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E2F transcription factor 2 variants as predictive biomarkers for recurrence risk in patients with squamous cell carcinoma of the oropharynx

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Abstract

Because E2F transcription factor 2 (*E2F2*) promoter polymorphisms have been implicated in carcinogenesis and prognosis, we investigated associations between genetic variants in five *E2F2* promoter polymorphisms and recurrence risk of squamous cell carcinoma of the oropharynx (SCCOP) in 1,008 patients. A log-rank test and multivariable Cox models were used to assess the associations. Compared with patients with variant genotypes of *E2F2*-rs2742976 and *E2F2*-rs3218123, patients with common homozygous genotypes had better disease-free survival (both log-rank, $p < 0.001$) and lower SCCOP recurrence risk (HR, 0.4, 95% CI, 0.3–0.6 and HR, 0.3, 95% CI, 0.2–0.5, respectively) after multivariable adjustment. Furthermore, among patients with HPV16–positive tumors, those with common homozygous genotypes of *E2F2*-rs2742976 and *E2F2*-rs3218123 had better disease-free survival rates (both log-rank, $p < 0.001$) and lower recurrence risk (HR, 0.1, 95% CI, 0.1–0.4 and HR, 0.1, 95% CI, 0.0–0.2, respectively) than patients with variant genotypes. However, no significant differences were found for the other three polymorphisms. After combining the risk genotypes of the 5 polymorphisms and using the high-risk group (2–5 risk genotypes) as the reference group, we found that the low-risk groups (0 or 1

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risk genotype) had significantly lower recurrence risk among all patients (HR, 0.4, 95% CI, 0.3–0.6) and among HPV16-positive patients (HR, 0.2, 95% CI, 0.1–0.5). Our findings suggest that *E2F2* polymorphisms may individually or jointly modify SCCOP recurrence risk, particularly for SCCOP patients with HPV16-positive tumors.

Keywords

E2F2 variant genotypes; recurrence; predictive biomarkers; human papillomavirus; oropharyngeal cancer

Introduction

The rates of squamous cell carcinoma of the oropharynx (SCCOP) recurrence and of patient survival after definitive treatment differ among patients with similar lifestyles and clinical factors, which suggests that genetic factors may contribute to interindividual differences. Thus, understanding the effect of genetic variations on disease-free and overall survival may improve clinical and therapeutic decision-making and thereby improve patient survival and quality of life [1,2].

Between 25% and 60% of SCCOP cases are associated with an oncogenic human papillomavirus (HPV) infection, and HPV has a stronger effect on the prognosis of SCCOP than other known risk factors. Although epidemiologic, histologic, and clinical data from SCCOP patients have been used to predict survival outcomes, several genetic factors that interact with HPV have not been investigated. Adding to our previous reports of prognostic biomarkers from several molecular pathways for HPV type 16 (HPV16)-associated SCCOP, we investigated *E2F* transcription factor 2 (*E2F2*) as a potential predictive biomarker for recurrence risk in this study. *E2F2* belongs to the activators of the family that controls the transition between G1 and S phase through various upstream signals [3]. *E2F2* is critical to many cell processes, including the cell cycle, proliferation, differentiation, and cancer development [4–6]. Because many important checkpoints and regulatory pathways control the cell cycle and ensure the fidelity of DNA replication and chromosome segregation [7–10], dysregulation of the cell cycle may lead to carcinogenesis. Outcomes vary widely among patients [11], indicating that genetic alterations may affect these genes' expression and SCCOP prognosis.

Genetic variants or polymorphisms in the promoter of *E2F2* affect the expression of *E2F2* [12,13]; such alterations of expression may also affect cell-cycle control and response to radiotherapy, thus leading to susceptibility to SCCOP recurrence. We and others have previously shown that *E2F2* promoter polymorphisms, which affect the expression of *E2F2* [12,13], are significantly associated with increased risk of many cancers [14–16] and that the combined risk genotypes of *E2F1* and *E2F2* may modify the risk of SCCOP, but not the risk of other subtypes of squamous cell carcinoma of the head and neck [17]. To our knowledge, no studies have assessed the associations of *E2F2* promoter polymorphisms with risk of SCCOP recurrence. Given the important roles of *E2F2* in cell-cycle regulation and the interaction between *E2F2* and HPV, we hypothesized that *E2F2* promoter polymorphisms may affect *E2F2* expression and cause interindividual variations in responses to

radiotherapy, leading to different risk of SCCOP recurrence. In the current study, we assessed the associations of common and variant genotypes of five *E2F2* promoter polymorphisms, both individually and jointly, with the risk of SCOP recurrence in a cohort of 1,008 patients with SCCOP and the subgroup of patients with HPV16(+) SCCOP (n=324).

Patients and Methods

Study participants

Patients with SCCOP were prospectively enrolled for a molecular profiling study and were treated at The University of Texas MD Anderson Cancer Center (Houston, TX) from May 1995 through April 2010, as we described previously [2]. In the original study, all patients had newly diagnosed, histopathologically confirmed, previously untreated SCCOP and were enrolled regardless of age at diagnosis, sex, ethnicity, or clinical stage. Patients with distant metastases at presentation were excluded. Before participating in the original molecular profiling study, all patients agreed to sign a consent form. That study was approved by the Institutional Review Board of MD Anderson Cancer Center. That study achieved an approximately 95% enrollment rate by recruited patients. Details of the patients' follow-up and clinical data were previously reported [1,2].

In the present study, we clearly distinguished SCCOP recurrence from second primary tumors to minimize the overestimation or underestimation of associations. Patients were typically followed and monitored through their treatment and post-treatment course with scheduled regular clinical and radiographic examinations. Patients were considered disease free if absence of disease was documented at the date of the last visit with the head and neck surgeon, head and neck radiation oncologist, or head and neck medical oncologist. There were no universal standards for imaging. Typically patients had either routine serial imaging, or follow-up imaging on the basis of symptoms or findings on physical examinations. Recurrent disease was defined as appearance of a new lesion of the same histology verified by biopsy (incisional, excisional, or needle biopsy), reappearance of any lesion that had disappeared, or development of tumor-related symptoms. Based on modified criteria of Warren and Gates, second primary malignancies (SPMs) were considered if the second lesions were different histopathologic type, or if they occurred more than 5 years following treatment for the index tumor, and/or clearly separated by normal epithelium based on clinical and radiographic assessment. Pulmonary lesions were considered as a SPM if they had a non-squamous histology; or if they were isolated squamous lesions greater than 5 years from initial SCCHN and felt to be SPM by the thoracic oncologist and thoracic surgeon. If there was discrepancy or differing of opinions regarding the origin of the tumor (i.e., SPM vs. recurrence), the second lesion were classified as a local recurrence rather than a SPM. We determined disease stage at presentation for all study patients using the sixth edition of the American Joint Committee on Cancer TNM staging system. Definitive radiotherapy was categorized as radiotherapy alone or radiotherapy in combination with other therapeutic modalities. Patients with comorbidities were categorized into two groups—none to mild and moderate to severe—using a modification of the Kaplan-Feinstein comorbidity index (Adult Comorbidity Evaluation 27) [18].

Genotyping

DNA was extracted from patient blood samples before treatment and was genotyped for five single nucleotide polymorphisms of *E2F2* (*E2F2*-rs6667575, *E2F2*-rs3218121, *E2F2*-rs2742976, *E2F2*-rs3218123, and *E2F2*-rs3218148) as we previously described [17]. Both positive and negative controls were included for genotyping. For quality control, approximately 10% of samples were selected for retesting, and the repeated test results demonstrated 100% concordance with the original results. The patients were categorized into two risk groups. The high-risk group included patients with 2–5 risk genotypes of the two polymorphisms and the low-risk group included patients with 0–1 risk genotype of either polymorphism.

Tumor HPV16 status and E2F2 protein expression

As described previously, to determine tumor HPV16 status, we subjected DNA from paraffin-embedded tissue specimens to polymerase chain reaction and in situ hybridization [19]. We retested a subset of samples (5%) for tumor HPV16 status for quality control. The retested results were 100% concordant with the findings from the original assays. A total of additional 64 SCCOP patients were selected with tumor specimens available for E2F2 immunohistochemistry (IHC). Paraffin blocks from SCCOP specimens were sectioned into 4- μ m-thick sections. For IHC, these sections were immunostained for E2F2 protein with primary antibodies against E2F2 (Epitomics, Burlingame, CA) and immunostained sections were evaluated according to previous study.

Statistical analysis

For this study, SCCOP recurrence was the primary endpoint. Time to recurrence was from the date of SCCOP diagnosis to the date of recurrence. Patients without known recurrence at the date of last follow-up, those lost to follow-up, and those who died of any cause were censored. A Student *t*-test was used to compare mean age at diagnosis and follow-up time between patients with and without SCCOP recurrence. The association of each demographic, epidemiologic, or clinical variable with time to SCCOP recurrence was evaluated using both univariate and multivariate Cox regression models. The estimates of association of each variable with disease-free survival (DFS)/recurrence status were assessed using a log-rank test. Associations of *E2F2* polymorphisms with risk of SCCOP recurrence were estimated using hazard ratios (HRs) and 95% confidence intervals (CIs) among both the whole patients and patients with HPV16(+) tumors, while in this study we did not include similar analyses for patients with HPV16(–) tumors owing to the relatively small sample size and the rarity of SCCOP recurrence in this subgroup. The multivariable Cox model included variables adjusted for major confounders, including demographic variables (age at diagnosis, sex, and ethnicity); epidemiologic factors (smoking status and alcohol use status); and clinical factors (comorbidity group, tumor stage, and treatment). For all analyses, *p* values less than 0.05 were considered statistically significant, and all tests were two-sided. All statistical analyses were performed using SAS software (version 9.2.3; SAS Institute).

Results

The overall SCCOP recurrence rates, the 5-year recurrence rate, and the associations between DFS and age at diagnosis, sex, ethnicity, smoking, alcohol use, comorbidity group, index cancer stage, and treatment were previously reported [2]. The median follow-up time and mean age at diagnosis among all patients, patients with SCCOP recurrence, and patients without recurrence were also previously reported [2]. We included 1,008 patients in our final analysis [2], and Kaplan-Meier univariate analysis results showed that the 5-year rate of recurrence was significantly different between groups divided according to age at diagnosis, ethnicity, smoking status, alcohol use, comorbidity group, and treatment (all $p < 0.05$) but was not significantly different between the groups for sex and index cancer stage (both $p > 0.05$).

SCCOP patients with the common homozygous *E2F2*-rs2742976 GG genotype and *E2F2*-rs3218123 GG genotype had significantly better DFS than those with the other two corresponding genotypes (log-rank, $p < 0.001$ for both polymorphisms; Fig. 1). We further estimated the recurrence rates of 324 patients with HPV16(+) SCCOP and found that DFS was significantly shorter in patients carrying the *E2F2*-rs2742976 GT/TT and *E2F2*-rs3218123 GT/TT genotypes than in those carrying the corresponding common homozygous GG genotypes (log-rank for both, $p < 0.001$; Fig. 1). However, significant differences in DFS were not found for the other three polymorphisms: *E2F2*-rs6667575 (log-rank, $p = 0.594$); *E2F2*-rs3218121 (log-rank, $p = 0.100$); or *E2F2*-rs3218148 (log-rank, $p = 0.060$).

We performed a multivariable Cox proportional hazards regression analysis to assess the associations of genotypes of the five single nucleotide polymorphisms with recurrence risk in patients with SCCOP. As shown in Table 2, compared with patients with the *E2F2*-rs2742976 GT/TT and *E2F2*-rs3218123 GT/TT variant genotypes, patients with the *E2F2*-rs2742976 GG and *E2F2*-rs3218123 GG common homozygous genotypes had significantly lower risk of SCCOP recurrence (HR, 0.4, [95% CI, 0.3–0.6 and HR, 0.3, 95% CI, 0.2–0.5, respectively). However, no significant differences were observed for the other three polymorphisms: *E2F2*-rs6667575 (HR, 0.9, 95% CI, 0.7–1.2); *E2F2*-rs3218121 (HR, 1.3, 95% CI, 0.8–1.9); or *E2F2*-rs3218148 (HR, 1.3, 95% CI, 0.9–1.8).

Given the important role of HPV and *E2F2* in regulating proliferation, cell-cycle progression, and tumorigenesis, we also evaluated the associations of genotypes of the five polymorphisms with risk of recurrence at 5 years among the subgroup of patients with HPV16(+) SCCOP. As shown in Table 3, patients with HPV16(+) tumors and the *E2F2*-rs2742976 GG and *E2F2*-rs3218123 GG common homozygous genotypes had significantly lower risk of recurrence (approximately 90%) than those with HPV16(+) tumors and the *E2F2*-rs2742976 GT/TT and *E2F2*-rs3218123 GT/TT variant genotypes (HR, 0.1, 95% CI, 0.1–0.4 for *E2F2*-rs2742976 and HR, 0.1, 95% CI, 0.0–0.2 for *E2F2*-rs3218123). However, no significant differences were observed for the other three polymorphisms: *E2F2*-rs6667575 (HR, 0.9, 95% CI, 0.5–1.7); *E2F2*-rs3218121 (HR, 1.6, 95% CI, 0.6–4.1); or *E2F2*-rs3218148 (HR, 1.3, 95% CI, 0.7–2.5).

Finally, we explored the joint effect of risk group (based on number of risk genotypes) on risk of recurrence among all patients and the subgroup of those with HPV16(+) SCCOP. As

shown in Figure 2, patients in the low-risk group had lower rates of recurrence in both the whole group and the HPV16(+) subgroup (all log-rank $p < 0.001$). Furthermore, after combining risk genotypes of the five polymorphisms and using the high-risk group as the reference group, the low-risk groups were significantly associated with lower risk of recurrence among the whole patients (HR, 0.4, 95% CI, 0.3–0.6) and among the patients with HPV16(+) tumors only (HR, 0.2, 95% CI, 0.1–0.5; Table 4), respectively.

To further characterize the potentially functional relevance of *E2F2* promoter polymorphisms, we performed correlation analyses between tumor E2F2 expression by E2F2 immunohistochemistry (IHC) and genotypes of *E2F2* polymorphisms among a subset of 64 SCCOP patients. Among 64 SCCOP tissue specimens, we found that 50 patients had HPV16- positive tumors and 14 patients had HPV16-negative tumors. In all patients, we found that the *E2F2*-rs2742976 GG genotype had a significantly higher E2F2 protein expression than *E2F2*-rs2742976 GT/TT genotypes ($P = 0.028$) and *E2F2*-rs3218123 GG genotype had a borderline significantly higher E2F2 protein expression than *E2F2*-rs3218123 GT/TT genotypes ($P = 0.062$), while no significant associations of other *E2F2* SNPs with E2F2 protein expression ($P = 0.345$ for *E2F2*-rs6667575, $P = 0.653$ for *E2F2*-rs3218121, and $P = 0.768$ for *E2F2*-rs3218148, respectively). In HPV16-negative cases, the E2F2 protein expression was not significantly different between genotypes of any *E2F2* SNPs.

Furthermore, in HPV-positive patients only, the patterns of above associations of these SNPs of *E2F2* with E2F2 protein expression remained similar. *E2F2*-rs2742976 GG genotype had a significantly higher E2F2 protein expression than *E2F2*-rs2742976 GT/TT genotypes ($P = 0.013$) and *E2F2*-rs3218123 GG genotype had a borderline significantly higher E2F2 protein expression than *E2F2*-rs3218123 GT/TT genotypes ($P = 0.057$), while no significant associations of other *E2F2* SNPs with E2F2 protein expression ($P = 0.567$ for *E2F2*-rs6667575, $P = 0.687$ for *E2F2*-rs3218121, and $P = 0.877$ for *E2F2*-rs3218148, respectively). In HPV16- negative cases, no any significant associations were found for any SNPs.

In this study, we did not perform the survival analysis by E2F2 protein expression because there were few outcome events (e.g., death, recurrence etc) among these 64 SCCOP patients. However, these findings will be validated in our future study with larger patients whose tumor specimens become available.

Discussion

We found that the *E2F2*-rs2742976 and *E2F2*-rs3218123 variants were significantly associated with the risk of SCCOP recurrence, particularly in patients with HPV16(+) tumors. Furthermore, we found that patients with more than 1 risk genotype among the five *E2F2* polymorphisms had higher risk of SCCOP recurrence, especially in patients with HPV16(+) SCCOP tumors. These findings suggest that certain *E2F2* polymorphisms may individually or jointly predict risk of SCCOP recurrence, particularly in patients with HPV16(+) tumors.

Although the exact mechanism by which these *E2F2* promoter variants modify cancer recurrence remains unknown, it is likely that these variants could alter expression of *E2F2* and thus might affect susceptibility of response to radiotherapy, leading to different clinical outcomes. Therefore, *E2F2* variants could be potential predictive biomarkers for SCCOP recurrence and could help identify patients for individualized therapies. Binding of E7 to phosphorylated RB leads to activation of E2F2, which induces cell-cycle progression and p16 expression. Thus, it is biologically plausible that *E2F2* promoter polymorphisms might affect E2F2 protein expression, which, in turn, could affect p16 expression level, thereby influencing cancer prognosis. So far, no investigations on functional relevance of these *E2F2* promoter polymorphisms have been reported. Since E2F2 regulates expression of p16 through *Rb*-mediated pathways and these *E2F2* polymorphisms are within the functional regions of the *E2F2*'s promoter, it is likely that these *E2F2* polymorphisms may have potentially functional effect on expression levels of p16, thus leading to better survival. In fact, in this study, we found that *E2F2*-rs2742976 polymorphism significantly affected expression of E2F2 in SCCOP patients, particularly HPV16-positive patients but not in HPV16-negative cases in SCCOP tumor specimens. Although the functional relevance of the *E2F2*-rs2742976 polymorphism has not yet been elucidated, our results could partially suggest a functional correlation between this polymorphism and E2F2 expression, which may provide preliminary evidence of biological plausibility for the observed association in the current study. As we found from IHC staining, individuals with *E2F2*-rs2742976 GG genotype had significantly higher E2F2 expression than those with GT/TT genotypes in SCCOP patients, particularly HPV16-positive SCCOP patients, implying that binding of HPV16 E7 to Rb might lead to release of transcription factor E2F2 in HPV-associated SCCOP patients. Therefore, genetic variations, such as *E2F2*-rs2742976 polymorphism, may affect promotion of cell cycle progression and induction of p16 expression, thereby leading to better survival of SCCOP. However, all these findings need to be validated in future large and well-designed studies.

Several studies have shown the associations of *E2F2* promoter polymorphisms with risk of various types of human cancer [20–22], but no studies have investigated the modifying effect of *E2F2* polymorphisms on SCCOP recurrence risk, especially for HPV-associated SCCOP. Chen *et al.* reported that *E2F2* was a potential marker for the prognosis of patients with non-small cell lung cancer [12]. Similarly, an association of ovarian cancer risk with *E2F2*-rs760607 and *E2F2*-rs3218203 polymorphisms was reported [20]. However, Lu *et al.* did not find a main effect of *E2F2* variants or other variant genotypes on risk of squamous cell carcinoma of the head and neck [17], and Justenhoven *et al.* found that the *E2F2*_-5368_A>G (rs760607) promoter polymorphism was not significantly associated with risk of breast cancer [21]. In our study, we found a significant association between the *E2F2*-rs2742976 or *E2F2*-rs3218123 variant genotypes and an increased risk of SCCOP recurrence; however, we did not observe significant differences for the other three *E2F2* polymorphisms.

The inconsistent results regarding the associations of *E2F2* promoter polymorphisms with cancer risk and prognosis suggest that these effects might also depend on other factors, such as cancer type, genetic background, environmental factors, sample size, disease stage, treatment, adequacy of adjustment for other confounding factors, and different study

populations [1]. The retinoblastoma-E2F pathway and other molecular pathways involved in cell-cycle control, DNA repair, and apoptosis [23,24] and/or interactions between functional genetic variants and therapeutic agents may also cause interindividual differences in prognosis. Tumor HPV status also significantly affected risk of SCCOP recurrence: patients with HPV16(+) SCCOP had a better prognosis than those with HPV(–) SCCOP. Patients with HPV(+) SCCOP, who have few somatic mutations, appear to have a better response to radiotherapy than those with HPV(–) SCCOP, whose disease is mainly driven by smoking and who have common somatic mutations, such as those in p53. Therefore, tumor HPV status significantly predicts SCCOP prognosis, and future studies of factors potentially related to SCCOP prognosis and recurrence should consider stratified analysis by tumor HPV status.

We also assessed the effect of *E2F2* promoter variants on recurrence risk in patients with HPV16(+) SCCOP. The modifying effect of *E2F2*-rs2742976 and *E2F2*-rs3218123 variant genotypes on SCCOP recurrence risk was higher in SCCOP patients with HPV16(+) tumors than in those with common homozygous genotypes, suggesting that *E2F2*-rs2742976 and *E2F2*-rs3218123 variant genotypes interact with HPV status to affect SCCOP recurrence risk. Although the molecular mechanisms underlying these findings in patients with HPV16(+) SCCOP remain incompletely understood, the variant genotypes of these two polymorphisms may affect DNA repair capacity or apoptosis through controlling the cell cycle [22]. We also found that patients with more than one risk genotype had a significantly higher risk of SCCOP recurrence, particularly patients with HPV16(+) tumors, which supports the notion that SCCOP is mainly driven by HPV [25,26]. Thus, *E2F2* variants may affect susceptibility to radiotherapy and lead to different recurrence outcomes. However, this hypothesis should be investigated in future studies.

Our study has several strengths including its relatively large sample size, inclusion of tumor HPV status, and analysis of a homogeneous tumor site (SCCOP only). However, we did not have data about the exact dosage and duration of radiotherapy for our patients; detailed information on radiotherapy should be collected in future studies. Second, because there were relatively small numbers of patients with HPV16(+) tumors and recurrence, our results could have been due to chance. Third, most of our patients were non-Hispanic whites recruited at a single cancer center; thus, our results should be validated in other racial and ethnic populations. Lastly, SCCOP recurrence was identified retrospectively without a strictly defined screening or follow-up regimen, and our study likely had recall bias and selection bias.

Taken together, our findings suggest that the *E2F2* promoter variants might individually or in combination modulate the risk of SCCOP recurrence, especially in patients with HPV16(+) tumors who have received definitive radiotherapy. However, the prognostic utility of these promoter variants should be validated, and further exploration of the molecular mechanisms underlying our findings is warranted.

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Abbreviations

CI	confidence interval
DFS	disease-free survival
E2F2	E2F transcription factor 2
HPV	human papillomavirus
HPV16	HPV type 16
HPV16(+)	HPV–positive
HR	hazard ratio
SCCOP	squamous cell carcinoma of the oropharynx.

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Novelty and impact statements

E2F2 promoter variants individually or jointly modify the risk of SCCOP recurrence, and may be a predictive biomarker for recurrence risk of SCCOP, particularly in HPV-positive SCCOP patients.

Figure 1.

Kaplan-Meier estimates for DFS of patients according to the *E2F2*-rs2742976 and *E2F2*-rs3218123 genotypes among all patients and those with human papillomavirus (HPV)-positive SCCOP.

Figure 2.

Kaplan-Meier estimates for the DFS of patients according to the combined risk genotypes of the 5 polymorphisms of E2F2 among all patients and those with human papillomavirus (HPV)-positive SCCOP. The high-risk group included patients with 2–5 risk genotypes, and the low-risk group included patients with 0–1 risk genotype.

Table 1.

Characteristics of patients with SCCOP (N = 1,008).

Variable	No. of patients (n, %)	No of patients with recurrence	5-year recurrence rate (%)	p^I value
No. of patients	1008 (100)	181	0.20	
Age				
57 years	621 (61.6)	85	0.15	<0.0001
> 57 years	387 (38.4)	96	0.27	
Sex				
Male	872 (86.5)	161	0.20	0.3110
Female	136 (13.5)	20	0.19	
Ethnicity				
Non-Hispanic white	913 (90.6)	146	0.17	<0.0001
other	95 (9.4)	35	0.41	
Smoking				
Never	388 (38.5)	51	0.14	0.0004
Ever	620 (61.5)	130	0.23	
Alcohol use				
Never	247 (24.5)	26	0.10	0.0005
Ever	761 (75.5)	155	0.23	
Comorbidity				
None or mild	913 (90.6)	157	0.19	0.0370
Moderate to severe	95 (9.4)	24	0.27	
Index cancer stage				
1 or 2	72 (7.1)	11	0.19	0.5280
3 or 4	936 (92.6)	170	0.20	
Treatment				
X/XC/SX	947 (93.9)	166	0.19	0.0030
SXC	61 (6.1)	15	0.32	

X, radiotherapy; C, chemotherapy; and S, surgery.

 p^I : Log-rank test for DFS between the two groups.

Table 2.

Association between *E2F2* genotypes and SCCOP recurrence in patients with SCCOP (*N*=1,008).

Genotype	No. of recurrence/no. of patients	5-year recurrence rate	Log-rank <i>p</i> values	aHR ^{<i>I</i>} , 95% CI
<i>E2F2</i> -rs6667575				
GG(Ref.)	88/474	0.17	0.594	1.0
GA+AA	93/534	0.18		0.9 (0.7-1.2)
<i>E2F2</i> -rs3218121				
GG(Ref.)	152/885	0.18	0.100	1.0
GA+AA	29/123	0.24		1.3 (0.8-1.9)
<i>E2F2</i> -rs2742976				
GT/TT(Ref.)	138/603	0.24	<0.001	1.0
GG	43/405	0.11		0.4 (0.3-0.6)
<i>E2F2</i> -rs3218123				
GT/TT(Ref.)	128/461	0.30	<0.001	1.0
GG	53/547	0.09		0.3 (0.2-0.5)
<i>E2F2</i> -rs3218148				
AA(Ref.)	63/415	0.16	0.060	1.0
AG+GG	118/593	0.21		1.3 (0.9-1.8)

aHR: adjusted hazard ratio.

^{*I*} Adjusted for age, sex, ethnicity, smoking status, alcohol use status, stage, comorbidity, and treatment.

Ref.:Reference group.

Table 3.

Association between *E2F2* genotypes and HPV-positive SCCOP recurrence in patients with SCCOP (N = 324).

Genotype	No. of recurrence/no. of patients	5-year recurrence rate	Log-rank <i>p</i> values	aHR ^I , 95% CI
<i>E2F2</i> -rs6667575				
GG(Ref.)	24/157	0.16	0.464	1.0
GA+AA	21/167	0.14		0.9 (0.5-1.7)
<i>E2F2</i> -rs3218121				
GG(Ref.)	40/296	0.15	0.451	1.0
GA+AA	5/28	0.20		1.6 (0.6-4.1)
<i>E2F2</i> -rs2742976				
GT/TT(Ref.)	41/196	0.21	<0.001	1.0
GG	<u>4/128</u>	0.04		0.1 (0.1-0.4)
<i>E2F2</i> -rs3218123				
GT/TT(Ref.)	39/146	0.31	<0.001	1.0
GG	6/178	0.04		0.1 (0.0-0.2)
<i>E2F2</i> -rs3218148				
AA(Ref.)	17/135	0.14	0.554	1.0
AG/GG	28/189	0.16		1.3 (0.7-2.5)

aHR, adjusted hazard ratio.

^IaHR, adjusted Hazard ratio for age, sex, ethnicity, smoking status, alcohol use status, stage, comorbidity, and treatment.

Ref: Reference group.

Table 4.

Association between combined *E2F2* genotypes and recurrence risk in overall and tumor HPV-positive patients with SCCOP.

<i>Combined genotypes</i>	<i>No. of recurrence/no. of patients</i>	<i>5-year recurrence rate</i>	<i>Log-rank <i>p</i> values</i>	<i>aHR^I, 95% CI</i>
All patients (<i>N</i> =1008)				
High risk group [*] (Ref.)	73/247	0.32	< 0.001	1.0
Low risk group	108/761	0.14		0.4 (0.3-0.6)
HPV-positive SCCOP patients (<i>N</i> =324)				
High risk group(Ref.)	26/82	0.40	< 0.001	1.0
Low risk group	19/242	0.08		0.2 (0.1-0.5)

^{*} high-risk group: individuals carrying 2-5 genotypes; Low-risk group: individuals carrying 0-1 genotype.

^I aHR, adjusted hazard ratio for age, sex, ethnicity, smoking status, alcohol use status, stage, comorbidity, and treatment.

Ref: Reference group.