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Biology of Human Tumors

Clinical Cancer Research

TGFβ1 Genetic Variants Predict Clinical Outcomes of HPV-Positive Oropharyngeal Cancer Patients after Definitive Radiotherapy

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Abstract

Purpose: TGFβ1 plays a critical role in inflammation and immune responses and treatment response and survival. *TGFβ1* variants may affect its expression level or functional efficiency, thus modifying tumor status and survival in human papillomavirus (HPV)–positive squamous cell carcinoma of the oropharynx (SCCOP).

Experimental Design: We determined tumor HPV16 status and genotyped three *TGFβ1* polymorphisms in 564 incident SCCOP patients treated with radiotherapy or chemoradiation. Univariate and multivariable Cox models were used to evaluate the associations between the three polymorphisms and survival.

Results: Overall, 85% of patients (482 of 564) had HPV16-positive SCCOP. We found that *TGFβ1* rs1982073 had statistically significant associations with survival, whereas *TGFβ1* rs1800469 and *TGFβ1* rs1800471 did not. Patients with *TGFβ1* rs1982073 CT/CC variant genotypes had significantly better overall, disease-specific, and disease-free survival compared with those with the corresponding common homozygous TT genotype (all log-rank: $P < 0.001$). Furthermore, these genotypes were significantly associated with an approximately 5 times reduced risk of overall death, death owing to disease, and recurrence after multivariable adjustment. Moreover, the stratified analyses by tumor HPV status indicated that the significant effects of *TGFβ1* rs1982073 polymorphism on survival were found among HPV16-positive SCCOP patients only. Finally, the functional relevance of these variants was further characterized.

Conclusions: Our findings support that the *TGFβ1* rs1982073 polymorphism plays a significant role in the prognosis of SCCOP, especially in HPV16-positive SCCOP patients treated with chemoradiation. Prospective studies with larger sample sizes are needed to confirm these findings. *Clin Cancer Res; 24(9); 2225–33. ©2018 AACR.*

Introduction

Squamous cell carcinomas of the oropharynx (SCCOP) are a subset of squamous cell carcinomas of the head and neck (SCCHN) that arise within epithelial tissues and are characterized by local tumor aggressiveness, high local recurrence rates, and a high frequency of second primary tumors. The overall incidence rate of SCCOP has remained stable over the past several decades at approximately 1.5 per 100,000 population, with approximately 48,330 new cases and 9,570 deaths in 2016 (1, 2). Tobacco and alcohol exposure remain significant risk factors for SCCOP; however, in recent years, human papillomavirus (HPV) has accounted for a growing proportion of cases, particularly among young adults (3–6). Because of this, the incidence of SCCOP has been rising, whereas the overall incidence of head and neck cancer has been declining (1), and patients with SCCOP now present at a younger age than do patients with other head and neck cancers (median age, 61 vs. 64 years; refs. 6, 7).

The current treatment of choice for SCCOP is radiotherapy or chemoradiation, and these treatment modalities have increasingly replaced surgery over the last few decades (8–10). Early-stage disease is most often treated with radiotherapy alone, whereas advanced-stage disease is more often treated with chemoradiation (11). Specifically, studies have shown that although greater toxicity is observed with chemoradiation than with radiotherapy alone, both disease-free survival (DFS) and overall survival (OS) tend to be better with concomitant therapy (8). Despite improvements in diagnostic techniques, up to 70% of patients are still diagnosed with late-stage SCCOP, and consequently, the overall 5-year survival rate has not significantly risen (1, 8, 9, 12, 13). In particular, the 5-year survival rate is 50% for regional-stage tumors and 30% for distant-stage tumors (1). In light of these low survival rates, it is important to identify factors such as genetic variants that lead to improved outcomes for patients with SCCOP.

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Translational Relevance

Human papillomavirus (HPV)–positive squamous cell carcinoma of the oropharynx (SCCOP) is a distinct subgroup with different etiology, treatment response, and survival. HPV status is highly relevant to SCCOP prognosis, although there is significant heterogeneity in the outcomes of HPV-positive SCCOP. The identification of molecular biomarkers, such as *TGFβ1* genetic variants, which determine HPV status and thus may further stratify SCCOP patients who could benefit from reduction of treatment intensity, is critical to the delivery of more effective and less morbid treatment and tailored follow-up strategies for improved survival and a better quality of life.

Prior attempts to predict the prognosis of SCCOP patients have been hampered in part by underpowered studies, differences between SCCOP tumor types, underlying heterogeneity among the different studies, and failure to fully adjust for important confounders. Clinically, the clinicians decide the treatment for SCCOP patients primarily based on tumor tumor–node–metastasis classification. More recently, HPV status has become a key clinically prognostic predictor, whereas even SCCOP patients with the same tumor HPV status can have significantly different clinical prognosis. For example, HPV-positive SCCOP is generally associated with better survival, except among smokers, who in some studies have outcomes similar to those of patients with HPV-negative SCCOP. Thus, molecular biomarkers might be more effective than clinical and epidemiologic prognostic factors for survival prediction in SCCOP. At present, no molecular biomarkers have been found to accurately predict outcomes in SCCOP patients.

TGFβ is an inhibitor of cell growth that in human cancers may act as both a tumor suppressor and a potent stimulator of tumor progression, local invasion, and metastasis (14). As such, TGFβ1's effects on survival can vary. On the one hand, it contributes to inflammation and immune responses that promote HPV clearance and escape from immune surveillance and may contribute to genetic susceptibility to HPV16 infection, but on the other hand, it may improve treatment response to HPV16 treatment. Early in tumor development, TGFβ acts to suppress growth; however, at later stages of tumorigenesis, genetic and biochemical changes allow TGFβ to stimulate tumor progression (14). Functional TGFβ is found in many human tumors, but mutations in the TGFβ signaling pathway leading to loss of function may also promote tumor progression. Excess TGFβ has been associated with poor prognosis, as TGFβ may slow tumor growth but increase invasive and metastatic potential (14). Circulating plasma levels of TGFβ1 have been explored as a potential prognostic indicator, and increased levels have been associated with a worse prognosis (15, 16).

Three isoforms of TGFβ have been identified: TGFβ13. TGFβ1 is highly conserved in mammals, is expressed in several types of cells, and is the most upregulated isoform in cancer cells (14, 17, 18). The role of TGFβ1 in tumor suppression and progression stems from several functions, among them inhibition of cell-cycle progression leading to uncontrolled growth; regulation of cellular adhesion, motility, and the extracellular matrix, resulting in increased invasiveness and metastatic potential; control of apoptosis; direct stimulation of angiogenesis; and inhibition of proliferation and activation of inflammatory cells, thereby allowing escape of cancer cells from host immunosurveillance (14, 17, 19).

Several potentially functional *TGFβ1* polymorphisms have been identified, and some of them may modulate plasma levels of TGFβ1. Plasma levels were almost twice as high for *TGFβ1* rs1800469:C509T, which has a variant T allele in the promoter region of *TGFβ1*, than in *TGFβ1* with the wild-type C allele (20). In the signal sequence of *TGFβ1*, the SNPs rs1982073:T869C and rs1800471:G915C result in nonsynonymous amino acid changes and were also associated with variability in TGFβ1 levels. The T869C SNP at codon 10 of exon 1 results in a leucine to proline change, whereas G915C at codon 25 results in an arginine to proline change. Previous studies have demonstrated that increased production of TGFβ1 is associated with the variant C allele of T869C (21, 22) and with the wild-type G allele of G915C (23). Although several studies have investigated the association between HPV and TGFβ1 expression levels in cervical cancer using both *in vitro* and *in vivo* methods (24–26) and assessed their influence on primary risk (21, 22, 27–29), few have evaluated their effects on survival (28, 30). In addition, we previously have reported that *TGFβ1* rs1982073 was significantly associated with HPV16-positive SCCOP tumors (31). However, no studies have investigated whether *TGFβ1* polymorphisms are associated with survival of SCCOP patients, particularly HPV16-positive SCCOP patients. We hypothesized that *TGFβ1* polymorphisms are associated with survival of SCCOP patients after radiotherapy or chemoradiation.

We investigated whether genetic variation in *TGFβ1* had an effect on disease-specific survival (DSS), DFS, and OS of SCCOP to further identify factors that may be used to improve outcomes in these patients and found that individual variation in these genes may be used to predict survival.

Materials and Methods

Study patients

A total of 564 patients with incident SCCOP were recruited consecutively as part of an ongoing molecular epidemiology study of squamous cell carcinoma of the head and neck at The University of Texas MD Anderson Cancer Center from January 2000 to May 2013. All patients were newly diagnosed, histopathologically confirmed, and untreated SCCOP and recruited without restrictions regarding age, sex, ethnicity, cancer stage, or histology. Before enrollment, all patients provided informed consent, and the study was approved by the Institutional Review Board of MD Anderson Cancer Center. At study enrollment, the patients completed an epidemiologic questionnaire to provide information on demographic and risk factors including smoking and alcohol status and had 30 mL of blood taken for genotyping. The details of such study patients on enrollment, epidemiologic, and clinical information have been described in our previously study (31).

HPV16 detection in tumor specimens

DNA was extracted from paraffin-embedded tumor tissues of SCCOP patients and analyzed for the presence of HPV16 using polymerase chain reaction and ISH methods described in our previous studies (31, 32). For some SCCOP patients, tumor HPV16 status was determined by *in situ hybridization* and p16

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immunohistochemical analysis from HPV data in the patient's clinical records, as the pathology laboratory at MD Anderson had begun classifying all SCCOP specimens as a standard clinical practice.

TGFβ1 genotyping

The blood samples were used for *TGFβ1* genotyping. The genotyping and selection criteria for the *TGFβ1* polymorphisms were previously reported (33). The reasons for choosing these three polymorphisms were described as our previous studies (31, 33).

Measuring of serum TGFβ1

Plasma was stored at −80°C until use. Plasma levels of TGFβ1 were measured using eBioscience Human Th1/Th2 11plex FlowCytomix Kit (eBioscience) following manufacturing instruction for sample collection, storage, and assay procedure. Each sample was tested in duplicate, and the mean of tests was used for analysis. Furthermore, 10% of samples were randomly chosen and retested for quality assurance.

Patient follow-up

The patients were followed up and monitored throughout their treatment and posttreatment courses through regularly scheduled clinical and radiographic examinations. Patients were considered disease free if there was no disease documented on 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 from physical examination. Clinical data, including stage of the index tumor at presentation, site of the index tumor, and treatment, were obtained at initial presentation and through follow-up examinations. Index cancer stage was dichotomized into early-stage (clinical stage I and II) and late-stage (clinical stage III and IV) disease. We divided treatment into two categories: radiotherapy only and radiotherapy plus chemotherapy. Medical comorbidities were classified according to a modification of the Kaplan–Feinstein comorbidity index (Adult Comorbidity Evaluation 27), which categorizes related comorbidities as none, mild, moderate, or severe.

The primary endpoints of the study were overall deaths, deaths due to disease, and recurrence. We investigated differences in DFS, DSS, and OS among the SCCOP patients. Time to recurrence was computed from the date of the end of treatment to the date of last follow-up or clinical detection of recurrent cancer (local, regional, or distant). Participants who were recurrence free or lost to follow-up were censored. OS was defined as the time from first appointment to death from any cause or date of last follow-up. DSS was defined as the time from first appointment to death from disease or date of last follow-up. For both OS and DSS calculations, participants who were alive at the end of the study period or lost to follow-up were censored.

Statistical analysis

Statistical Analysis System software (Version 9.4; SAS Institute) was used for all of the statistical analyses. In the univariate analysis, we evaluated epidemiologic variables assessed at the time of diagnosis, including age in years, ethnicity, sex, smoking and alcohol status, HPV status, and clinical characteristics such as index tumor stage, comorbidity, and treatment. Although the univariate prognostic analysis was not statistically significant for some variables, these variables were retained in the main-effects and final multivariable model owing to epidemiologic and clinical considerations in building the model. We also used the Kaplan–Meier method to compare survival between patients with different genotypes and calculated the log-rank statistic to test the hypothesis that there was a difference in survival between the genotyping groups. Then, we investigated how genotypes modulated survival and whether the genotypes were statistically associated with survival among SCCOP patients by fitting a Cox proportional hazards model that included age, sex, ethnicity, smoking history, alcohol consumption, disease stage, comorbidity, and treatment as covariates. We also performed a similar multivariable analysis stratified by tumor HPV status. The unpaired *t* test or Z test was used for comparison of TGFβ1 expression levels between the different groups. For all analyses, statistical significance was set at $P < 0.05$, and all tests were two-sided.

Results

Patient characteristics

The characteristics of the 564 patients are summarized in Table 1. The median age at diagnosis was 55 years, with a range of 28 to 82 years. The majority of patients were non-Hispanic white (91.8%), and there were 491 males (87.1%) and

Table 1. Characteristics of study patients with SCCOP ($N = 564$)

Characteristics	Number (%) of SCCOP patients ($N = 564$)
Age	
≤57 years	343 (60.8)
>57 years	221 (39.2)
Sex	
Male	491 (87.1)
Female	73 (12.9)
Ethnicity	
Non-Hispanic white	518 (91.8)
Other	46 (8.2)
Smoking	
Never	280 (49.7)
Ever	284 (50.3)
Alcohol use	
Never	187 (33.2)
Ever	377 (66.8)
Index cancer stage	
I or II	36 (6.4)
III or IV	528 (93.6)
Comorbidity	
None or mild	511 (90.6)
Moderate to severe	53 (9.4)
Treatment	
Radiotherapy only	133 (23.6)
Radiochemotherapy	431 (76.4)
Death, all causes	
Yes	78 (14.0)
No	485 (86.0)
Death, owing to disease	
Yes	44 (8.0)
No	519 (92.0)
Recurrence	
Yes	64 (11.4)
No	500 (88.6)
Tumor HPV status	
Positive	482 (85.0)
Negative	82 (15.0)

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73 females (12.9%). There were 284 (50.3%) patients who reported a history of smoking, whereas 377 (66.8%) had a history of alcohol drinking. Approximately 85% of these SCCOP patients were tumor HPV16 positive. Most of the patients had late-stage disease ($n = 528$; 93.6%), whereas only 36 (6.4%) presented with early-stage disease. All patients received radiotherapy, with 133 (23.6%) receiving radiation alone and 431 (76.4%) receiving radiation plus chemotherapy. At a median follow-up time of 34.8 months, 78 patients have died from any cause (44 died from SCCOP), and 64 patients have experienced disease recurrence.

Association between TGFβ1 genotypes and SCCOP survival

The genotype distributions for the three polymorphisms and associated survival among the patients are shown in Table 2. The CT/CC variant genotypes of *TGFβ1* rs1982073 were significantly associated with better OS, DSS, and DFS than the corresponding common homozygous TT genotype (log-rank $P < 0.001$ for OS, DSS, and DFS, Fig. 1). With the other two polymorphisms of *TGFβ1*, rs1800469 and rs1800471, survival did not vary by genotype (log-rank $P > 0.05$, Table 2). Cox regression analysis was performed to adjust for other important confounders, including age, sex, ethnicity, smoking, alcohol, disease stage, comorbidity, and HPV status and treatment (Table 2). After adjustment for these confounders, the patients with CT/CC variant genotypes of *TGFβ1* rs1982073 had risks of overall death [HR, 0.2; 95% confidence interval (CI), 0.1–0.5], death due to disease (HR, 0.2; 95% CI, 0.1–0.4), and disease recurrence (HR, 0.2; 95% CI, 0.1–0.4) after chemoradiation that were approximately 5 times lower than in patients with the T variant. There were no significant associations between survival and the *TGFβ1* rs1800469 (HR, 1.2, 95% CI, 0.8–2.0 for OS; HR, 1.6, 95% CI, 0.8–3.0 for DSS; and HR, 1.5, 95% CI, 0.9–2.6 for DFS) and *TGFβ1* rs1800471 (HR, 1.0, 95% CI, 0.6–1.6 for OS; HR, 1.1, 95% CI, 0.6–2.0 for DSS; and HR, 1.4, 95% CI, 0.8–2.4 for DFS) polymorphisms.

Association between TGFβ1 genotypes and SCCOP survival, stratified by tumor HPV16 status

TGFβ1 rs1982073 was significantly associated with the tumor HPV status of SCCOP patients (OR, 2.1; 95%CI, 1.2–3.9), which is consistent with the finding we previously reported (OR, 1.97; 95%CI, 1.03–3.76; ref. 31). The other two polymorphisms were not associated with HPV status. Because of the majority of HPV16-positive SCCOP cases and few outcome events in the HPV16-negative SCCOP patients in our patient cohort, our analysis of the effect of *TGFβ1* genetic variants on survival was focused on HPV16-positive cases only. As shown in Fig. 2, the univariate survival analysis demonstrated that SCCOP patients carrying the *TGFβ1* rs1982073 CT/CC variant genotypes had significantly better OS, DSS, and DFS than the patients carrying the corresponding common homozygous TT genotype (log-rank $P < 0.001$), but significant genotype differences were not observed for the *TGFβ1* rs1800469 and *TGFβ1* rs1800471 polymorphisms. Furthermore, the multivariable analysis on associations between the *TGFβ1* polymorphisms and OS, DSS, and DFS among 482 HPV16-positive SCCOP patients and 82 HPV16-negative patients was shown in Table 3. These estimates of associations were adjusted for potential confounders, including age, sex, ethnicity, smoking and alcohol status, disease stage, comorbidity, and treatment. Compared with SCCOP patients with the *TGFβ1* rs1982073 TT genotype, the patients with the *TGFβ1* rs1982073 CT/CC variant genotypes had a 5- to 10-times lower risk of overall death, death from SCCOP, and recurrence (HR, 0.1, 95% CI, 0.1–0.3 for OS; HR, 0.1, 95% CI, 0.1–0.3 for DSS; and HR, 0.2, 95% CI, 0.1–0.3 for DFS; Table 3). For the *TGFβ1* rs1800469 and *TGFβ1* rs1800471 polymorphisms, no significant associations were observed. Furthermore, in HPV-negative SCCOP patients, no significant associations between the three *TGFβ1* polymorphisms and survival were found.

Characterization of functional relevance of TGFβ1 rs1982073

To further characterize the potentially functional relevance of *TGFβ1* rs1982073 polymorphism, we determined serum expression level of TGFβ1 in another subset of 162 incident SCCOP patients, who were recently recruited and their serum samples were available. We compared the circulating expression levels of TGFβ1 before treatment between tumor HPV16–positive and –negative patients, as well as between patients with different genotypes of *TGFβ1* rs1982073 among tumor HPV16–positive patients only. As shown in Table 4, we found that the expression of TGFβ1 was significantly higher in tumor HPV16–positive patients than the HPV16-negative cases ($P = 0.045$). Furthermore, the expression of TGFβ1 was significantly higher in the patients with variant genotypes of *TGFβ1* rs1982073 (CT + CC) than the patients with the corresponding wild-type homogenous TT genotype among all patients ($N = 162$; $P = 0.021$) and HPV16-positive patients only ($N = 120$; $P = 0.048$), whereas the similarly significant differences were not observed for other two *TGFβ1* polymorphisms (*TGFβ1* rs1800469 and *TGFβ1* rs1800471).

Table 2. Association of *TGFβ1* variants with OS, DSS, and DFS of SCCOP patients ($N = 564$)

	OS			DSS			DFS		
Genotypes	Overall death/total	Log-rank *P* value	aHR[a] (95% CI)	Death, owing to disease/total	Log-rank *P* value	aHR[a] (95% CI)	Rec.[b]/total	Log-rank *P* value	aHR[a] (95% CI)
TGFβ1 rs1800469		0.559			0.370			0.145	
CC[c]	47/371		1.0	25/371		1.0	36/371		1.0
CT + TT	31/193		1.2 (0.8–2.0)	19/193		1.6 (0.8–3.0)	28/193		1.5 (0.9–2.6)
TGFβ1 rs1982073		<0.001			<0.001			<0.001	
TT[c]	41/170		1.0	27/170		1.0	37/170		1.0
CT + CC	37/394		0.2 (0.1–0.5)	17/394		0.2 (0.1–0.4)	27/394		0.2 (0.1–0.4)
TGFβ1 rs1800471		0.689			0.974			0.192	
GG[c]	56/433		1.0	30/433		1.0	42/433		1.0
CG + CC	22/131		1.0 (0.6–1.6)	14/131		1.1 (0.6–2.0)	22/131		1.4 (0.8–2.4)

[a]HR adjusted for age, sex, ethnicity, smoking status, alcohol use status, stage, comorbidity, HPV status, and treatment.
[b]Rec.: Recurrence.
[c]Reference group.

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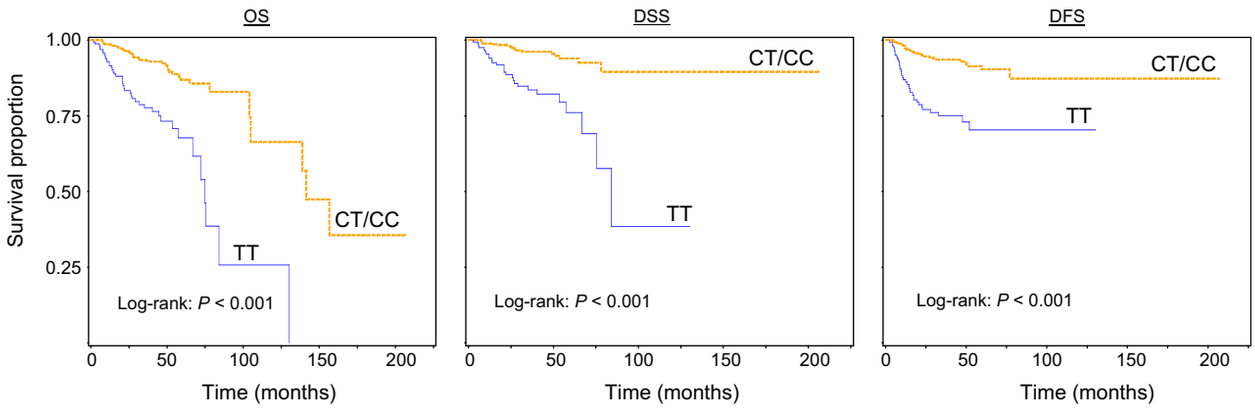

Figure 1.
Survival by *TGFβ1* rs1982073 genotypes in all SCCOP patients ($N = 564$).

Discussion

In this study, 564 patients with incident SCCOP treated with radiotherapy or chemoradiotherapy were evaluated for differences in survival associated with three selected polymorphisms in the *TGFβ1* gene. *TGFβ1* rs1982073 was significantly associated with OS, DSS, and DFS, particularly in HPV16-positive SCCOP patients, whereas there were no such significant associations for the *TGFβ1* rs1800469 and *TGFβ1* rs1800471 polymorphisms. Moreover, no significant associations were observed for all three *TGFβ1* polymorphisms in HPV-negative SCCOP patients.

To date, only two small studies from the same research team have reported that *TGFβ1* rs1982073 genetic variant modifies clinical outcomes of SCCHN (30, 34). However, all these studies were performed with either mixed tumor sites or small sample

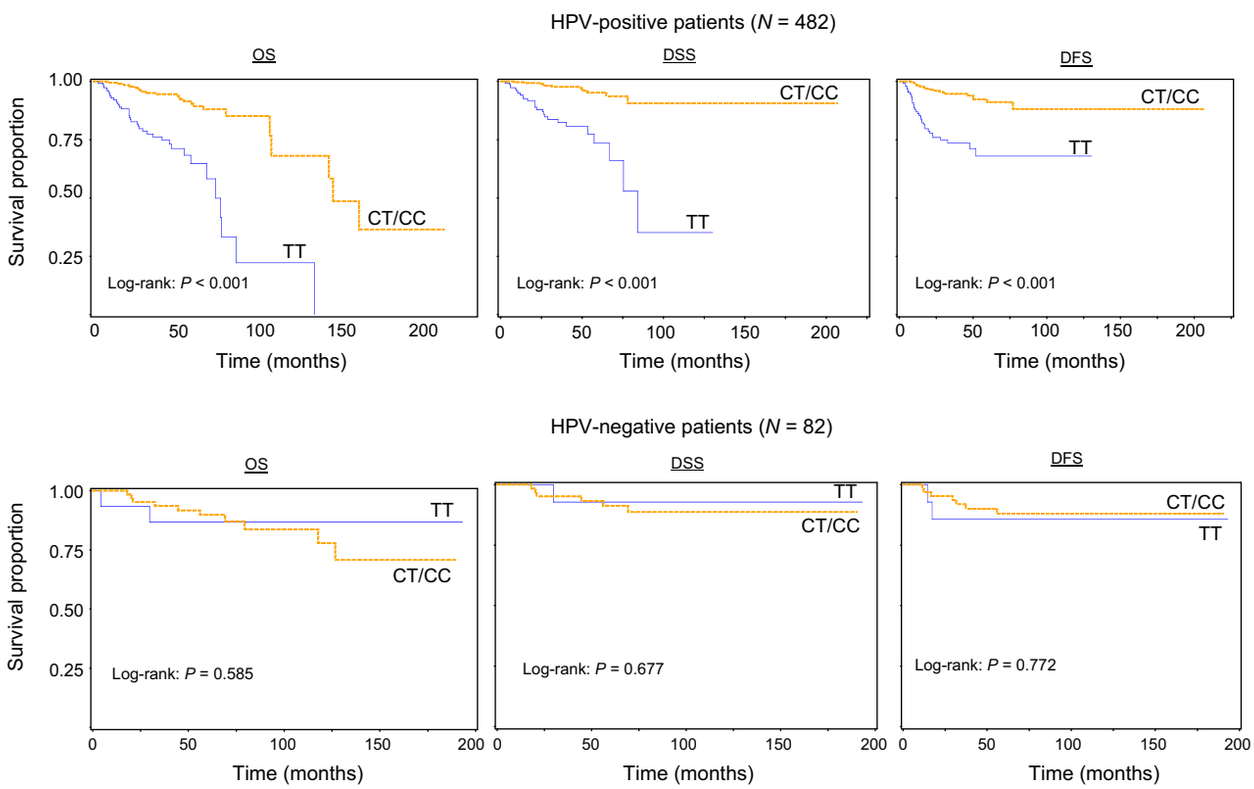

Figure 2.
Survival by *TGFβ1* rs1982073 genotypes in SCCOP patients stratified by tumor HPV16 status: HPV16-positive SCCOP patients ($N = 482$) and HPV16-negative SCCOP patients ($N = 82$).

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Table 3. Association of *TGFβ1* variants with OS, DSS, and DFS of SCCOP patients stratified by tumor HPV16 status

	OS			DSS			DFS		
Genotypes	Overall death/total	Log-rank *P* value	aHR[a] (95% CI)	Death, owing to disease/total	Log-rank *P* value	aHR[a] (95% CI)	Rec.[b]/total	Log-rank *P* value	aHR[a] (95% CI)
Tumor HPV–positive SCCOP patients ($N = 482$)									
TGFβ1 rs1800469		0.876			0.450			0.192	
CC[c]	41/318		1.0	21/318		1.0	31/318		1.0
CT + TT	25/164		1.1 (0.6–1.8)	16/164		1.4 (0.7–2.8)	24/164		1.5 (0.8–2.5)
TGFβ1 rs1982073		<0.001			<0.001			<0.001	
TT[c]	39/155		1.0	26/155		1.0	35/155		1.0
CT + CC	27/327		0.1 (0.1–0.3)	11/327		0.1 (0.1–0.3)	20/327		0.2 (0.1–0.3)
TGFβ1 rs1800471		0.496			0.600			0.234	
GG[c]	48/376		1.0	26/376		1.0	36/376		1.0
CG + CC	18/106		0.8 (0.5–1.5)	11/106		0.9 (0.4–1.8)	19/106		1.4 (0.8–2.6)
Tumor HPV–negative SCCOP patients ($N = 82$)									
TGFβ1 rs1800469		0.277			0.657			0.551	
CC[c]	6/53		1.0	4/53		1.0	5/53		1.0
CT + TT	6/29		2.8 (0.7–10.4)	3/29		3.8 (0.5–27.2)	4/29		4.2 (0.4–47.7)
TGFβ1 rs1982073		0.585			0.677			0.772	
TT[c]	2/15		1.0	1/15		1.0	2/15		1.0
CT + CC	10/67		2.1 (0.4–11.4)	6/67		4.2 (0.3–56.4)	7/67		0.8 (0.1–4.7)
TGFβ1 rs1800471		0.540			0.433			0.841	
GG[c]	8/57		1.0	4/57		1.0	6/57		1.0
CG + CC	4/25		1.6 (0.4–6.0)	3/25		2.3 (0.4–13.5)	3/25		1.0 (0.2–4.5)

[a]HR adjusted for age, sex, ethnicity, alcohol use status, stage, comorbidity, and treatment.
[b]Rec.: Recurrence.
[c]Reference group.

sizes. More importantly, these studies did not include HPV information for further risk stratification assessment as HPV is known to have distinct prognostic effect on SCCOP outcome. We did have also previously reported that variant genotypes of *TGFβ1* rs1982073 were significantly associated with tumor HPV16–positive SCCOP patients (31), which is consistent with our finding from this study that the patients with *TGFβ1* rs1982073 CT + CC variant genotypes had significantly better OS, DSS, and DFS than those with the TT genotype. Unlike what has been done in most previous studies (30, 34), in the current study, the identified *TGFβ1-related* biomarkers were fully adjusted for other potentially important prognostic parameters (e.g., age, sex, ethnicity, smoking, pack years of smoking, alcohol consumption, tumor HPV status, stage, treatment, and comorbidity) to minimize potential confounding effects. Moreover, unlike previous studies, this study was restricted to cancers originating from the oropharynx to avoid an admixture of different diseases (e.g., nonoropharyngeal head and neck cancers).

Our results demonstrate that patients with *TGFβ1* rs1982073 CT+CC genotypes had significantly lower risks of death and disease recurrence than did patients with the corresponding common homozygous TT genotype. These results may help physicians make decisions about individualized treatment. If the association between the *TGFβ1* rs1982073 genotypes and survival outcome is confirmed, clinicians could use these genotyping profiles as a biomarker to identify an important subgroup of patients who are at high risk of death or disease recurrence. It is likely that future targeted therapies will be designed (in part) to counteract the effects of adverse genotype as well as be administered in an individualized way to SCCOP patients, particularly HPV-positive SCCOP patients. For example, if the adverse genotype is identified prior to treatment start, a more aggressive treatment strategy may be developed. If the genotype is identified immediately after treatment, adjuvant therapy could be added to the treatment plan. And if the patient is found to have a high risk of recurrence at follow-up, an intensified workup for treatable recurrent disease may be needed. However, each of these potential therapeutic options would have to be tested in clinical trials before it is used in clinics. Nevertheless, the earlier the increased risk of death or recurrent disease is detected, the greater the chances of successful treatment. Eventually, the findings of this study may improve prognostication and facilitate individualized treatment, thus hopefully improving clinical outcomes.

The role of *TGFβ1* in human cancer is multifactorial, and functions may both suppress and promote tumorigenesis. *TGFβ1* is highly polymorphic, and some have been identified that modulate the risk and prognosis for cancers such as those of the breast, lung, bladder, and head and neck (15, 22, 28–30). We chose to study the *TGFβ1* polymorphisms rs1800469, rs1982073, and rs1800471 because they appear to affect *TGFβ1* levels. *TGFβ1* rs1800469 is located in the promoter region of the *TGFβ1* and is associated with differences in circulating TGFβ1 plasma levels. It has been shown that when cytosine is encoded, activator protein 1 (AP1) binds to it, resulting in the downregulation of TGFβ1 (35). The polymorphism *TGFβ1* rs1982073 results in a nonsynonymous change from leucine to proline within the signal peptide sequence of *TGFβ1*. This region of the gene is responsible for directing TGFβ1 into the extracellular matrix and the wild-type T allele has been associated with lower levels of secretion and serum levels of TGFβ1 compared with the variant C allele (18). *TGFβ1* rs1800471 is located in exon 1 and results in an arginine to proline change. *TGFβ1* polymorphisms have been investigated as predictors of cancer, and several studies have found that the 869C allele increases the risk for breast cancer (21, 22, 36). In a large Breast Cancer Association Consortium study, the proline encoding allele (C) of T869C was found to have a dose-dependent association with increased risk for invasive breast cancer among a group of primarily European women (35). Another large European study found that both the TT genotype versus CT/CC of C509T and the CC genotype versus TC/TT of T869C increased the

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Table 4. Correlation of serum *TGFβ1* expression levels with tumor HPV16 status and *TGFβ1* genotypes in 162 SCCOP patients

Variables	Number of patients	Serum *TGFβ1* level (Mean ± SD, pg/mL)	*P* (by unpaired *t* test)
All patients ($N = 162$)			
Tumor HPV16 status			0.045
Negative	42	10.2 ± 8.1	
Positive	120	14.2 ± 11.9	
TGFβ1 rs1800469			0.161
CC[a]	107	12.5 ± 10.2	
CT + TT	55	10.2 ± 9.1	
TGFβ1 rs1982073			0.021
TT[a]	48	9.9 ± 8.2	
CT + CC	114	13.9 ± 10.6	
TGFβ1 rs1800471			0.276
GG[a]	124	13.1 ± 11.3	
CG + CC	38	10.9 ± 9.2	
HPV16-positive patients ($N = 120$)			
TGFβ1 rs1800469			0.657
CC[a]	79	14.7 ± 10.3	
CT + TT	41	13.8 ± 10.9	
TGFβ1 rs1982073			0.048
TT[a]	39	12.0 ± 8.4	
CT + CC	81	16.3 ± 12.1	
TGFβ1 rs1800471			0.544
GG[a]	93	15.2 ± 11.1	
CG + CC	27	13.7 ± 11.9	

[a]Reference.

risk for invasive breast cancer (21). Similar findings have been observed for colon cancer but not ovarian cancer (37, 38). Few studies have investigated *TGFβ1* rs1800471 polymorphism, but Castillejo and colleagues found no association between this polymorphisms and bladder cancer risk (28). Likewise, no association was seen for risk of radiation pneumonitis among non–small cell lung cancer patients, whereas the association was found for *TGFβ1* rs1982073 polymorphism (33).

Several studies have evaluated the association of *TGFβ1* rs1982073 variant with survival of patients with SCCHN and lung cancer (30, 34, 39) with radiochemotherapy. They found that *TGFβ1* rs1982073 CT + CC variant genotypes were independent favorable prognostic factors for survival in SCCHN (30, 34), and our current study showed a similarly significant association between these variant genotypes and better survival of HPV16-positive SCCOP patients, whereas Yuan and colleagues found an opposite effect of these variant genotypes of *TGFβ1* rs1982073 on distant metastasis-free survival of lung cancer (39). Although we do not know how this *TGFβ1* rs1982073 variant modifies survival differently in these different types of cancer, it is biologically plausible that these variants may be either functional or in linkage disequilibrium with other functional variants of *TGFβ1*, thereby altering the function of *TGFβ1*, or with alleles at other nearby susceptibility loci. Moreover, because *TGFβ1* has been shown to enhance the lethal effects of DNA-damaging agents (e.g., radiation, cisplatin), the central components of radiochemotherapy in SCCHN (40), the patients carrying the C allele had a better survival after radiochemotherapy, supporting that C allele had increased serum level of *TGFβ1* to sensitize cancer cells to radiochemotherapy as compared with the T allele (20). The more susceptible to radiochemotherapy might also be enhanced by induction or differentiation of cancer stem cells due to the increased *TGFβ1* levels (30).

The increased *TGFβ1* expression levels by CT + CC variant genotypes of *TGFβ1* rs1982073 may also affect the regulation of the immune and inflammatory responses. For example, *TGFβ1* rs1982073 CT + CC genotypes might alter regulation in these pathways which might not enable many HPV-infected cells to escape or counterattack against the immune system, leading to more likely HPV-positive tumors. Because HPV16-positive SCCOP patients typically have better survival, our current finding that patients with *TGFβ1* rs1982073 CT+CC variant genotypes had favorable prognosis in HPV16-positive SCCOP patients is consistent with our previous finding of a significant association of these CT + CC variant genotypes with HPV16-positive SCCOP tumors (31). Generally, HPV-positive SCCOP patients lack somatic genetic changes (e.g., intact p53), whereas patients with HPV-negative SCCOP, which is mostly driven by smoking, have the most common p53 mutations. Such p53 mutations seem to be correlated with a poor response to radiotherapy, partially due to inactivation of the p53-mediated apoptotic pathway. When HPV-positive SCCOP patients undergo radiotherapy or chemoradiotherapy, tumor cells harboring intact p53 may induce apoptosis. We expect that HPV-positive SCCOP patients with *TGFβ1* rs1982073 CT+CC variant genotypes will have higher apoptotic efficacy than will patients with the corresponding common TT genotype; and subsequently experience a better response to chemoradiotherapy, leading to a better prognosis. However, these hypotheses need to be verified in future well-designed prospective studies.

Although the CT + CC variant genotypes of *TGFβ1* rs1982073 had a better survival after chemoradiotherapy as compared with the TT genotype, the results were different than those observed in lung cancer (39). The mechanism for the association could be mediated by the infiltration promoting and metastasis-enhancing effects of *TGFβ1* (41), but the apparent inconsistency between our current findings in SCCOP and those in lung cancer could be due to distinct modes of cancer progression in different types of cancer. For example, SCCHN typically advances locally as compared with lung cancer, which often progresses by distant metastasis. The local advancement of SCCHN may lead to less morbidity than distant metastasis of lung cancer. These findings indicate that *TGFβ1* has complex roles and conflicting effects on tumor growth, progression, and metastasis in different cancers.

Several studies on functional relevance of this *TGFβ1* rs1982073 polymorphism have been reported (20–22), supporting that variant genotypes of *TGFβ1* rs1982073 were associated with increased expression of *TGFβ1*. In this study, we did find significant higher expression levels of *TGFβ1* in serum among HPV16-positive patients than among HPV16-negative patients. Furthermore, the patients with the variant genotypes of *TGFβ1* rs1982073 are significantly correlated with increased expression of *TGFβ1* in serum as compared with those with the corresponding common TT genotype; and this similar correlation was also seen among HPV16-positive patients only. Although the functional relevance of this polymorphism has not yet been elucidated, our results might partially suggest a functional correlation between this polymorphism and expression of *TGFβ1*, providing preliminary evidence of biological plausibility for the observed association in this study.

Some inherent limitations exist in this study. As the majority (>90%) of the SCCOP cases in our study were non-Hispanic whites, our current results for generalizability to other ethnic populations may be limited. Because of relatively limited

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number of outcome events (death or recurrence) and duration of follow-up in this study, the significant associations could be found by chance. Moreover, some significant findings in the stratified analysis may have limited the interpretation due to the relatively small subgroup numbers in each subgroup. Finally, it is possible that misclassification of HPV status occurred in the HPV-negative group due to presence of other high-risk HPV types, leading to a bias of our results away from the null. However, this is unlikely to have occurred as we did not find a significant association in the HPV-negative group.

In conclusion, our findings suggest that *TGFβ1* polymorphisms might be useful prognostic biomarkers in SCCOP patients, particularly HPV16-positive SCCOP patients. Because the *TGFβ1* rs1982073 variant was significantly associated with survival in HPV16-positive SCCOP, *TGFβ1* rs1982073 together with other significant markers may help stratify HPV16-positive SCCOP patients for appropriate treatment strategies. However, in order to validate our findings, larger, well-designed prospective studies may be required to more accurately evaluate the clinical validity and utility of this biomarker before implementation. In addition, future studies for exploring the molecular mechanisms underlying the observed associations are also needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: Y. Tao, E.M. Sturgis, Z. Huang, Q. Wei, G. Li
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E.M. Sturgis, Q. Wei, G. Li
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Tao, E.M. Sturgis, Z. Huang, Y. Wang, P. Wei, J.R. Wang, G. Li
Writing, review, and/or revision of the manuscript: Y. Tao, E.M. Sturgis, Z. Huang, P. Wei, J.R. Wang, Q. Wei, G. Li
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Tao, Z. Huang, J.R. Wang, Q. Wei, G. Li
Study supervision: Y. Tao, Z. Huang, Q. Wei, G. Li

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