ORIGINAL RESEARCH

Associations of GWAS-Identified Risk Loci with Progression, Efficacy and Toxicity of Radiotherapy of Head and Neck Squamous Cell Carcinoma Treated with Radiotherapy

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Background: Head and neck squamous cell carcinoma (HNSCC) ranks the sixth most common cancer worldwide. This study aims to evaluate the associations of GWAS-identified HNSCC risk loci with progression, efficacy and toxicity of radiotherapy of HNSCC treated with radiotherapy.

Methods: Six GWAS-identified risk loci were genotyped and evaluated. Multivariate logistic regression was used to determine the associations of these SNPs with progression, efficacy and toxicity of radiotherapy of HNSCC treated with radiotherapy.

Results: We found that rs259919 was significantly associated with higher TNM stage (allele A vs G: OR=1.49; 95% CI: 1.09–2.03; P=0.012), while rs3135001 was significantly associated with better efficacy of radiotherapy (allele T vs C: OR=1.80, 95% CIs=1.19–2.73, P=0.005). Both SNP rs1265081 (allele A vs C: OR=1.41, 95% CIs=1.08–1.86, P=0.012) and rs3135001 (allele T vs allele C: OR=0.53, 95% CIs=0.35–0.79, P=0.002) were significantly associated with the occurrence of grade 3–4 oral mucositis.

Conclusion: We identified that three GWAS-identified HNSCC risk loci were significantly associated with progression, efficacy and toxicity of radiotherapy of HNSCC. Our findings strengthen the understanding of the essential role of genetic background in the progression and therapeutic effects of HNSCC.

Keywords: progression, genetic, radiotherapy, HNSCC, efficacy, toxicity

Introduction

Head and neck squamous cell carcinoma (HNSCC), a group of malignant tumors originating in the head and neck, ranks the sixth most common cancer worldwide.[1] Unlike the increased rates of HPV infection in the oropharynx in the United States and Western Europe, the high incidence of HNSCC in Southeast Asia and Australia is associated with the consumption of specific carcinogenic-containing products.[2] The main methods of treatment for localized or locally limited HNSCC are resection, radiotherapy and systemic therapy.[3] Despite the use of aggressive treatment, only about 40% of patients with the most common histologically altered type-HNSCC, could survive more than 5 years.[4,5] Further, it is characterized by considerable heterogeneity in disease course and treatment outcome.[6,7] Therefore, an in-depth understanding of the factors influencing the progression and therapeutic effects of HNSCC is urgently needed to support biomarkers development, early warning and personalized patient treatment.

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There is a growing interest in finding genetic factors that can potentially help identify subgroups of patients at higher risk of disease progression and therapeutic effects. Against this backdrop, studies identified that there was a solid connection between single nucleotide polymorphisms (SNPs) of candidate genes/loci and progression of HNSCC.[8–11] Recently, a two-phase genome-wide association study (GWAS) identified six loci, including 6p22.1, 18q22.2, 2p23.1, 5p15.33, 6p21.32, and 6p21.33, were associated with risk of HNSCC.[12] These findings suggested that the immunologic mechanism was implicated in the etiology of HNSCC. It is assumed that these loci might explain inter-individual differences in the disease progression and sensitivity to standard anticancer treatment. However, whether these loci contribute to the progression and therapeutic effects of HNSCC was still unexplored, especially in Chinese population. The purpose of this study was to evaluate associations of these GWAS-identified HNSCC risk loci with progression, efficacy and toxicity of radiotherapy of HNSCC treated with radiotherapy.

Patients and Methods
Patient and Clinical Data
Totally included in this study were 500 newly diagnosed, histologically confirmed HNSCC cases treated with radiotherapy (RT) alone or in combination with chemotherapy (CHT). At recruitment, five milliliter of blood sample from the patients was collected. Written informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committee of Xiangyang Central Hospital.

Treatment Efficacy and Toxic Reaction
All patients were treated with radical external radiotherapy with or without cisplatin-based chemotherapy or both. Treatment efficacy were evaluated with magnetic resonance imaging (MRI) directly after finishing radiotherapy in line with the Response Evaluation Criteria in Solid Tumors (RECIST), which defined endpoint of treatment efficacy as complete remission (CR). Radiation-induced oral mucositis were evaluated according to the radiation toxicity grading criteria of the Radiation Therapy Oncology Group or European Organization for Research and Efficacy of Cancer (RTOG/EORTC). Patients were defined as grade 0–2 group and grade 3–4 group.

DNA Extraction and Genotyping
Germline DNA was extracted from 200 μL peripheral blood samples of patients using a commercial extraction kit (Tiangen Biotech Corporation, Beijing, China). Genotyping of six top signal SNPs (rs259919, rs3135001, rs1265081, rs142021700, rs10462706, and rs4318431) was performed using the Sequenom iPLEX MassARRAY system (Sequenom, Inc., San Diego, CA, USA). For quality control, a 10% random sample was repeated, and 100% concordance was achieved for all SNPs. All laboratory genotyping personnel were blind to the clinical outcomes of the patients' samples.

Statistical Analysis
Continuous data was presented as mean ± standard deviation, while categorical data was presented as frequency and percentage. Univariate logistic regression was performed to determine the association of the GWAS-identified risk loci with the progression, efficacy and toxicity of radiotherapy of HNSCC treated with radiotherapy. Statistical Analysis System software (Version 9.4; SAS Institute, Cary, NC) was used for all of the statistical analyses.

Results
Characteristics of Study Patients
Table 1 presents the baseline demographics and clinical information of the 500 HNSCC cases. The mean age was 50.4 (SD=9.7) with 407 males (81.5%), and a mean BMI of 23.4 (SD=3.9). There were 203 smokers (40.6%) and 167 alcohol drinkers (33.4%). The tumor site included oropharynx (30.6%), hypopharynx (19.0%), and larynx (50.4%). Among them, 54.2% of the patients received radiotherapy alone. The TNM stage distribution of all HNSCC patients was 176 cases (35.2%) in stage I or II, 324 cases (64.8%) in stage III or IV, respectively. Overall, 349 (69.8%) patients reached CR after radiotherapy. Of the toxic reactions, 237 (47.4%) patients developed grade 3–4 oral mucositis.

Associations Between GWAS-Identified Risk Loci and the TNM Stage in HNSCC Patients
Table 2 presents the associations between GWAS-identified risk loci and the TNM stage in HNSCC patients. Only five variants (rs259919, rs3135001, rs1265081, rs10462706, and rs4318431) were analyzed, because the

Table 1 Characteristics of the Patients

Variables	HNSCC Cases (n=500)
Age at diagnosis, years	50.4±9.7
Gender	
Male	407 (81.5%)
Female	93 (18.5%)
Drinking status	
Never	297 (59.4%)
Ever	203 (40.6%)
Smoking status	
Never	333 (66.6%)
Ever	167 (33.4%)
BMI, kg/m^2	23.4±3.9
Tumor site	
Oropharynx	153 (30.6%)
Hypopharynx	95 (19.0%)
Larynx	252 (50.4%)
Treatment	
Radiochemotherapy	229 (45.8%)
Radiotherapy	271 (54.2%)
TNM stage	
I–II	176 (35.2%)
III–IV	324 (64.8%)
Complete remission	
Yes	349 (69.8%)
No	151 (30.2%)
Grade 3–4 Oral mucositis	
Yes	237 (47.4%)
No	263 (52.6%)

Table 2 Association Between GWAS-Identified Risk Loci and the TNM Stage in HNSCC Patients

Variants	TNM (III–IV) (N=324)	TNM (I–II) (N=176)	OR (95% CIs) *	P value
rs259919				
GG	146	98	1.00 (Reference)	
AG	153	71	1.50 (1.01–2.25)	0.046
AA	25	7	2.49 (1.06–5.85)	0.036
A vs G			1.49 (1.09–2.03)	0.012
rs1265081				
CC	115	60	1.00 (Reference)	
CA	148	83	0.93 (0.62–1.4)	0.731
AA	61	33	0.96 (0.57–1.63)	0.893
A vs C			0.97 (0.75–1.27)	0.839
rs3135001				
CC	257	144	1.00 (Reference)	
CT	61	28	1.22 (0.75–2.00)	0.426
TT	6	4	0.84 (0.23–3.02)	0.790
T vs C			1.11 (0.73–1.7)	0.615
rs10462706				
CC	135	74	1.00 (Reference)	
CT	136	82	0.91 (0.61–1.35)	0.636
TT	53	20	1.45 (0.81–2.61)	0.211
T vs C			1.12 (0.86–1.47)	0.399
rs4318431				
CC	268	149	1.00 (Reference)	
CT	52	27	1.07 (0.65–1.78)	0.791
TT	4	0	–	–
T vs C			1.23 (0.77–1.97)	0.395

Note: *Age, gender, BMI, smoking, drinking, and tumor site.

Associations Between the GWAS-Identified Risk Loci and Grade 3–4 Radiation-Induced Oral Mucositis

Table 4 presents the associations between GWAS-identified risk loci and grade 3–4 radiation-induced oral mucositis. Both SNP rs1265081 (allele A vs C: OR=1.41, 95% CIs=1.08–1.86, P=0.012) and rs3135001 (allele T vs allele C: OR=0.53, 95% CIs=0.35–0.79, P=0.002) were significantly associated with the occurrence of grade 3–4 oral mucositis.

Discussion

In the past decades, combined radiotherapy and chemotherapy have been recognized as feasible in HNSCC treatment.[13] However, its progression, efficacy and toxicity of radiotherapy was full of uncertainty. In the current study, we explored the associations between several

minor allele frequency of rs142021700 was small than 5%. We found rs259919 was significantly associated with higher TNM stage (allele A vs G: OR=1.49; 95% CI: 1.09–2.03; P=0.012). A significantly higher rs259919 AG/AA genotype distribution in the TNM (III–IV) group than in the TNM (I–II) subgroup was detected.

Associations Between GWAS-Identified Risk Loci and the Efficacy of Radiotherapy

Table 3 presents the associations between GWAS-identified risk loci and the efficacy of radiotherapy in HNSCC patients. We found rs3135001 was significantly associated with better efficacy of radiotherapy (allele T vs C: OR=1.80, 95% CIs=1.19–2.73, P=0.005).

Table 3 Association Between GWAS-Identified Risk Loci and the Efficacy of Radiotherapy in HNSCC Patients (CR: Complete Remission)

Variants	CR (N=151)	Non-CR (N=349)	OR (95% CIs)*	P value
rs259919				
GG	82	162	1.00 (Reference)	
AG	62	162	0.76 (0.51–1.12)	0.165
AA	7	25	0.55 (0.23–1.32)	0.182
A vs G			0.77 (0.57–1.05)	0.095
rs1265081				
CC	58	117	1.00 (Reference)	
CA	70	161	0.88 (0.58–1.34)	0.542
AA	23	71	0.65 (0.37–1.15)	0.139
A vs C			0.81 (0.62–1.07)	0.141
rs3135001				
CC	112	289	1.00 (Reference)	
CT	33	56	1.58 (0.96–2.61)	0.073
TT	6	4	4.03 (1.24–13.04)	0.020
T vs C			1.80 (1.19–2.73)	0.005
rs10462706				
CC	69	140	1.00 (Reference)	
CT	57	161	0.72 (0.47–1.09)	0.120
TT	25	48	1.06 (0.60–1.86)	0.848
T vs C			0.94 (0.71–1.25)	0.675
rs4318431				
CC	128	289	1.00 (Reference)	
CT	23	56	0.93 (0.55–1.57)	0.779
TT	0	4	–	–
T vs C			0.82 (0.50–1.34)	0.424

Note: *Age, gender, BMI, smoking, drinking, tumor site, treatment, and TNM stage.

Table 4 Association Between GWAS-Identified Risk Loci and Grade 3–4 Radiation-Induced Oral Mucositis

Variants	Grade 3–4 (N=263)	Grade 0–2 (N=237)	OR (95% CIs)*	P value
rs259919				
GG	125	119	1.00 (Reference)	
AG	116	108	1.02 (0.71–1.47)	0.904
AA	22	10	2.09 (0.96–4.55)	0.062
A vs G			1.18 (0.90–1.56)	0.234
rs1265081				
CC	79	96	1.00 (Reference)	
CA	129	102	1.60 (1.06–2.41)	0.025
AA	55	39	1.78 (1.06–2.99)	0.028
A vs C			1.41 (1.08–1.86)	0.012
rs3135001				
CC	224	177	1.00 (Reference)	
CT	36	53	0.54 (0.34–0.85)	0.008
TT	3	7	0.34 (0.09–1.25)	0.104
T vs C			0.53 (0.35–0.79)	0.002
rs10462706				
CC	108	101	1.00 (Reference)	
CT	119	99	1.12 (0.77–1.64)	0.547
TT	36	37	0.91 (0.53–1.55)	0.728
T vs C			0.99 (0.77–1.28)	0.951
rs4318431				
CC	222	195	1.00 (Reference)	
CT	40	39	0.90 (0.56–1.46)	0.671
TT	1	3	0.29 (0.03–2.48)	0.260
T vs C			0.83 (0.53–1.28)	0.398

Note: *Age, gender, BMI, smoking, drinking, tumor site, treatment, and TNM stage.

GWAS-identified HNSCC risk loci with progression, efficacy and toxicity of radiotherapy of HNSCC. We revealed that: (1) rs259919 was significantly associated with higher TNM stage; (2) rs3135001 was significantly associated with better efficacy of radiotherapy; (3) both SNP rs1265081 and rs3135001 were significantly associated with the occurrence of grade 3–4 oral mucositis. Taking together, these loci might be useful biomarkers for predicting efficacy and toxicity of radiotherapy for HNSCC patients.

HNSCC is a heterogeneous disease, differing not only in clinical presentation and course, but also in genetic variation.[14] Using candidate gene approach, Zhang et al have identified several loci for risk and progression of HNSCC.[9–11] The study of these genetic variants has revealed not only underlying mechanisms but also clinically useful biomarkers that contribute to the personalization of treatment. In current study, 3 of the five GWAS-identified HNSCC risk loci was identified to be

associated with either progression, efficacy or toxicity of radiotherapy of HNSCC. Among them, rs259919 not only increases the occurrence of the disease but also is related to the progress of HNSCC. SNP rs259919 was located in the lncRNA ZNRD1-AS1 region, which have reported to associated with bladder cancer, nasopharyngeal carcinoma, breast cancer, glioma, endometrial cancer, hepatocellular carcinoma, lung cancer, and cervical cancer.[15–24] Interestingly, we found rs3135001 was significantly associated with better efficacy of radiotherapy and fewer grade 3–4 oral mucositis. Using RegulomeDB 2.0, we found rs3135001 was located in a TF binding or DNase peak region.[25] SNP rs3135001 was located in the HLA-DQB1 domain, which exhibit a larger degree of allelic polymorphism than usually recognized by routine serology.[26] It has been linked many diseases, including cancer, cardiovascular diseases, and autoimmune diseases.[27–30] In addition, we also found rs1265081 was significantly associated with the occurrence of grade 3–4 oral mucositis. SNP

rs1265081 was located in the CCHCR1 domain, and GTEX portal also showed allele A of rs1265081 was associated with higher expression level of CCHCR1 in whole blood and many tissues.[31] CCHCR1 was identified to up-regulate in skin cancer and associated with EGFR expression.[32]

Although we have relative moderate sample size and systematic follow-up endpoint collection, several inherent limitations in this study need to be addressed. First, because of study time, funding, and staffing constraints, we are not currently following up on overall survival, just some short- to medium-term endpoints. Second, sample size was limited in relation to our stratification analysis. Third, further large-scale analysis with in-depth functional experiments are needed. Nevertheless, our results still provided new evidence and ideas for the prognostic study of HNSCC.

In conclusion, we identified three GWAS-identified HNSCC risk loci were significantly with progression, efficacy and toxicity of radiotherapy of HNSCC. Our findings strengthen the understanding of the essential role of genetic background in the progression and therapeutic effects of HNSCC. Further investigations of the underlying molecular mechanisms to explain how these polymorphisms affect disease progression, and response to radiotherapy are needed.

Disclosure

The authors declare that they have no conflicts of interest.

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