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Original Article

XRCC1 polymorphisms rs25487 and rs1799782 and radiotherapy sensitivity in esophageal squamous cell carcinoma patients

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Abstract: X-ray repair cross complementing group 1 (XRCC1) is a key mediator of single-strand break DNA repair. However, XRCC1 polymorphisms in esophageal squamous cell carcinoma (ESCC) and the prognostic value for radiotherapy sensitivity are still not fully understood. This study aimed to investigate the association between radiotherapy sensitivity of ESCC and XRCC1 polymorphisms rs25487 and rs1799782. Peripheral blood samples were collected from 97 patients with ESCC and genomic DNA was extracted using phenol-chloroform method. Genotyping was performed using SNaPshot® Multiplex Kit. Associations between genotypes and clinicopathological characteristics were analyzed with Pearson's Chi-Square test. No significant association was observed between rs25487 or rs1799782 genotype and the age, gender, tumor differentiation, treatment and response of the patients. rs1799782 genotype was associated with tumor size (P=0.029). The patients who had one or both of rs25487 or rs1799782 mutant type showed better response to radiotherapy than patients with wild-type alleles (total effective rate, P=0.048). In conclusion, ECSS patients with one or both of rs25487 and rs1799782 mutation have better response to radiotherapy than patients with wild-type of both SNPs. XRCC1 polymorphisms rs25487 and rs1799782 may be used as prognostic biomarkers for ESCC.

Keywords: X-ray repair cross complementing group 1, esophageal squamous cell carcinoma, polymorphism, radiotherapy

Introduction

Esophageal cancer (EC) is the eighth most common and aggressive cancers in the world and is highly prevalent in China [1, 2]. EC is pathologically classified as esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EADC) [3]. ESCC is the predominant pathologic type of EC in China, accounting for 90% [4]. Genetic factors are known to have crucial impacts on the incidence and development of ESCC. Recent genome-wide association studies have shown that multiple single nucleotide polymorphisms (SNPs) are associated with ESCC [5-9].

Radiotherapy (RT) is an important treatment for ESCC, but the outcomes remain unsatisfactory with a 5-year survival rate of about 10% [10]. RT alone has limited success [11]. Chemoradiotherapy (CRT) is the main treatment for advanced ESCC, and cisplatin and 5-fluorouracil (5-FU) are commonly used currently. The platinum-based compound is able to form intramolecular or intermolecular DNA cross-links, which can damage DNA by leading to single and double strand breaks [12].

X-ray repair cross complementing group 1 (XRCC1) is a key mediator of single-strand break DNA repair, including nucleotide excision repair and base excision repair [13]. In the past decade, a number of studies have suggested an association between polymorphisms in codons 194 (base C to T, rs1799782) and 399 (base G to A, rs25487) of XRCC1 and cisplatin resistance [14-16]. However, the relationship of XRCC1 polymorphisms and short-term efficacy of RT or CRT in Chinese ESCC patients is still not fully understood. This study aimed to exam-

Table 1. Clinicopathologic characteristics of 97 patients

Characteristics	n	rs25487			*P*	rs1799782			P
		AA	AG	GG		TT	CT	CC	
Age (years)					0.670				0.296
≤60	37	1	15	21		1	19	17	
>60	60	3	28	29		6	24	30	
Gender					0.748				0.812
Male	67	3	28	36		5	31	31	
Female	30	1	15	14		2	12	16	
Differentiation					0.462				0.248
Well	4	0	3	1		0	3	1	
Mod	28	2	14	12		1	9	18	
Poor	65	2	26	37		6	31	28	
Tumor size (cm)					0.313				0.029
<2	10	0	3	7		0	7	3	
2~5	60	4	29	27		2	24	34	
>5	27	0	11	16		5	12	10	
Treatment					0.087				0.888
Radiotherapy	63	4	31	28		5	27	31	
Chemoradiotherapy	34	0	12	22		2	16	16	
Response evaluation					0.817				0.888
PD	13	0	4	9		0	7	6	
SD	3	0	1	2		0	1	2	
PR	17	1	7	9		2	7	8	
CR	64	3	31	30		5	28	31	

PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response. *P* value was measured with Pearson's Chi-Square test.

ine XRCC1 polymorphisms rs25487 and rs1799782 in ESCC patients who were treated with RT or CRT. Furthermore, we investigated the relationship between XRCC1 polymorphisms and the clinicopathological variables and short-term efficacy of treatment.

Patients and methods

Participants

Total 97 patients with ESCC who visited Jiangsu Cancer Hospital (Nanjing, China) between January 2011 and July 2012 were included in this study. Patients with distant metastasis or other malignant diseases were excluded. 63 patients were treated with radiotherapy and 34 patients were treated with chemoradiotherapy. The standard dose of radiotherapy was a total of 60-64 Gy given in 2-Gy daily fractions. During radiotherapy, 34 patients received combination chemotherapy with cisplatin (25-30 mg/m^2 on day 1-3) and 5-FU (450-500 mg/m^2 on days 1-5) every 4 weeks for a total of 8 weeks. Evaluation of the response was based on the Response Evaluation Criteria in Solid Tumors (RECIST 1.1). Blood samples were collected before RT and CRT. All patients gave written informed consent. The study was approved by Ethics Committees of Jiangsu Cancer Hospital.

Genotyping

DNA extraction was carried out using the phenol-chloroform extraction method as described previously [17]. Genotyping was performed using SNaPshot® Multiplex Kit (Life Technologies) according to the manufacturer's protocol on the Applied Biosystems 3730 DNA Analyzer. The sequences of the primers were as follows: rs25487 forward 5'-CCCTCAGATCACACCTAACTG-3', reverse 5'-CATTGCCCAGCACAGGATAAG-3', and extending 5'-TTTTTTTGGCGTGTGAGGCCTTACCTC-3'; rs1799782 forward 5'-TAAGCTGTACCTGTCACTCCC-3', reverse 5'-TCAACCCTACTCACTCAGGAC-3', and extending 5'-TTTTCTGGGGATGTCTTGTTGATCC-3'.

Statistical analysis

Statistical analyses were performed with SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Deviations from Hardy-Weinberg equilibrium were evaluated by the Chi-Square test. Associations between genotypes and clinicopathological characteristics or radiotherapy responses were measured with Pearson's Chi-Square test. Statistical significance was set at $P<0.05$.

Results

Characteristics of the study population

97 ESCC patients were included in this study. Clinical characteristics and rs25487 and rs1799782 genotypes of all 97 patients were presented in **Table 1**. Both SNPs conformed to Hardy-Weinberg equilibrium. rs25487 genotypic frequencies in different age, gender, tumor differentiation, tumor size, treatment and response evaluation groups showed no significant difference. Similar results were observed for rs1799782 except that it showed significant difference in patients with different tumor size (P=0.029).

Table 2. Relation of rs25487 with total effective rate/desease control rate in 97 patients

Genotype	Total effective rate			Desease control rate		
	Responder (CR+PR)	Non-responder (SD+PD)	P	Responder (CR+PR+SD)	Non-responder (PD)	P
A/A	4	0	0.269	4	0	0.341
A/G	38	5		39	4	
G/G	39	11		41	9	
A/A+A/G	42	5	0.132	43	4	0.170
G/G	39	11		41	9	

PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response. *P* value was measured with Pearson's Chi-Square test.

Table 3. Relation of rs1799782 with total effective rate/desease control rate in 97 patients

Genotype	Total effective rate			Desease control rate		
	Responder (CR+PR)	Non-responder (SD+PD)	P	Responder (CR+PR+SD)	Non-responder (PD)	P
T/T	7	0	0.465	7	0	0.495
C/T	35	8		36	7	
C/C	39	8		41	6	
T/T+C/T	42	8	0.892	43	7	0.858
C/C	39	8		41	6	

PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response. *P* value was measured with Pearson's Chi-Square test.

Table 4. Combined effort of rs25487 and rs1799782 and total effective rate/desease control rate in 97 patients

Genotype	Total effective rate			Desease control rate		
	Responder (CR+PR)	Non-responder (SD+PD)	P	Responder (CR+PR+SD)	Non-responder (PD)	P
Mutant type	68	10	0.048	70	8	0.065
G/G+C/C	13	6		14	5	

PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response. Mutant type: one or both of rs25487 and rs1799782 had mutant type. *P* value was measured with Pearson's Chi-Square test.

XRCC1 genotype and therapy efficacy

In all 97ESCC patients, no significant association was found between rs25487 (**Table 2**), rs1799782 (**Table 3**) and the total effective rate or disease control rate (all P>0.05). However, when we tested the combined effort of the two SNPs, the results showed that patients who had one or both rs25487 and rs1799782 mutant type responded better than patients with both wild-type alleles (**Table 4**: total effective rate, P=0.048; disease control rate, P=0.068).

Discussion

Currently, RT remains one of the most effective and well-established treatments for ESCC. However, radioresistance is observed in some patients [18]. A variety of factors such as tumor suppressors, cell cycle regulators, angiogenic factors, signaling molecules and DNA repair molecules are correlated with the prediction of the response of ESCC patients to RT [19].

DNA repair plays important role in maintaining the integrity and stability of human genome as well as avoiding genetic mutations. DNA repair pathways mainly include base excision repair, DNA double-strand-break repair, mismatch repair and nucleotide excision repair [20]. Inhibition of these pathways can result in genetic instability and predisposition to cancer [21]. XRCC1 is involved in efficient repair of DNA single-strand breaks induced by ionizing

radiation and alkylating agents [22]. XRCC1 functions by interacting with other components such as DNA glycosylases and Ligase III [23].

Recent studies have shown that genetic variants of XRCC1 are related to the risk of lung cancer [22, 24], thyroid cancer [25], breast cancer [26], urothelial cancer [27] and esophageal cancer [28]. Srivastava et al. found that XRCC1 polymorphisms rs25487 and rs1799782 conferred low risk for gallbladder cancer in North Indian population [29]. In Chinese, XRCC1 polymorphism rs25487 may increase the risk of breast cancer [30] and glioma [31]. Zhou et al. investigated a set of 97 patients with ESCC and found that rs25487 (base G to A) or rs1799782 (base C to T) was not associated with overall survival [21]. However, they did not show the combine effects of the variant genotypes on clinicopathologic characteristics and short-term efficacy.

In this study we observed that rs1799782 genotype was related with tumor size of ESCC patients, suggesting that rs1799782 may act as a predicator for the malignancy of ESCC. Interestingly, the two SNPs had combined effects. Patients who had one or both rs25487 or rs1799782 mutant type responded better than patients who had both wild-type alleles. These results suggest that each mutation in one gene may affect only a part of DNA repair function [32]. Moreover, combined DNA repair gene polymorphisms are likely to determine ESCC patients' responses to DNA damaging treatment. Therefore, further identification of XRCC1 SNPs may help predict therapeutic outcomes in ESCC patients and develop effective therapy strategies [33].

In conclusion, our data present clinical relevance of XRCC1 polymorphisms rs25487 and rs1799782 in Chinese ESCC patients. rs25487 and rs1799782 genotypes have combined effects on radiotherapy sensitivity in these patients, and may be used as prognostic biomarkers in ESCC.

Disclosure of conflict of interest

None.

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References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.

[2] Tang WR, Chen ZJ, Lin K, Su M, Au WW. Development of esophageal cancer in chaoshan region, China: association with environmental, genetic and cultural factors. Int J Hyg Environ Health 2015; 218: 12-8.

[3] Vizcaino AP, Moreno V, Lambert R, Parkin DM. Time trends incidence of both major histologic types of esophageal carcinomas in selected countries, 1973-1995. Int J Cancer 2002; 99: 860-8.

[4] Zhang P, Wang J, Lu T, Wang X, Zheng Y, Guo S, Yang Y, Wang M, Kolluri VK, Qiu L, Shen F, Fan L, Li J, Wang Y, Wei Q, Jin L, Wang J, Wang M. miR-449b rs10061133 and miR-4293 rs12-220909 polymorphisms are associated with decreased esophageal squamous cell carcinoma in a Chinese population. Tumour Biol 2015; 36: 8789-95.

[5] Kang M, Sang Y, Gu H, Zheng L, Wang L, Liu C, Shi Y, Shao A, Ding G, Chen S, Tang W, Yin J. Long noncoding RNAs POLR2E rs3787016 C/T and HULC rs7763881 A/C polymorphisms are associated with decreased risk of esophageal cancer. Tumour Biol 2015; 36: 6401-8.

[6] Zhu S, Shao B, Hao Y, Li Z, Liu H, Li H, Wang M, Wang K. No association of single nucleotide polymorphisms involved in GHRL and GHSR with cancer risk: a meta-analysis. Cancer Biomark 2015; 15: 89-97.

[7] Li WQ, Pfeiffer RM, Hyland PL, Shi J, Gu F, Wang Z, Bhattacharjee S, Luo J, Xiong X, Yeager M, Deng X, Hu N, Taylor PR, Albanes D, Caporaso NE, Gapstur SM, Amundadottir L, Chanock SJ, Chatterjee N, Landi MT, Tucker MA, Goldstein AM, Yang XR. Genetic polymorphisms in the 9p21 region associated with risk of multiple cancers. Carcinogenesis 2014; 35: 2698-705.

[8] Wu C, Wang Z, Song X, Feng XS, Abnet CC, He J, Hu N, Zuo XB, Tan W, Zhan Q, Hu Z, He Z, Jia W, Zhou Y, Yu K, Shu XO, Yuan JM, Zheng W, Zhao XK, Gao SG, Yuan ZQ, Zhou FY, Fan ZM, Cui JL, Lin HL, Han XN, Li B, Chen X, Dawsey SM, Liao L, Lee MP, Ding T, Qiao YL, Liu Z, Liu Y, Yu D, Chang J, Wei L, Gao YT, Koh WP, Xiang YB, Tang ZZ, Fan JH, Han JJ, Zhou SL, Zhang P, Zhang DY, Yuan Y, Huang Y, Liu C, Zhai K, Qiao Y, Jin G, Guo C, Fu J, Miao X, Lu C, Yang H, Wang C, Wheeler WA, Gail M, Yeager M, Yuenger J, Guo ET, Li AL, Zhang W, Li XM, Sun LD, Ma BG, Li Y, Tang S, Peng XQ, Liu J, Hutchinson A, Jacobs K, Giffen C, Burdette L, Fraumeni

JF Jr, Shen H, Ke Y, Zeng Y, Wu T, Kraft P, Chung CC, Tucker MA, Hou ZC, Liu YL, Hu YL, Liu Y, Wang L, Yuan G, Chen LS, Liu X, Ma T, Meng H, Sun L, Li XM, Li XM, Ku JW, Zhou YF, Yang LQ, Wang Z, Li Y, Qige Q, Yang WJ, Lei GY, Chen LQ, Li EM, Yuan L, Yue WB, Wang R, Wang LW, Fan XP, Zhu FH, Zhao WX, Mao YM, Zhang M, Xing GL, Li JL, Han M, Ren JL, Liu B, Ren SW, Kong QP, Li F, Sheyhidin I, Wei W, Zhang YR, Feng CW, Wang J, Yang YH, Hao HZ, Bao QD, Liu BC, Wu AQ, Xie D, Yang WC, Wang L, Zhao XH, Chen SQ, Hong JY, Zhang XJ, Freedman ND, Goldstein AM, Lin D, Taylor PR, Wang LD, Chanock SJ. Joint analysis of three genome-wide association studies of esophageal squamous cell carcinoma in Chinese populations. Nat Genet 2014; 46: 1001-6.

[9] Zhao H, Zheng L, Li X, Wang L. FasL gene -844 T/C mutation of esophageal cancer in South China and its clinical significance. Sci Rep 2014; 4: 3866.

[10] Liu Q, Cai XW, Wu B, Zhu ZF, Chen HQ, Fu XL. Patterns of failure after radical surgery among patients with thoracic esophageal squamous cell carcinoma: implications for the clinical target volume design of postoperative radiotherapy. PLoS One 2014; 9: e97225.

[11] Zhu LL, Yuan L, Wang H, Ye L, Yao GY, Liu C, Sun NN, Li XJ, Zhai SC, Niu LJ, Zhang JB, Ji HL, Li XM. A meta-analysis of concurrent chemoradiotherapy for advanced esophageal cancer. PLoS One 2015; 10: e0128616.

[12] Yamaguchi M, Sugio K. Current status of induction treatment for N2-stage III non-small cell lung cancer. Gen Thorac Cardiovasc Surg 2014; 62: 651-9.

[13] Xu W, Wang S, Chen Q, Zhang Y, Ni P, Wu X, Zhang J, Qiang F, Li A, Røe OD, Xu S, Wang M, Zhang R, Zhou J. TXNL1-XRCC1 pathway regulates cisplatin-induced cell death and contributes to resistance in human gastric cancer. Cell Death Dis 2014: 5: e1055.

[14] Kudo K, Gavin E, Das S, Amable L, Shevde LA, Reed E. Inhibition of Gli1 results in altered c-Jun activation, inhibition of cisplatin-induced upregulation of ERCC1, XPD and XRCC1, and inhibition of platinum-DNA adduct repair. Oncogene 2012; 31: 4718-24.

[15] Siddiqui-Jain A, Bliesath J, Macalino D, Omori M, Huser N, Streiner N, Ho CB, Anderes K, Proffitt C, O'Brien SE, Lim JK, Von Hoff DD, Ryckman DM, Rice WG, Drygin D. CK2 inhibitor CX-4945 suppresses DNA repair response triggered by DNA-targeted anticancer drugs and augments efficacy: mechanistic rationale for drug combination therapy. Mol Cancer Ther 2012; 11: 994-1005.

[16] Jin ZY, Zhao XT, Zhang LN, Wang Y, Yue WT, Xu ST. Effects of polymorphisms in the XRCC1, XRCC3, and XPG genes on clinical outcomes of platinum-based chemotherapy for treatment of non-small cell lung cancer. Genet Mol Res 2014; 13: 7617-25.

[17] Potluri K, Mahas A, Kent MN, Naik S, Markey M. Genomic DNA extraction methods using formalin-fixed paraffin-embedded tissue. Anal Biochem 2015; 486: 17-23.

[18] Kim JJ, Tannock IF. Repopulation of cancer cells during therapy: an important cause of treatment failure. Nat Rev Cancer 2005; 5: 516-25.

[19] Okumura H, Uchikado Y, Setoyama T, Matsumoto M, Owaki T, Ishiqami S, Natsugoe S. Biomarkers for predicting the response of esophageal squamous cell carcinoma to neoadjuvant chemoradiation therapy. Surg Today 2014; 44: 421-8.

[20] Khanna A. DNA damage in cancer therapeutics: a boon or a curse? Cancer Res 2015; 75: 2133-8.

[21] Zhou Q, Zou BW, Xu Y, Xue JX, Meng MB, Liu FJ, Deng L, Ma DY, Ao R, Lu Y. DNA repair gene polymorphisms and clinical outcome of patients with primary small cell carcinoma of the esophagus. Tumour Biol 2015; 36: 1539-48.

[22] Gu AQ, Wang WM, Chen WY, Shi CL, Lu JH, Han JQ. XRCC1 genetic polymorphisms and sensitivity to platinum-based drugs in non-small cell lung cancer: an update meta-analysis based on 4708 subjects. Int J Clin Exp Med 2015; 8: 145-54.

[23] Robertson AB, Klungland A, Rognes T, Leiros I. DNA repair in mammalian cells: base excision repair: the long and short of it. Cell Mol Life Sci 2009; 66: 981-93.

[24] Wang Y, Ni J, Sun Z, Chen S, Jiao Y, Bai C. The influence of XRCC1 genetic variants on lung cancer susceptibility in Chinese Han population. Gene 2015; 556: 127-31.

[25] Wang X, Zhang K, Liu X, Liu B, Wang Z. Association between XRCC1 and XRCC3 gene polymorphisms and risk of thyroid cancer. Int J Clin Exp Pathol 2015; 8: 3160-7.

[26] Seibold P, Behrens S, Schmezer P, Helmbold I, Barnett G, Coles C, Yarnold J, Talbot CJ, Imai T, Azria D, Koch CA, Dunning AM, Burnet N, Bliss JM, Symonds RP, Rattay T, Suga T, Kerns SL, Bourgier C, Vallis KA, Sautter-Bihl ML, Claßen J, Debus J, Schnabel T, Rosenstein BS, Wenz F, West CM, Popanda O, Chang-Claude J. XRCC1 polymorphism associated with late toxicity after radiation therapy in breast cancer patients. Int J Radiat Oncol Biol Phys 2015; 92: 1084-92.

[27] Chiang CI, Huang YL, Huang CY, Shiue HS, Chen WJ, Pu YS, Lin YC, Hsueh YM. XRCC1 polymorphisms and urinary 8-hydroxydeoxyguanine levels are associated with urothelial carcinoma. PLoS One 2015; 10: e0124066.

[28] Wei B, Han Q, Xu L, Zhang X, Zhu J, Wan L, Jin Y, Qian Z, Wu J, Gao Y, Zhou J, Chen X. Effects of JWA, XRCC1 and BRCA1 mRNA expression on molecular staging for personalized therapy in patients with advanced esophageal squamous cell carcinoma. BMC Cancer 2015; 15: 331.

[29] Srivastava A, Srivastava K, Pandey SN, Choudhuri G, Mittal B. Single-nucleotide polymorphisms of DNA repair genes OGG1 and XRCC1: association with gallbladder cancer in north Indian population. Ann Surg Oncol 2009; 16: 1695-703.

[30] Zhu G, Wang L, Guo H, Lu L, Yang S, Wang T, Guo H, Wang H, Min J, Yang K, Chen X, Liu Y, Wang Z, Su H. DNA repair genes XRCC1 and ERCC1 polymorphisms and the risk of sporadic breast cancer in Han women in the gansu province of China. Genet Test Mol Biomarkers 2015; 19: 387-93.

[31] Wang L, Jiang YQ, Zhou MD, Jiang Z. Association between XRCC1 Arg399Gln polymorphism and glioma risk in a Chinese population: a case-control study. Int J Clin Exp Med 2015; 8: 10026-30.

[32] Peasland A, Wang LZ, Rowling E, Kyle S, Chen T, Hopkins A, Cliby WA, Sarkaria J, Beale G, Edmondson RJ, Curtin NJ. Identification and evaluation of a potent novel ATR inhibitor, NU6027, in breast and ovarian cancer cell lines. Br J Cancer 2011; 105: 372-81.

[33] Santarpia L, Iwamoto T, Di Leo A, Hayashi N, Bottai G, Stampfer M, André F, Turner NC, Symmans WF, Hortobágyi GN, Pusztai L, Bianchini G. DNA repair gene patterns as prognostic and predictive factors in molecular breast cancer subtypes. Oncologist 2013; 18: 1063-73.