TNF rs1799964 as a Predictive Factor of Acute Toxicities in Chinese Rectal Cancer Patients Treated With Chemoradiotherapy

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Abstract: Acute toxicity is the main dose-limiting factor in the chemoradiotherapy of rectal cancer patients and depends on several pro-inflammatory factors, including interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-α). It is unknown whether genetic factors, such as single-nucleotide polymorphisms (SNPs) in the IL-1, IL-6, and TNF genes, are also associated with acute toxicity in the process. We genotyped 5 potentially functional SNPs in these 3 genes (TNF rs1799964, TNF rs1800629, IL-6 rs1800796, and IL-1 rs1143623, IL-1 rs1143627) and estimated their associations with severe acute radiation injury (grade ≥ 2) in 356 rectal cancer patients. We found a predictive role of the TNF rs1799964 T variant allele in the development of acute injury (for CT vs CC: adjusted odds ratio [OR] = 4.718, 95% confidence interval [CI] = 1.152–19.328, P = 0.031; for TT vs CC: adjusted OR = 4.443, 95% CI = 1.123–17.581, P = 0.034). In the dominant model, for CT/TT vs CC, the adjusted OR = 4.132, 95% CI = 1.069–15.966, and P = 0.04. Our results suggested that genetic variants in the TNF gene may influence acute injury in rectal cancer patients treated with chemoradiotherapy and may be a predictor for personalized treatment. Additional larger and independent studies are needed to confirm our findings. (Medicine 94(45):e1955)

Abbreviations: 5-FU = 5-fluorouracil, AEs = adverse events, CHB = Chinese Han Beijing, CIs = confidence intervals, IL-1 = interleukin-1, IMRT = intensity modulated radiation therapy, LD = linkage disequilibrium, MAF = minor allele frequency, ORs = odds ratios, SNPs = single-nucleotide polymorphisms, TNF-α = tumor necrosis factor-alpha, TRG = tumor response grade.

INTRODUCTION

Rectal cancer is one of the most common malignancies and often presents with a poor prognosis.1 Radiotherapy with or without concurrent chemotheraphy is a major modality in the treatment of rectal cancer.2-5 Radiotherapy reduces local recurrence and possibly improves overall survival but with increased radiation-related morbidity,6-11 because of the damage to the surrounding normal tissues that primarily manifests as radiation intestinal injury, including acute toxicities and chronic fibrosis. Mucositis, vomiting, diarrhea, pain, tenesmus, bleeding, and hematologic dysfunction are the most common acute adverse effects.6-10 Many studies have focused on late radiation-induced injury.11,12 However, severe acute toxicities also impair the quality of life in rectal cancer patients, in addition to chronic complications. We were particularly interested in early normal tissue injury and attempted to explore additional molecular markers that predict acute chemo radiation-induced injury in rectal cancer patients.

Acute reactions to radiotherapy either are related to inflammation or occur through target cell depletion. Genes that affect early processes in the DNA repair or inflammation pathways may lead to a wide range of acute reactions after radiotherapy.13-15 The associations between DNA repair and radiation injury have been extensively investigated,16,17 though with inconsistent results, and recent studies have increasingly focused on the relationship between inflammation-related factors and radiation-induced injury.18 Ionizing radiation can activate the pro-inflammatory signal, which is then amplified by the recruitment and transmigration of monocytes and activation of resident mast cells and result in the production of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α).19,20

However, only a subset of patients develops severe radiation injuries, and little information is available to identify such individuals. A predictive tool to identify radiosensitive patients based on host factors such as genetic variants may be beneficial to personalized cancer treatment. Those genetic variants in key inflammatory-related genes may modulate the balance of inflammation and result in a change in radiation-induced normal tissue injury. Previous studies have demonstrated that variations in the circulating levels of IL-1, IL-6, and TNF-α were associated with the risk of radiation-induced pneumonitis and toxicities in breast cancer and head and neck cancer.21-22 Hence, we hypothesized that inter-individual variability in inflammatory cytokines may modulate the phenotype of radiosensitivity in rectal cancer patients.
This study was designed to determine whether the genotypes of the inflammatory-related genes IL-1, IL-6, and TNF were predictive of acute adverse events in patients with rectal cancer treated with pelvic radiotherapy.

MATERIALS AND METHODS

Study Subjects

The patients recruited in this study had received pelvic irradiation between January 2012 and October 2013 at Fudan University Shanghai Cancer Center (Shanghai, China). Totally, there were 398 eligible patients during the timespan. However, there were 42 patients whose blood samples were not collected. Thus, this study included 356 rectal cancer patients. The eligible patients were histopathologically confirmed with rectal adenocarcinoma, and other histological types and all metastases to the rectum were excluded. Blood samples of all patients were collected and processed by the Institutional Tissue Bank at Shanghai Cancer Center. Written informed consent was obtained from each patient. This study was approved by the Institutional Review Board of Fudan University Shanghai Cancer Center.

Treatment and Toxicity Evaluation

All patients received pelvic radiation with 6-MV (million volt) X-rays from linear accelerators (Elekta, Stockholm, Sweden; Varian, Palo Alto, CA). The intensity-modulated radiation therapy (IMRT) technique was used in all patients, and the IMRT plans were generated using the inverse planning module. A total dose ranging from 45 to 55 Gy was given with 1.8 or 2 Gy per fraction, 5 days a week. More than half of the patients had undergone pre-operation radiotherapy (Table 1). Over 90% of the patients also received concurrent chemotherapy, which was 5-fluorouracil (5-FU)/capecitabine or combined with oxaliplatin or irinotecan. Monotherapeutic capcitabine and 5-FU were administered at a dose of 825 mg/m2 twice a day by p.o. (per os) and 1000 mg/m2 5 days per week during the course of irradiation, respectively. In the Capcitabine + Oxaliplatin group, patients were administered oxaliplatin at a dose of 50 mg/m2 by 2-hour intravenous infusion once a week, and capcitabine was administered p.o. at 625 mg/m2 bid for 5 days per week. In the Capcitabine + Irinotecan group, irinotecan was administered at 50 to 70 mg/m2 by intravenous infusion once a week. All of the patients, who received surgery in our center, were treated by total mesorectal excision (TME) procedure.

Acute adverse events, including gastrointestinal (vomiting, diarrhea and incontinence), dermatitis, and hematologic toxicity were evaluated weekly. The Common Terminology Criteria (CTC) toxicity criteria version 3.0 scale was used to score acute adverse events (AEs) as follows: grade 0 means no AE, grade 1 indicates mild AE without the need for therapeutic intervention, grade 2 to 4 suggests AE with the need for therapeutic intervention, and grade 5 represents the most severe toxicity of adverse event-related death. The patients were grouped into 2 subgroups according to the CTC v3.0 score system: grade ≥2 patients were categorized as the case group (obvious toxicity), and grade 0 to 1 patients were categorized as the control group (mild toxicity). Grade 2 was set as a cutoff value because developing grade 2 toxicity was considered to impair quality of the patient’s life. The total toxicity grade indicated the highest toxicity grade among the hematologic toxicity, vomiting, diarrhea, incontinence (anal reaction), and dermatitis.

SNP Selection and Genotyping

The single-nucleotide polymorphisms (SNPs) were screened and selected from the NCBI dbSNP (http://www.ncbi.nlm.nih.gov/snp) and HapMap databases (http://www.hapmap.org) for potentially functional SNPs in the IL-1, IL-6, and TNF genes on the basis of 3 criteria: at the 2 ends of these genes, such as near the 5’-end, 3’-end, 5’untranslated regions (UTRs), or 3’UTR, which may be the regulatory regions of the genes; minor allele frequency (MAF) ≥5% in Chinese Han Bejing (CHB) descendants; and potentially affect functions as predicted by SNPinfo (http://snpinfo.niehs.nih.gov/), such as transcription factor binding site (TFBS) and microRNA
(miRNA) binding site activity. In addition, linkage disequi- 
brum (LD) analysis was performed to optimize SNP selection 
using the Haploview software (http://www.broadinstitute.org/ 
scientific-community/science/programs-medical-and-population-
genetics/haplovie/haploview). As a result, 5 SNPs (rs1799964 
and rs1800629 in TNF, rs1800796 in IL-6, rs1143626, and 
rs1143627 in IL-1β) met these criteria and were selected for 
genotyping. SNPs, TNFRs1799964C/T and rs1800629A/G, IL-6 
rs1800796C/G, and IL-1 rs1143626C/G and rs1143627G/A locate 
near the 3′-end of the genes that are predicted to affect TFBS 
activity.

Blood samples of all patients were collected before treat-
ment and processed by the Institutional Tissue Bank at Shanghai 
Cancer Center. Genomic DNA was extracted from white blood 
cells. DNA purity and concentration were determined using a 
UV spectrophotometer (BioTek, Winooski, VT). The Taqman 
universal PCR master mix and SNP-genotyping probes were 
purchased from Applied Biosystems by Life Technologies 
(Applied Biosystems, Foster City, CA), and the genotyping 
was performed using the Taqman real-time PCR method on a 
7900HT sequence detector system of ABI. A strict quality 
control procedure was used in each 384-well plate, in which 
4 duplicated positive controls (duplicated 4 wells) and 4 nega-
tive controls (ddH2O instead of DNA), and the concordant rate 
was 100%.

**TNF Gene Expression by Genotypes in the 
HapMap Data**

The biological plausibility of our findings was further 
investigated by assessing the correlation between genotypes 
and the mRNA expression levels of the same gene in 270 
lymphoblastoid cell lines. In the present study, the TNF 
rs1799964 SNP was obviously associated with chemoradiother-
apy-induced total toxicity; thus, the mRNA expression levels 
were further evaluated for their associations with the genotype 
data from the HapMap website (http://www.hapmap.org), and 
the mRNA expression information was retrieved using the 
SNPexp online tool (http://app3.titan.uio.no/biotools/help. 
pp?app=snpexp)” for the genotype–phenotype association 
analysis. The genotyping data in the HapMap phase II release 23 
data set were used, which was consisted of 3.96 million SNP 
genotypes of 270 individuals from 4 populations (Utah residents 
with ancestry from northern and western Europe: 90 cases; Han 
Chinese in Beijing: 45 cases; Japanese in Tokyo: 45 cases; 
Yoruba in Ibadan, Nigeria: 90 cases). The mRNA expression 
data were from the same 270 individuals.

**TNF mRNA Expression in Tumor Tissues of Rectal 
Cancer Patients**

Further, 212 primary tumor tissues by biopsy from rectal 
cancer patients before treatment were available at our institu-
tional bank. These patients had received pre-operative rectal 
irradiation at Fudan University Shanghai Cancer Center 
between September 2007 and January 2014. The patients under-
go conventional pelvic radiation with concurrent 5-fluorour-
acil-based chemotherapy and were re-evaluated 4 to 6 weeks 
after irradiation using magnetic resonance imaging (MRI). 
Consequently, 156 patients received primary cancer resection 
at Fudan University Shanghai Cancer Center. We evaluated the 
pathologic tumor response using the AJCC TRG (tumor 
response grade) score system, in which grade 0 indicates a 
complete response without viable cancer cells, and grade 3 
indicates a poor response with a minimal evidence of a tumor 
response. According to the TRG score, 31 of the 156 patients 
reached a pathologically complete response (CR; TRG = 0), 
and 34 achieved a poor response (TRG = 3). We extracted 
the total RNA of biopsy tumor tissue from patients with TRG 0 
and TRG 3, and compared the different TNF mRNA expression 
levels between these 2 tumor response groups. We used TRI 
Reagent (Sigma Co., St. Louis, MO) to extract RNA, and 
synthesized cDNA using the PrimeScript RT Master Mix 
system (TAKARA, Osaka, Japan) according to the manufac-
turer’s instructions. Quantitative SYBR Green PCR was carried 
out using a Light Cycle 480 Real-Time PCR System (Roche 
applied Science, Mannheim, Germany), with β-actin as an 
internal reference gene. The primers used were as follows: 
s′–TTGTATACAGGAAGTCTCG–3′ (forward) and 5′– 
ATGCTATACTCCTCCCTGTGT–3′ (reverse) for β–actin 
and 5′–GAAGATAGGGTGCTGTGGCACA–3′ (forward) and 
5′–TTAGCCCTGAGGTGTCTGGCACA–3′ (reverse) for TNF. 
The mRNA expression level of TNF was calculated using 
2−ΔΔCT (ΔCT = CTNF – CTβ-actin, CT: threshold cycle).

**Statistical Methods**

Patients were categorized according to their genotyping 
results. Chi-squared (χ²) test was used to compare clinical 
characteristics and frequencies of genotypes between the 2 
groups. Univariate and multivariate logistic regression analyses 
were used to calculate the odds ratios (ORs) and 95% confi-
dence intervals (95% CIs), in order to assess the associations 
between severe radiation injury and genotypes. Multivariate 
adjustments were made for age, sex, drinking and smoking 
status, radiation dose, and concurrent chemotherapy. Student t-
test was used to compare the mRNA expression levels between 
the groups. The trend of the transcript expression levels in 
genotypes was evaluated by general linear regression model. 
Statistical significance was established at P < 0.05, and all tests 
were 2-sided and were performed using SPSS 20.0 software.

**RESULTS**

**Clinical Characteristics**

The baseline clinical characteristics of the patients are 
summarized in Table 1. The analysis included 356 rectal cancer 
cases with a median age of 55 years (range: 18–87 years). More 
than 50% of the patients received radiation before surgery, 
approximately 30% of the patients received postsurgery radio-
therapy, and the others received radiotherapy because of local 
recurrence after surgery but without previous pelvic radiation. 
Over 90% of the patients had received concurrent chemotherapy, 
which primarily comprised 2-drug regimens and included fluoro-
ouracil or capecitabine. The fluorouracil-based regimens con-
tained capecitabine + oxaliplatin or fluorouracil + oxaliplatin, 
and the irinotecan-containing regimens comprised irinotecan + 
capecitabine. The radiation dose was also comparable between 
the case and control groups. There were also no significant 
differences (P > 0.05) in the distributions of age, sex, smoking, 
drinking, the pattern of radiation, and chemotherapy between 
the groups based on the total toxicity.

**Association Between Genotypes and Radiation-
Induced Acute Toxicity**

The associations of SNPs in IL-1, IL-6, and TNF genes 
with the occurrence of normal tissue injuries were examined. 
The crude ORs (95% CI), P values, and the corrected data for 
age, sex, radiation dose, radiation pattern, smoking, drinking, 
and the regimens of concurrent chemotherapy were obtained.
The genotyping of several SNPs was not successfully examined in some samples, so the total cases used to analyze the association between the genotypes and toxicities, as shown in the subsequent tables, did not reach 356. The allele and genotype frequencies of the 5 SNPs are summarized in Table 2. For TNF rs1799964, a positively and statistically significant association was found between the presence of 1 or both variant alleles and the risk of obvious (grade ≥2) total toxicity (CT vs CC: adjusted OR = 4.718, 95% CI = 1.152–19.328, \( P = 0.031 \); TT vs CC: adjusted OR = 4.433, 95% CI = 1.123–17.581, \( P = 0.034 \)). Assuming a dominant model (the combination of heterozygous and variant homozygous genotypes vs the wild-type genotype),

| Variant | Genotypes | Grade ≥2 No. (%) (N = 264) | Grade 0–1 No. (%) (N = 92) | \( P \) | Crude OR (95% CI) | \( P \) | Adjusted OR (95% CI) | \( P \) |
|---------|------------|-----------------------------|-----------------------------|-----|-------------------|-----|-------------------|-----|
| TNF rs1799964 | CC 4(1.5) 5(5.6) | 1.00 | 1.00 | 0.114 | 3.884(0.975–15.470) | 0.054 | 4.718(1.152–19.328) | 0.031 |
| | CT 87(33.5) 28(31.1) | 3.076(0.962–14.276) | 0.057 | 4.443(1.123–17.581) | 0.034 |
| | TT 169(65.0) 57(63.3) | 0.776 | 0.776 | 4.443(1.123–17.581) | 0.034 |
| | Dominant model CT/TT 256(98.5) 85(94.4) | 0.052 | 0.052 | 4.132(1.069–15.966) | 0.040 |
| | CC/CT 91(35.0) 33(36.7) | 1.00 | 1.00 | 0.056 | 1.076(0.648–1.787) | 0.766 |
| | TT 169(65.0) 57(63.3) | 3.765(0.988–14.341) | 0.052 | 4.132(1.069–15.966) | 0.040 |
| | Allele C 95(18.3) 38(21.1) | 0.402 | 0.402 | 1.000 | 1.000 |
| | TT 169(65.0) 57(63.3) | 0.776 | 0.776 | 1.076(0.648–1.787) | 0.766 |
| | Allele T 425(81.7) 142(78.9) | 1.197(0.785–1.825) | 0.403 | 1.207(0.787–1.931) | 0.388 |
| | NTN rs1800629 | 0.446 | 0.446 | 1.000 | 1.000 |
| | AA 2(0.8) 1(1.1) | 0.000 | 0.000 | 1.000 | 1.000 |
| | AG 37(14.2) 18(19.6) | 1.028(0.087–12.098) | 0.983 | 0.923(0.076–11.150) | 0.950 |
| | GG 222(85.1) 73(79.6) | 1.521(0.136–17.014) | 0.734 | 1.454(0.126–16.750) | 0.764 |
| | Dominant model AG/GG 259(99.2) 85(94.4) | 0.052 | 0.052 | 4.132(1.069–15.966) | 0.040 |
| | AA/AG 39(14.9) 19(20.7) | 1.000 | 1.000 | 1.000 | 1.000 |
| | Recessive model TT 169(65.0) 57(63.3) | 1.521(0.136–17.014) | 0.734 | 1.454(0.126–16.750) | 0.764 |
| | Allele C 95(18.3) 38(21.1) | 0.402 | 0.402 | 1.000 | 1.000 |
| | TT 169(65.0) 57(63.3) | 0.776 | 0.776 | 1.076(0.648–1.787) | 0.766 |
| | Allele T 425(81.7) 142(78.9) | 1.197(0.785–1.825) | 0.403 | 1.207(0.787–1.931) | 0.388 |

TABLE 2. Logistic Regression Analysis of Association Between the Genotypes and Total Toxicity in Rectal Cancer Patients

The results were in bold, if the 95% CI on one side of I or \( P \) < 0.05. CI = confidence interval, OR = odds ratio.

\*Chi-square test for genotype distributions between cases and controls.

\^Adjusted for age, sex, smoking and drinking status, total radiation dose, the pattern of radiation, and the regimen of concurrent chemotherapy in logistic regression models.

\&For dominant genetic models (the combination of heterozygous and variant homozygous genotypes vs wild-type genotype).

\$For recessive genetic models (the variant homozygous genotypes vs variant heterozygous combined with wild-type genotype).

\|At allelic level.
analyzed whether the naive TNF expression in tumor tissues would indicate the treatment response of radiotherapy, we tested the TNF mRNA expression levels in tumor tissues biopsied from patients with pre-treatment primary tumors. The response of tumors to radiotherapy was graded by pathologists in postsurgery tumor tissues according to the extent of alive tumor cells. The TNF mRNA expression in 31 patients who achieved a complete response was lower than that in 34 patients who had a poor response to radiotherapy ($P = 0.041$, Fig. 1B).

**DISCUSSION**

It has long been recognized that cancer patients exhibit a heterogeneous response to chemoradiotherapy and that these responses manifest as variations in normal tissue toxicity. Genes that affect early processes in DNA repair or inflammation pathways are expected to be responsible for early chemoradiation-induced reactions. As inflammation-related factors, IL-1, IL-6, and TNF-α are known to be extensively involved in acute inflammation reactions. In this study, we investigated 5 SNPs in the IL-1, IL-6, and TNF genes for their associations with radiation-induced acute normal tissue injury in rectal cancer patients treated with pelvic radiotherapy. We considered grade $\geq 2$ toxicity as the toxicity group and grade 0 to 1 as the control group because developing grade $\geq 2$ toxicity will influence the patients’ quality of life. We found for the first time that variant genotypes of rs1799964 of TNF were associated with acute injury in rectal cancer patients with pelvic radiation—that is, patients with the variant genotypes were more likely to develop grade $\geq 2$ toxicity, which may influence the patients’ quality of life.

**TABLE 3. TNF mRNA Expression by the Genotype of rs1799964 Using Data from the Hapmap**

| Genotype | No | Mean ± SD | $P^*$ | $P^{trend}$ |
|----------|----|-----------|-------|-------------|
| All population | 270 | 6.29 ± 0.07 |  |  |
| rs1799964$^d$ |  |  |  |  |
| CC | 4 | 6.26 ± 0.09 |  |  |
| CT | 80 | 6.30 ± 0.07 | 0.575 | 0.041 |
| TT | 166 | 6.29 ± 0.08 | 0.595 | 0.192 |
| CT + TT | 246 | 6.29 ± 0.07 | 0.607 |  |
| CC + CT | 84 | 6.30 ± 0.07 | 0.706 |  |
| CHB | 6.31 ± 0.05 |  |  |  |
| rs1799964 |  |  |  |  |
| CC | 2 | 6.21 ± 0.01 |  |  |
| CT | 16 | 6.33 ± 0.05 | 0.082 |  |
| TT | 25 | 6.30 ± 0.05 | 0.054 | 0.002 |
| CT + TT | 41 | 6.31 ± 0.05 | 0.064 |  |
| CC + CT | 18 | 6.32 ± 0.06 | 0.214 |  |

TNF rs1799964 genotyping data and mRNA expression levels for TNF by genotypes were obtained from the HapMap phase II release 28 data from EBV-transformed lymphoblastoid cell lines from 270 individuals, including 45 Han Chinese in Beijing (CHB).

$^d$ Two-sided Student $t$ test.

$^*$ $P$ values for the trend test of TNF mRNA expression among 3 genotypes for SNP rs1799964 from a general linear model.

$^{trend}$ There were missing data because genotyping data for 20 individuals were not available.
The incidence of severe radiation-induced injury (grade ≥ 2) in our study was 74.2%. Some publications have suggested that combined chemotherapy will influence the toxicity of radiation. In our study, for patients who received radiotherapy alone, 6.1% developed grade ≥ 2 toxicity, whereas more patients developed grade ≥ 2 toxicity (12%) in the group with a single or combined chemotherapy; however, the results did not reach statistical significance (P = 0.184). Because TNF-α, IL-1, and IL-6 participate in the radiation response, associations between the variants of these genes and risk of developing radiation injury are biologically plausible. Although little is known concerning the relationship between TNF-α, IL-1, and IL-6 variants and risk of chemoradiation injury in rectal cancer, several studies examining the association between TNF-α, IL-1, and IL-6 variants and radiosensitivity have been published, and their results can be used for comparison.

Our findings demonstrated that patients with the variant genotypes (CT or TT) of TNF rs1799964 had a higher probability of developing severe total toxicity than those with the wild-type (CC) genotype. TNF-α is a pro-inflammatory cytokine and is produced by activated macrophages. It is well known that TNF-α has both beneficial and harmful effects, playing a role as a “double-edged sword.” The pro-inflammatory cytokine TNF-α may be responsible for initiating inflammation in response to tissue injury. The importance of TNF-α in inflammation has made this cytokine one of the most widely studied molecules in relation to its genetic variants and inflammation. The molecular mechanisms involved in the process of radiation-induced inflammation are somewhat similar to infectious or wound inflammation; as a result, TNF-α is considered very important in radiation-induced injury.

In some reports, elevated levels of TNF-α were documented in the acute-phase response after the radiation of head and neck tumors, which suggests that TNF-α might be useful for monitoring this acute response in patients receiving radiotherapy. It was found that the variants in TNF were associated with radiation-induced esophagitis and pneumonitis in nonsmall cell lung cancer patients who received thoracic radiotherapy. It was also reported that SNPs near TNF were associated with radiation toxicity in breast cancer. These studies suggest that TNF variants may play a role in both the efficacy and side effects of radiotherapy. In addition to radiotherapy, TNF-α was associated with severe chemotherapy-induced gastrointestinal toxicity.

More than 90% of the patients in our study received concurrent chemoradiotherapy. Thus, TNF-α may be an effective indicator of toxicity in patients who receive chemoradiotherapy.

We could not detect any correlation between SNPs in IL-1 or IL-6 and radiation-induced injury in our study, although it was reported that early variations of circulating IL-6 levels were significantly associated with the risk of radiation inflammation. In another study, IL-6 rs1800795 was shown to be associated with an increased risk of esophagitis in nonsmall cell lung cancer patients with thoracic radiation, and 2 SNPs in IL-1 were associated with a higher risk of pneumonitis in Caucasian patients. The cause may be that SNPs in IL-1 and IL-6 are organ and histology-specific regarding radiation-induced injury; alternatively, our study may not have had sufficient study power to detect the weaker difference in Chinese patients.

Whether SNPs in IL-1, IL-6, and TNF genes influence the expression level of mRNA and protein in serum and tissue is controversial. A meta-analysis did not find a relationship between the variant and IL-6 serum levels. We analyzed the TNF mRNA expression by different genotypes of SNP rs1799964 using data from the HapMap database, including 270 individuals from different populations. Although there was no difference in the mRNA expression levels in all 270 individuals, TNF mRNA expression tended to be higher in subjects with the CC genotype than in those with the CC genotype in 45 Chinese populations. Combined with our result that patients with the CT and TT genotypes have an increased risk of developing acute injury, the underlying mechanism may be that the CT and TT genotypes of SNP rs1799964 may positively modulate the mRNA expression level of TNF and consequently increase the acute injury after radiotherapy. The results of the present study are consistent with our current opinion that the acute inflammatory factor TNF-α may increase acute radiation-induced injury.

Several studies have investigated the toxicity as radiosensitivity, considering that severe toxicity indicated increased sensitivity to radiation, partly because it is difficult to evaluate the sensitivity to radiation in postsurgery radiotherapy without the gross tumor volume. We examined the TNF mRNA expression levels in tumor tissues before radiotherapy and analyzed the correlation between TNF mRNA expression and the tumor response to radiotherapy. The TNF mRNA expression level was found to be lower in the complete response group than in the poor response group. In addition, the result seemed to be in contrast to previous results. However, it should be clarified whether tumor tissues and normal tissues show a consistent response to radiotherapy. Furthermore, the present result should be confirmed in further studies with a larger sample size and different ethnicities.

Over half of the patients in our study received preoperative radiotherapy, which is considered the standard treatment for T3 to T4 rectal cancers. Whether radiotherapy combined with chemotherapy improves survival further is controversial. The results of clinical trials FFCD9203 and EORTC22921 showed that preoperative chemoradiotherapy had no impact on overall survival but significantly improved local control with moderate increased acute toxicity. Adding oxaliplatin to fluorouracil-based preoperative chemoradiotherapy did not affect the primary tumor response but significantly increased the toxicity in the STAR-01 and ACCORD12/0405 trials. However, in our center and another cancer center in China, the combination with oxaliplatin increased the pathological response in local advanced rectal cancer patients, although some patients showed obvious toxicity. If TNF rs1799964 is validated as an indicator for predicting acute toxicity in Chinese populations, it may determine which patients will benefit from additional oxaliplatin therapy without severe toxicity and which group of patients is not suited for 2-drug concurrent chemoradiotherapy. Furthermore, the treatment intensity for elderly patients is controversial. Although it was reported that elderly patients with rectal cancer could be safely treated with radiotherapy or chemoradiotherapy, toxicities must be carefully monitored. TNF rs1799964 may be an additional predictor for acute toxicities that can allow treatments to be carefully considered in elderly patients.

A limitation of our study is that only a few potentially functional SNPs were analyzed. Therefore, we might omit SNPs such as intronic SNPs that may also be functional and thus associated with the risk of acute injury after chemoradiotherapy by modifying the mRNA and protein expression. In addition, our results should be validated in larger samples and at multicenter institutes. Finally, the ethnicities included in the study should also be considered. The SNPs reported in Caucasian or...
Japanese populations may not always be consistent with those in Chinese populations.

In summary, we found that SNP rs1799964 in TNF was associated with the risk of total acute chemoradiotherapy-induced injury in Chinese rectal cancer patients. Alternatively, a patient possessing the variant CT or TT genotype of SNP rs1799964 may be an ideal candidate for radiotherapy because this genetic variant may render this patient’s cancer radio-sensitive. It is possible that such an individual could be treated using a lower radiotherapy dose or lower intensity of concurrent chemotherapy that is less likely to cause injury. For patients, who do not possess such genetic variants, higher radiation doses or combined 2-drug chemotherapy may improve their curable chance without injury to the surrounding normal tissues. In this regard, once validated, genotyping of TNF variants among rectal cancer patients may help identify individuals who are at a high or low risk of radiation injury, which in turn allows for the design of personalized radiotherapy with maximal doses to the tumor and minimal toxicity to normal tissues. Therefore, our findings may support the value of these SNPs in assessing the risk of development radiation-induced normal tissue injury. However, our findings require further validation in subsequent studies.

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