Translational Oncology
www.transonc.com

Volume 11 Number 3 June 2018 pp. 633–638 633

The Modifying Effect of a Functional Variant at the miRNA Binding Site in *E2F1* Gene on Recurrence of Oropharyngeal Cancer Patients with Definitive Radiotherapy

Hua Zhang[*,†,1], Erich Sturgis[*,‡], Lijun Zhu[*,§,1], Zhongming Lu[*,¶], Ye Tao[*,#], Hongliang Zheng[*,] and Guojun Li[*,‡]**

[*]Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, USA; [†]Department of Otolaryngology-Head and Neck Surgery, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai,China; [‡]Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, USA; [§]Department of Oral and Maxillofacial Surgery, Guangdong General Hospital and Guangdong Academy of Medical Sciences, Guangzhou, China; [¶]Department of Otolaryngology-Head and Neck Surgery, Guangdong General Hospital and Guangdong Academy of Medical Sciences, Guangzhou, China; [#]Department of Otolaryngology-Head and Neck Surgery, the 2nd affiliated hospital of Anhui Medical University, Hefei, China; [**]Department of Otorhinolaryngology-Head and Neck Surgery, Changhai Hospital, Second Military Medical University, Shanghai, 200433, China

Abstract

Human papillomavirus (HPV) activates *E2F1*-driven transcription via the E7-RB-*E2F1* pathway. A polymorphism in the 3′ UTR of *E2F1* gene may disrupt a binding site for miRNA and may affect its transcription level, thus modifying the susceptibility to radiotherapy and outcomes through this pathway. We evaluated the association of a polymorphism at the 3′UTR miRNA binding site of *E2F1* gene (rs3213180) with risk of recurrence of SCCOP in a cohort of 1008 patients. Log-rank test and univariate and multivariable Cox models were used to evaluate the associations. Compared with patients with *E2F1*-rs3213180 GG homozygous genotype, the patients with *E2F1*-rs3213180GC + CC variant genotypes had significantly better disease-free survival (log-rank $P < .001$) and decreased risk of SCCOP recurrence (HR, 0.4, 95% CI, 0.3–0.5) after multivariable adjustment. Furthermore, among patients with HPV16-positive tumors, the patients with *E2F1*-rs3213180 GC + CC variant genotypes had significantly better disease-free survival rates (log-rank $P < .001$) and lower recurrence risk than those with *E2F1*-rs3213180 GG homozygous genotype (HR, 0.2, 95% CI, 0.1–0.4). Our findings suggest that *E2F1*-rs3213180 polymorphism may modulate the risk of recurrence in SCCOP patients, particularly for patients with HPV16-positive tumors of SCCOP. However, future larger population and functional studies are warranted to validate these results.

Translational Oncology (2018) 11, 633–638

Introduction

Oral squamous cell carcinoma (OSCC), a subsite of squamous cell carcinoma of the head and neck (SCCHN), includes squamous cell carcinoma of the oropharynx (SCCOP) and oral cavity squamous cell carcinoma (OCSCC). Over the past three decades, the incidence of SCCHN continues decreasing, partially due to the success of public health efforts in tobacco control [1,2], while the incidence of SCCOP

Address all correspondence to: Guojun Li, Department of Head and Neck Surgery, Unit 1445, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA or Hongliang Zheng, MD, PhD, Department of Otorhinolaryngology-Head and Neck Surgery, Changhai Hospital, Second Military Medical University, Shanghai, China, 200433. E-mail: zheng_hl2004@163.com
[1]These authors contributed equally to this work and are considered co-first authors.
Received 9 December 2017; Accepted 16 February 2018; Accepted 16 February 2018

1936-5233/18
https://doi.org/10.1016/j.tranon.2018.02.022

is increasing [3] due to the increasing infection of human papillomavirus (HPV). The patients with SCCOP are characterized with local tumor aggressiveness, the high frequency of second primary malignancies, the high recurrence rate moderately, and a high frequency of medical comorbidities [4]. Surgery, radiotherapy, and chemotherapy have been used individually or in combination to treat SCCOP successfully, however disease recurrence remains a major problem clinically for mortality. The recurrence rates of SCCOP are not consistent among patients who have similar clinical characteristics and similar therapeutic approaches. Clinicians would ensure appropriately individualized treatment if they could accurately predict the recurrence risk of individual patients with SCCOP for improved survival and better life quality.

As HPV has been considered as an etiologic risk factor for SCCOP, approximately 50–80% of SCCOP patients are likely to be HPV-positive [HPV (+)] [5,6]. Among the types of HPV, HPV16 is the most common high-risk type of HPV in SCCOP, accounting for approximately 90–95% of HPV (+) SCCOP [6,7]. Smoking and HPV are well known factors for prognosis of SCCOP, while the prognosis is different for SCCOP patients with similar clinical characteristics and similar treatment, suggesting other factors, such as polymorphism, may play a role in prognosis.

The *E2F1* gene is one of key members of the *E2F* family, locating on chromosome 20q.*E2F1*regulates expression of genes in many molecular pathways including cell cycle progression, apoptosis and DNA synthesis [8]. Previous studies showed that *E2F1* had been demonstrated as a key downstream target in the retinoblastoma (RB) pathway [9,10]. Binding of E7 to phosphorylated RB leads to activation of *E2F1*, which induces cell-cycle progression and p16 expression [11].Therefore, *E2F1 and* HPV may interact to activate gene transcription via the E7-RB-*E2F* pathway. Furthermore, down expression of *E2F1* enhanced tumor growth in HPV(+) oral cancer, while overexpression of *E2F1* decreased the clonogenicity of HPV(+) cancer [12].

MiRNA plays important roles in many ranges of biological processes. MiRNAs can bind to targeted mRNA in the 3' UTR to alter mRNA levels and expression of proteins. Thus genetic variants in the 3' UTR targeted by miRNAs can either create illegal binding sites or remove existing binding sites, affecting the regulation of target genes [13–15]. Such functional variants at the miRNA binding site in *E2F1* gene may alter its expression, thus subsequently influencing the response to radiotherapy and susceptibility to recurrence.*E2F1*-rs3213180, one of such single nucleotide polymorphisms (SNP), was recently reported to be located in the 3'UTR miRNA binding site of the *E2F1* gene. *E2F1*-rs3213180 was significantly associated with *E2F1* expression and might alter *E2F1* gene function [16], and our previous study has found that *E2F1*-rs3213180 polymorphism was associated with risk of HPV-associated OSCC, particularly for SCCOP [17]. While no large studies have examined the associations between the *E2F1*-rs3213180 polymorphism and risk of recurrence of patients with SCCOP, we hypothesized that *E2F1*-rs3213180 polymorphism at miRNA binding site in 3' UTR may cause interindividual variations in responses to radiotherapy, leading to different risk of SCCOP recurrence. Therefore, in the present study we evaluated the association in a cohort of 1008 incident patients with SCCOP.

Materials and Methods

Study Population

This study included 1008 patients with newly diagnosed, histopathologically confirmed, and untreated SCCOP. These cases had been recruited consecutively at the University of Texas MD Anderson Cancer Center as part of an ongoing molecular epidemiologic study of SCCHN. All patients provided written informed consent to be enrolled in the study, which was approved by The University of Texas MD Anderson Cancer Center's Institutional Review Board. All informed consent was obtained from all subjects. The committee approved all experiments performed in this study. In addition, all methods were performed in accordance with the relevant guidelines and regulations. Clinical data, including site and histologic subtype of the tumor, stage at presentation of the tumor and any comorbidity, recurrence and therapy, were obtained from review of the medical records. Patients who had smoked over 100 cigarettes during their lifetimes were categorized as 'ever-smokers' and others as 'never-smokers.' Those who had drunk alcoholic beverages at least once a week for more than 1 year were classified as 'ever-drinkers' and otherwise as 'never-drinkers.' During enrollment in the study, some participants were excluded for final analysis if they 1) had known distant metastases; 2) had any prior cancer except non-melanoma skin cancer; 3) had a salivary gland tumor, cervical metastases of unknown origin, a primary sinonasal tumor, or a tumor outside the upper aerodigestive tract; 4) had treatment outside of our institution; or 5) experienced only palliative treatment.

Patients were followed up throughout their treatment and posttreatment course, including scheduled regular clinical and radiographic examinations. Patients were considered disease free if there was no documented disease at the date last visiting with the head and neck surgeon, head and neck radiation oncologist, or head and neck medical oncologist. Recurrent disease was defined as appearance of a new lesion of the same histology verified by biopsy (excisional, incisional, or needle biopsy), reappearance of any lesion that had disappeared, or development of tumor-related symptoms. The sixth edition of the American Joint Committee on Cancer TNM staging system was used to determine disease stage at the time of presentation for all study patients. Patients with comorbidities were categorized into two groups-none or mild and moderate to severe using a modification of the Kaplan-Feinstein comorbidity index (Adult Comorbidity Evaluation 27), as described in our previously report [18].

E2F1-rs3213180 Genotyping

We extracted genomic DNA from peripheral blood leukocyte cell pellet using the QIAamp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions. The quantification of DNA was determined by a Nanodrop analyzer (ND-1000 spectrophotometer [Nano Drop Technologies, Inc., Wilmington, DE]). The SNP was genotyped using the TaqMan assay. The PCR amplification was run and the plate was read with the built-in Sequence Detection Software on an ABI-Prism 7900 instrument (Applied Biosystems, Foster City, CA). The analysis was repeated on a randomly selected subset of 10% of the samples, and the repeated test results demonstrated 100% concordance with the original analysis.

HPV16 Testing

Paraffin-embedded tumor tissue biopsy samples or specimens from SCCOP patients were used to extract DNA for HPV16 detection,

using the PCR-based, type-specific assays for the E6 and E7 regions, as we previously reported [19]. 10% of the samples were retested using restriction digestion of the PCR products with BanII and MspI to verify the presence of E6- and E7-specific fragments. The results of both methods were 100% concordant.

Statistical Analysis

Student t-test was used to detect the differences between mean age at diagnosis and follow-up time between patients with and without recurrence. The associations between demographic variables (age at diagnosis, sex, and ethnicity); epidemiologic factors (smoking status and alcohol drinking status); clinical factors (comorbidity group, tumor stage, and treatment); and time to recurrence were assessed using both univariate and multivariable Cox proportional hazards regression models. The estimates of association of each variable with disease-free survival (DFS)/recurrence status were assessed by a log-rank test. 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. We quantified the associations of *E2F1* polymorphisms with risk of SCCOP recurrence, using hazard ratios (HRs) and their 95% confidence intervals (CIs) among both overall patients and patients with HPV16 (+) tumors. While because of the relatively small sample size and the few recurrence events of SCCOP patients in HPV16 (–) subgroup, we did not include similar analyses for patients with HPV16 (–) tumors in this study. Statistical analyses were performed with SAS software (version 9.2; SAS Institute Inc., Cary, NC). All tests were two-sided, and $P < .05$ was considered significant.

Results

Demographics and Risk Factors for the Study Population

We had a total of 1226 SCCOP patients from May 1995 through October 2013, and 218 patients were excluded from the final analysis due to insufficient information available about follow-up and treatment or no blood samples available for genotyping. Therefore, our final analysis included 1008 incident patients with SCCOP. The overall recurrence rates of SCCOP, the 5-yr recurrence rate, and the associations between DFS and patient's characteristics including age at diagnosis, sex, ethnicity, smoking, alcohol use, comorbidity group, index cancer stage, and treatment, were previously reported [18]. 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 [18].As shown in Table 1, the Kaplan–Meier univariate analysis showed that the 5-yr rate of recurrence was significantly different between two groups categorized by age at diagnosis, ethnicity, smoking status, alcohol drinking, comorbidity group, and treatment (all $P < .05$), while no significant difference was found between the groups for sex and index cancer stage (both $P > .05$).

Joint Effect of E2F1-rs3213180 Polymorphism and HPV-16 Serology on the DFS of SCCOP

SCCOP patients with the common homozygous E2F1-rs3213180GC/CC genotypes had significantly better DFS than those with GG genotype (log-rank, $P < .001$; Figure 1). Furthermore, among 324 patients with HPV16 (+) SCCOP only, we found that the DFS was significantly better in patients carrying the E2F1-rs3213180 GC/CC genotypes than in those carrying the corresponding common homozygous GG genotype (log-rank, $P < .001$; Figure 1).

Table 1. Characteristics of Patients With SCCOP (N = 1008)

Variable	No. (%) of Patients	No. of Patients With Recurrence	5-year Recurrence Rate(%)	P^a
No. of patients	1008 (100)	181	0.20	
Age				
≤57 years	621 (61.6)	85	0.15	<.0001
>57 years	387 (38.4)	96	0.27	
Sex				
Male	872 (86.5)	161	0.20	.3110
Female	136 (13.5)	20	0.19	
Ethnicity				
Non-Hispanic white	913 (90.6)	146	0.17	<.0001
Other	95 (9.4)	35	0.41	
Smoking				
Never	388 (38.5)	51	0.14	.0004
Ever	620 (61.5)	130	0.23	
Alcohol drinking				
Ever	761 (75.5)	155	0.23	.0005
Never	247 (24.5)	26	0.10	
Comorbidity				
None or mild	913 (90.6)	157	0.19	.0370
Moderate to severe	95 (9.4)	24	0.27	
Index cancer stage				
1 or 2	72 (7.1)	11	0.19	.5280
3 or 4	936 (92.9)	170	0.20	
Treatment				
X/XC/XS/S	947 (93.9)	166	0.19	.0030
SXC	61 (6.1)	15	0.32	

P^a: Log-rank test for DFS between the two groups.
X, radiotherapy; C, chemotherapy; S, surgery.

Joint Effect of E2F1-rs3213180 Polymorphism and HPV-16 Serology on the Recurrence Risk of SCCOP

We further performed multivariable Cox proportional hazards regression analyses to assess the associations of *E2F1*-rs3213180 polymorphism with recurrence risk in patients with SCCOP. As shown in Table 2, compared with patients with the *E2F1*-rs3213180 GG common homozygous genotype, patients with the *E2F1*-rs3213180 GC/CC genotypes had significantly lower risk of SCCOP recurrence (HR, 0.4, 95% CI, 0.3–0.5).Given the important role of HPV and *E2F1* in the E7-RB-*E2F* pathway which is involved in regulation of apoptosis and proliferation, cell-cycle progression, and tumorigenesis, we further evaluated the associations of this polymorphism with risk of recurrence among the patients with HPV16 (+) SCCOP. As shown in Table 3, among HPV16 (+) patients, the *E2F1*-rs3213180 GC/CC variant genotype had significantly lower risk of recurrence than the *E2F1*-rs3213180 GG common homozygous genotype (HR, 0.2, 95% CI, 0.1–0.4).

Discussion

In this large cohort study of 1008 patients with incident SCCOP, we found that the *E2F1*-rs3213180 variant was significantly associated with the risk of SCCOP recurrence, especially in patients with HPV16 (+) tumors. The results suggested that *E2F1*-rs3213180 may predict the recurrence risk of SCCOP, particularly for those with HPV16 (+) tumors.

The exact mechanism by which this*E2F1*gene variant predicts the recurrence risk of SCCOP remains unclear, while it is likely that genetic variants within the functional regions of *E2F1*may result in the altered expression of this gene and thus affect susceptibility of response to treatment such as radiotherapy, thus leading to different

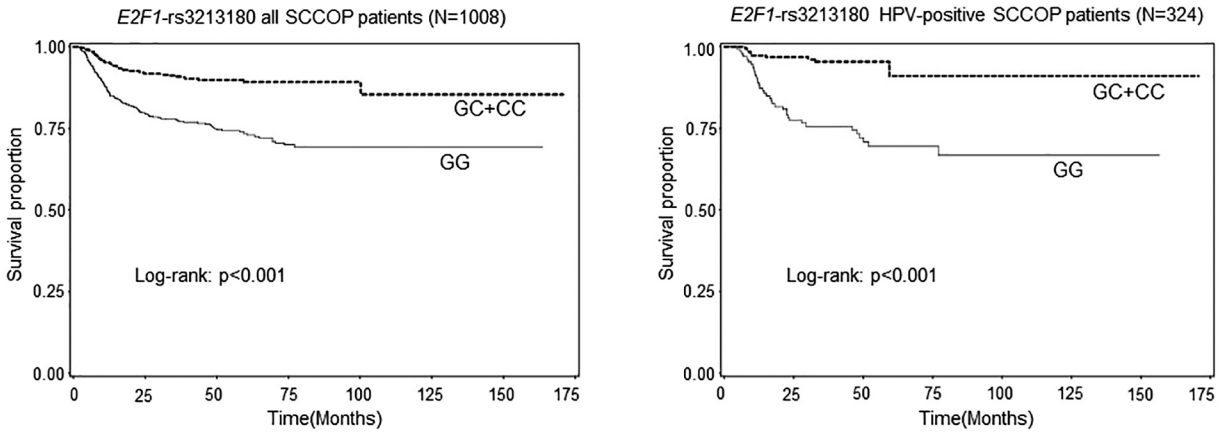

Figure 1. Kaplan–Meier estimates for the cumulative recurrence rates of patients according *E2F1*-rs3213180 genotypes (A, all SCCOP patients and B, HPV16-positive SCCOP patients).

clinical prognosis. Therefore, *E2F1* polymorphisms could be served as potential predictive biomarkers of recurrence in SCCOP patients, and it can help surgeons make individualize treatment which lead to an improved prognosis and better quality life for SCCOP patients.

The E7 gene of HPV can constitute complex formation with pRB1 in Rb gene families which have negative control function to the cell growth, and release *E2F1* transcription factors in the cells. Free *E2F1* can activate the expression of some host genes in the process of cell cycle; inactivate the corresponding proteins of p16 and Rb [20]. Thus, it is biologically plausible that genetic variants within the 3'-UTR of *E2F1* might affect expression of *E2F1*protein which, in turn, could affect p16 expression level, and thus affect susceptibility of response to radiotherapy. Although the functional relationship of the*E2F1*-rs3213180polymorphism has not been clarified yet, our association results can partially support a functional correlation between this polymorphism and *E2F1*function, which may provide preliminary evidence of biological plausibility. Thus the rs3213180 polymorphism may cause significant changes in the expression levels of *E2F1*, which result in the alternation of cell cycle progression and induction of p16 expression. Therefore, it is likely that this*E2F1*polymorphism may have potentially functional effect on p16 expression, thus leading to different recurrence risk. However, these findings should be investigated in future studies.

In recent years, growing evidence suggests that miRNA*s* directly binds to the sites in its 3'UTR and promoter of the *E2F1*, activating its transcription, which in turn modulates the translation of *E2F1* mRNAs [21]. The altered *E2F1* expression may affect the expression of genes involved in DNA repair, cell cycle progression, and apoptosis [8]. Zhao et al. [16] observed that *E2F1*-rs3213180 had a significant association with *E2F1* expression, implying that this variant could contribute in part to *E2F1* post-transcriptional regulation. Gorgoulis et al. [22] reported that increased *E2F1* positivity demonstrated a significant increase in their growth indexes and were associated with adverse prognosis in non-small cell lung carcinomas. Similarly, Gao, et al. [23] also reported that *E2F1*overexpression were significantly associated with tumor stage and poor prognosis in non-small cell lung carcinomas. Liang et al. [24] demonstrated that *E2F1* might function as a biomarker that can differentiate patients with recurrent and non-recurrent disease following radical prostatectomy, while no studies have investigated the modifying effect of *E2F1* polymorphisms on prognosis of SCCOP patients, especially for HPV-associated SCCOP. In our previous study, we found that certain selected SNPs within the *E2F1* genes had a combined effect on the risk of SCCHN in a genotype dose–response manner [25]. We also found that polymorphism at the 3'UTR miRNA binding site of *E2F1*-rs3213180 was associated with risk of HPV-associated OSCC, particularly for SCCOP [17]. In our current study, we found a significant association between the *E2F1*-rs3213180 variant genotypes and an increased risk of SCCOP recurrence. Although these studies have demonstrated the significant associations of *E2F1* polymorphisms with cancer risk and prognosis, we can't exclude the effects of other factors, such as genetic background, cancer type, environmental factors, tumor size, tumor stage, treatment, adequacy of adjustment for other confounding factors, and different study populations [26].

Except for the factors such as clinical, genetic, and lifestyle factors, affected risk of patients with SCCOP, tumor HPV status also influenced the risk of SCCOP recurrence. Patients with HPV16 (+) SCCOP had a better prognosis than those with HPV (–) SCCOP. Our previous study had found that *E2F1* was associated with risk of HPV-associated SCCOP [17]. We further explored the role of this *E2F1* polymorphism in recurrence risk in SCCOP patients who were stratified by tumor HPV16 status. We found that among patients with HPV16(+) tumors, the patients with variant genotypes of the *E2F1* polymorphism had better DFS and lower recurrence risk than those with homozygous genotype of this polymorphism, suggesting that *E2F1*-rs3213180 variant genotypes interact with HPV status partially via the E7-RB-*E2F1* pathway to affect SCCOP recurrence risk. Although the molecular mechanisms underlying this finding in patients with HPV16(+) SCCOP remain incompletely understood, the variant genotypes of the polymorphism may affect

Table 2. Association Between *E2F1*-rs3213180 Genotypes and Recurrence in Patients With SCCOP (N = 1008)

Genotype	No. of Recurrences/ No. of Patients	5-year Recurrence Rate	Log-Rank *P* value	aHR *, 95% CI
E2F1-rs3213180				
GG†	138/552	0.27	<.0001	1.0
GC + CC	43/456	0.11		0.4(0.3–0.5)

HR, hazard ratio.
† Reference group.
* Adjusted for age, sex, ethnicity, smoking status, alcohol use status, stage, comorbidity, and treatment.

Table 3. Association Between *E2F1*-rs3213180 Genotypes and HPV-Positive Recurrence in Patients With SCCOP (N = 324).

Genotype	No. of Recurrences/ No. of Patients	5-year Recurrence Rate	Log-rank *P* value	aHR*, 95% CI
E2F1-rs3213180				
GG†	36/131	0.33	<.0001	1.0
GC + CC	9/193	0.10		0.2(0.1–0.4)

HR, hazard ratio.
† Reference group.
* Adjusted for age, sex, ethnicity, smoking status, alcohol use status, stage, comorbidity, and treatment.

DNA repair capacity or apoptosis through controlling the cell cycle [27], which supports the notion that SCCOP is mainly driven by HPV [28,29].Thus, *E2F1*variants may affect susceptibility to radiotherapy and lead to different recurrence outcomes. However, this hypothesis should be investigated in future studies.

Our study reveals several strengths including its relatively large sample size, inclusion of tumor HPV status, and analysis of a homogeneous tumor site in SCCOP, while there are also several limitations inherited in this study including the lack of details on radiotherapy (e.g., dosage, treatment cycles, duration, etc.), the limited number of outcome events in HPV16 (+), and our cases recruited at a single cancer center with a majority of racial and ethnic group of non-Hispanic white. Taken together, our findings support a significant role of a functional variant of *E2F1*-rs3213180 at the miRNA binding site in *E2F1* gene in susceptibility to disease recurrence of SCCOP, especially in patients with HPV16 (+) tumors. However, large and prospective studies are needed to validate our results and further explore the molecular mechanisms underlying the observed associations.

Acknowledgments

The authors thank Margaret Lung, Kathryn L. Tipton, Liliana Mugartegui, and Angeli Fairly for their help with participant recruitment and Li-E Wang for laboratory management. This work was supported by start-up funds from The University of Texas MD Anderson Cancer Center (to E.M.S.), a National Institutes of Health Head and Neck Specialized Program of Research Excellence Career Development Award (P50CA097007 to E.M.S.), an Institutional Research Grant from The University of Texas MD Anderson Cancer Center (to E.M.S.), and other National Institutes of Health grants: grants ES011740 and CA131274 (to Q.W.), a Clinician Investigator Award (K12CA88084 to E.M.S.), a Cancer Center Support Grant to The University of Texas MD Anderson Cancer Center (CA16672), and grants CA135679, CA133099, and 1R03CA186261-01A1 (to G.L.).

Conflict of Interest

The authors declare no conflicts of interest.

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, and Forman D (2011). Global cancer statistics. *CA Cancer J Clin* **61**, 69–90.
[2] Siegel R, Naishadham D, and Jemal A (2012). Cancer Statistics, 2012. *CA Cancer J Clin* **62**, 10–29.
[3] Chaturvedi AK, Engels EA, Anderson WF, and Gillison ML (2008). Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. *J Clin Oncol* **26**, 612–619.
[4] Reuschenbach M, Wagner S, and Würdemann N (2016). Human papillomavirus and squamous cell cancer of the head and neck region: Prognostic, therapeutic and prophylactic implications. *HNO* **64**, 450–459.
[5] Sturgis EM and Cinciripini PM (2007). Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? *Cancer* **110**, 1429–1435.
[6] Marur S, D'Souza G, Westra WH, and Forastiere AA (2010). HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol* **11**, 781–789.
[7] Hammarstedt L, Lindquist D, and Dahlstrand H (2006). Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. *Int J Cancer* **119**, 2620–2623.
[8] DeGregori J and Johnson DG (2006). Distinct and overlapping roles for E2F family members in transcription, proliferation and apoptosis. *Curr Mol Med* **6**, 739–748.
[9] Julian LM, Palander O, Seifried LA, Foster JE, and Dick FA (2008). Characterization of an E2F1- specific binding domain in pRB and its implications for apoptotic regulation. *Oncogene* **27**, 1572–1579.
[10] Flowers S, Xu F, and Moran E (2013). Cooperative activation of tissue-specific genes by pRB and E2F1. *Cancer Res* **73**, 2150–2158.
[11] Polager S and Ginsberg D (2008). E2F- at the crossroads of life and death. *Trends Cell Biol* **18**, 528–535.
[12] Zhong R, Bechill J, and Spiotto MT (2015). Loss of E2F1 extends survival and accelerates oral tumor growth in HPV-positive mice. *Cancer* **7**, 2372–2385.
[13] Wang C, Zhao H, and Zhao X (2014). Association between an insertion/deletion polymorphism within 3'UTR of SGSM3 and risk of hepatocellular carcinoma. *Tumour Biol* **35**, 295–301.
[14] Du Y, Han X, Pu R, Xie J, Zhang Y, and Cao G (2014). Association of miRNA-122-binding site polymorphism at the interleukin-1 alpha gene and its interaction with hepatitis B virus mutations with hepatocellular carcinoma risk. *Front Med* **8**, 217–226.
[15] Thurlings I and de Bruin A (2016). E2F transcription factors control the roller coaster ride of cell cycle gene expression. *Methods Mol Biol* **1342**, 71–88.
[16] Zhao Y, Tang L, Nie W, Wang Z, and Guan X (2013). Functional variants at the miRNA binding sites of the E2F1 gene and its mRNA expression. *Oncol Lett* **5**, 398–402.
[17] Yuan Y, Sturgis EM, and Zhu L (2017). A functional variant at the miRNA binding site in E2F1 gene is associated with risk and tumor HPV16 status of oropharynx squamous cell carcinoma. *Mol Carcinog* **56**, 1100–1106.
[18] Zhang F, Sturgis EM, and Sun Y (2015). Apoptotic variants as predictors of risk of oropharyngeal cancer recurrence after definitive radiotherapy. *Int J Cancer* **137**, 2454–2461.
[19] Guan X, Sturgis EM, and Lei D (2010). Association of TGF-b1 genetic variants with HPV16- positive oropharyngeal cancer. *Clin Cancer Res* **16**, 1416–1422.
[20] Naqsh e Zahra S, Khattak NA, and Mir A (2013). Comparative modeling and docking studies of p16ink4/cyclin D1/Rb pathway genes in lung cancer revealed functionally interactive residue of RB1 and its functional partner E2F1. *Theor Biol Med Model* **10**(1).
[21] Emmrich S and Pützer BM (2010). Checks and balances: E2F-microRNA crosstalk in cancer control. *Cell Cycle* **9**, 2555–2567.
[22] Gorgoulis VG, Zacharatos P, and Mariatos G (2002). Transcription factor E2F-1 acts as a growth-promoting factor and is associated with adverse prognosis in non-small cell lung carcinomas. *J Pathol* **198**, 142–156.
[23] Gao Z, Shi R, Yuan K, and Wang Y (2016). Expression and prognostic value of E2F activators in NSCLC and subtypes: a research based on bioinformatics analysis. *Tumour Biol* **37**, 14979–14987.
[24] Liang YX, Lu JM, and Mo RJ (2016). E2F1 promotes tumor cell invasion and migration through regulating CD147 in prostate cancer. *Int J Oncol* **48**, 1650–1658.
[25] Lu M, Liu Z, and Yu H (2012). Combined effects of E2F1 and E2F2 polymorphisms on risk and early onset of squamous cell carcinoma of the head and neck. *Mol Carcinog* **51**(Suppl 1), E132-1.
[26] Zhang C, Sturgis EM, and Zheng H (2014). Genetic variants in TNF-α promoter are predictors of recurrence in patients with squamous cell carcinoma of oropharynx after definitive radiotherapy. *Int J Cancer* **134**, 1907–1915.
[27] Longworth MS, Wilson R, and Laimins LA (2005). HPV31 E7 facilitates replication by activating E2F2 transcription through its interaction with HDACs. *EMBO J* **24**, 1821–1830.

[28] Gillespie MB, Rubinchik S, Hoel B, and Sutkowski N (2009). Human papillomavirus and oropharyngeal cancer: what you need to know in 2009. *Curr Treat Options Oncol* **10**, 296–307.

[29] Begum S, Gillison ML, Nicol TL, and Westra WH (2007). Detection of human papillomavirus-16 in fine needle aspirates to determine tumor origin in patients with metastatic squamous cell carcinoma of the head and neck. *Clin Cancer Res* **13**, 1186–1191.