray irradiation on normal peripheral blood, which revealed that the DNA loading in homozygous 399Gln/Gln blood was decreased compared with the DNA loading in wild type and heterozygous DNA. The authors concluded that polymorphisms in repairing genes may affect the DNA repairing ability of an individual. Zhang *et al* (31) identified SNPs in hOGG1, XRCC1 and XRCC3 in the peripheral blood of 94 patients with ESCC using restriction fragment length polymorphism-PCR and revealed that polymorphisms at Arg399Gln of XRCC1 were clearly associated with radiosensitivity.

The present study demonstrated that the effective rate of radiotherapy is associated with patients with the 4th SNP locus mutation, and the effective rate of radiotherapy in these patients was significantly increased compared with non-mutation patients. In addition, the present results indicated that the 4th SNP locus mutation may be a marker for radiation efficacy in patients with esophageal cancer. The present study identified that the efficacy rate of radiation in patients with mutations at ≥1 SNP locus is increased compared with patients with no mutations. Although there is no significant difference in the efficacy rate of radiation between patients with no mutations and patients with mutations at the 1st, 2nd, 3rd or 4th SNP locus, additional study is required due to the limited sample size in the present study.

Based on the stratification analysis of patients with stage III ESCC, the efficacy rate of radiotherapy in patients with ≥1 SNP locus mutation was significantly increased compared with the rate in patients with no mutations. The effective rate of radiotherapy was not different in patients with mutations only at the 4th SNP locus or with other locus combining with the 4th SNP locus.

In conclusion, the sensitivity of radiotherapy to ESCC is a complex process that involves multiple genes. The present study suggests that SNP mutations are closely associated with the sensitivity of radiotherapy in patients with ESCC. Specifically, mutations in the 4th SNP locus (G28152A, Arg399Gln) of XRCC1 significantly improves the curative effect of radiotherapy. Therefore, the 4th SNP locus may be a promising marker for the sensitivity of radiotherapy. Mutations in the 1st SNP locus (rs3213245, C.-77, C>T), which has never been previously reported in the literature, is hypothesized to potentially increase the sensitivity of radiotherapy. Understanding the association between mutations of various SNPs and the sensitivity of radiotherapy may aid in the development of individualized radiotherapy for patients with ESCC.

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