Published in final edited form as:

Mol Carcinog. 2018 March ; 57(3): 361–369. doi:10.1002/mc.22760.

Mouse double minute 4 variants modify susceptibility to risk of recurrence in patients with squamous cell carcinoma of the oropharynx

Zhongming Lu[1,2], **Erich M. Sturgis**[2,3], **Lijun Zhu**[2,4], **Hua Zhang**[2,5], **Ye Tao**[2,6], **Peng Wei**[7], **Qingyi Wei**[8], and **Guojun Li**[2,3,*]

[1]Department of Otolaryngology-Head and Neck Surgery, Guangdong General Hospital and Guangdong Academy of Medical Sciences, Guangzhou, Guangdong 510080, China

[2]Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

[3]Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

[4]Department of Oral and Maxillofacial Surgery, Guangdong General Hospital and Guangdong Academy of Medical Sciences, Guangzhou, Guangdong 510080, China

[5]Department of Otolaryngology-Head and Neck Surgery, the Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong 264000, China

[6]Department of Otolaryngology & head and neck Surgery, the 2nd affiliated hospital of Anhui Medical University, Hefei, Anhui 230601, China

[7]Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

[8]Duke Cancer Institute, Duke University Medical Center, Durham, NC 27710, USA

Abstract

Given the crucial role of Mouse double minute 4 (MDM4) oncoprotein in p53 pathway, single nucleotide polymorphisms (SNPs) could serve as such biomarkers for prediction of SCCOP recurrence. Thus, we investigated associations between three tagging putatively functional variants of *MDM4*, two in the 3′ untranslated region of 3′ UTR [rs11801299 (NC_000001.10:g. 204529084G>A) and rs10900598(NC_000001.10:g.204525568G>T)] and one in intron 1 [rs1380576(NC_000001.10:g.204488278G>C)], and recurrence risk of SCCOP in 1,008 incident

*Corresponding author: Guojun Li, Department of Head and Neck Surgery, Unit 1445, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030-4009; phone: 713-792-0227; fax: 713-794-4662; gli@mdanderson.org.

Availability of data materials: Data and materials will be shared.

Authors' contributions: ZL, EMS, LZ, HZ, and GL carried out the majority of the experiment, data analysis, and wrote the manuscript. They were all helped by YT, AND QW. All authors reviewed and approved the final manuscript.

Competing interests Statement: The authors have no conflicts of interest to declare.

Consent for publication: All patients provided their written informed consent for their data and tissues for the study.

Ethics approval and consent to participate: The study protocol was approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center.

patients. A log-rank test and multivariable Cox models were used to assess associations. Patients with *MDM4*-rs10900598 GT/TT had a worse disease-free survival (DFS) compared with corresponding GG genotype, while those with rs11801299 AG/AA genotypes had a lower recurrence risk than the cases with rs11801299 GG genotype (both log-rank, P<0.001). Multivariable analysis showed that significantly different recurrence risk were found among patients with *MDM4*-rs10900598 GT/TT and rs11801299 AG/AA variant genotypes (HR, 2.0, 95% CI, 1.4–2.9 and HR, 0.4, 95% CI, 0.3–0.6, respectively) compared with their corresponding common homozygous genotypes. Furthermore, after combining the risk genotypes of the three SNPs, patients among low-risk group had a significantly lower risk of SCCOP recurrence than those in high-risk group (HR, 0.2, 95% CI, 0.1–0.3). The risk for both individual SNPs or combined risk genotypes was restricted to HPV-positive SCCOP patients. Our findings suggest that the *MDM4* polymorphisms may, individually or in combination, confer an independent risk of SCCOP recurrence, particularly in HPV-positive SCCOP patients. However, larger studies are needed to validate our findings.

Keywords

MDM4 polymorphism; recurrence; oropharyngeal neoplasm; biomarkers; human papillomavirus

Introduction

Squamous cell carcinoma of head and neck (SCCHN) is the sixth most common cancer in the world. SCCHN includes different anatomic tumor sites involving the oropharynx, hypopharynx, larynx and oral cavity. Smoking and alcohol consumption have been recognized as the most important cause of SCCHN for decades. Human papillomavirus (HPV) infection, in particular HPV type 16, is also involved and leads to the significant increasing incidence rate in a subset of SCCHN, mainly in squamous cell carcinoma of the oropharynx (SCCOP)[1,2]. HPV-associated SCCOP has been shown to have unique epidemiologic, molecular, biologic characteristics and better prognosis compared with non-HPV related SCCOP [1,3].

Recurrences of the primary tumor are the major cause of poor prognosis and mortality of SCCOP. A major challenge for the management of SCCOP is how to identify patients with high-risk recurrence for and optimally clinical treatment. Refining prognostic stratification is crucial to better individualize treatment in SCCOP for improved and less-toxic outcomes[4]. However, prognostic models based on current TNM staging system may not effectively predict the outcome of an increasing HPV-related SCCOP[5]. Outcomes for SCCOP patients with the similar TNM stage may vary significantly. Thus, identifying new prognostic biomarkers to better accurately predict the risk of recurrence of SCCOP may lead to better treatment and survival.

Many studies have revealed that single nucleotide polymorphisms (SNP), may modify genetic susceptibility to development or outcomes of SCCOP [6,7], and could serve as reliable and efficient prognostic biomarkers to allow accurately identify SCCOP patients with high-risk of recurrence [8,9]. p53, an important tumor suppressor protein, plays a

critical role in genome integrity, acting as “the guardian of genome”[10], is mutated in about half of all human cancers, especially in SCCHN[11,12]. p53 is mainly regulated by interaction with two negative modulators, mouse double minute 2(MDM2) and 4 (MDM4), which inhibit the tumor suppressor activity of p53[13-15]. As a homolog of MDM2, MDM4 is overexpressed in diverse human tumors, including SCCHN [11,15] and is one of major endogenous negative regulators of p53. Thus, MDM4 can not only directly binds to p53 and inhibits its transcriptional activity [16,17], but also bind to MDM2 and regulate its role in inhibiting the p53 activity [18,19]. Moreover, MDM4 plays an essential role in MDM2–MDM4–p53 regulatory circuit by enhancing the function of the E3 ubiquitin ligase of MDM2 and promoting degradation of p53 [20]. Amplification or overexpression of *MDM4* gene may contribute to tumor development and prognosis [21,22], individually or synergistically with *MDM2*. Recently, *MDM4* has showed the translational potential for predicting clinical outcomes and become an attractive therapeutic target for p53 reactivation cancer treatment[13].

MDM4 polymorphisms have been reported to be associated with the risk of developing gastric cancer [23], prostate cancer [24], and SCCHN [7], as well as HPV16-related SCCOP [7,25]. Among the three *MDM4* SNPs we studied, two [rs11801299 (NC_000001.10:g.204529084G>A) and rs10900598 (NC_000001.10:g.204525568G>T)] are located in the 3′-untranslated region (3′-UTR), whereas the other, rs1380576 (NC_000001.10:g.204488278G>C), is in the first intron. The 3′-UTR and intron1 of gene play vital role in gene-regulatory functions, affecting gene expression and tumor susceptibility through regulation of the mRNA stability and translational efficiency [26-29]. Hence, the levels of MDM4 expression could be significantly altered by these functional genetic changes in *MDM4*. Additionally, polymorphisms in the 3′-UTR of *MDM4* are useful predictors of the outcome in advanced lung cancer patients treated with chemotherapy[30]. However, no study to date has investigated the effects of *MDM4* polymorphisms the recurrence risk in SCCOP specifically. As the incidence of HPV-related SCCOP continues to increase, more efforts should be made to reduce the disease burden caused by SCCOP. In the present study, we evaluated the associations of 3 *MDM4* variants with the likelihood of recurrence among 1008 SCCOP patients.

Materials and methods

Study Subjects

Patients with SCCOP in the present study were recruited during May 1995 through April 2010 at The University of Texas (U.T.) M. D. Anderson Cancer Center in Houston, USA. All of the enrolled subjects matched the following criteria: 1) newly diagnosed, previously untreated, histologically proven primary SCCOP; 2) with complete clinical, epidemiological and follow-up data. After excluding patients who had history of other cancers, insufficient or outside institutional treatment, unavailable blood samples for genotyping or follow-up data, a total of 1008 incident SCCOP patients were included in this study. Prior to treatment, peripheral blood samples were collected from all enrolled patients for DNA extraction. Demographic, epidemiological, and clinical variables were obtained including age, sex, ethnicity, smoking status, alcohol drinking, TNM stage, and treatment. This study was

approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center. Written informed consent was obtained from all participants.

Subjects were confirmed to have recurrence disease after treatment if they had developed new lesions with the same pathological type as the original squamous cell carcinoma of the oropharynx proved by biopsy. Local recurrences were defined as recurrences which located in the same or adjacent place of the primary SCCOP tumors. Recurrences within cervical lymph nodes which routinely drained the primary tumor were classified as regional recurrences. Distant recurrences were defined as recurrences occurs in organs other than local or regional sites as a result of tumor metastases (e.g. lung, liver, bone, brain). In the present study, the recurrence is distinctly defined and distinguished from second primary malignancy. Disease stage of SCCOP patients was determined according to the seventh edition of the American Joint Committee on Cancer (AJCC) tumor node metastasis (TNM) classification system at the initial of presentation. Detailed information about treatment and comorbidities has been described previously [31]. “Ever drinkers” were defined as subjects who had drunk alcoholic beverages at least once a week for more than 1 year in their lifetime and the rest were categorized as “never drinkers.” Participants who had smoked over 100 cigarettes during their lifetime were classified as “ever smokers,” and otherwise as “never smokers.”[31]

Tag SNPs selection and genotyping

The public HapMap SNP database (http://www.hapmap.org/) was used to identify the *MDM4* tagging SNPs, and all SNPs either were directly genotyped or exceeded a threshold level of linkage disequilibrium (LD) value (r^2) with a genotyped SNP. The SNPs of *MDM4* gene were searched within an approximately 34-kb region on chromosome 1q32 from a European population (e.g, CEPH). The tagging SNPs for study were selected according to their pairwise LD with a 0.8 of r^2 threshold and ≥ 0.10 of minor allele frequency (MAF), as we previously reported [7]. Finally, we identified three tagging SNPs: rs11801299 (NC_000001.10:g.204529084G>A), rs10900598 (NC_000001.10:g.204525568G>T), and rs1380576 (NC_000001.10:g.204488278G>C). For these three identified SNPs, both rs11801299and rs10900598 are located in the 3′ UTR of the *MDM4* gene; and rs1380576 is located in intron 1 of the gene. Genomic DNA was extracted from 1 ml blood sample of each SCCOP patient with the Qiagen DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Genotyping of *MDM4* SNPs were carried out using the Applied Biosystems TaqMan genotyping platform as described previously [7]. Approximately 10% of the specimens were randomly double-checked for quality control, showing that genotype concordance rate was 100%.

To assess the combined effect of these three SNPs, we categorized all putative risk (aHRs > 1.0) genotypes of each polymorphism into a new variable according to the number of risk genotypes carried by an individual for each of the 3 polymorphisms (for the rs1380576 and rs11801299 genotypes, we reversed the reference group to reflect the protective effects of the variant genotypes of CG/GG for rs1380576 and AG/AA for rs11801299). Therefore, in this study, patients were further categorized into the two groups according to their number of risk genotypes on the basis of results of SCCOP recurrence risk associated with each

individual SNP: 1) a low-risk group (individuals carrying 0 risk genotypes) and 2) a high-risk group (individuals carrying 1-3risk genotypes).

Tumor HPV16 detection

Paraffin-embedded tumor tissue biopsies or specimens from enrolled patients were used to extract DNA for HPV16 detection using the specific PCR and in situ hybridization assay as described previously [32]. A subset of samples (5%) were checked for tumor HPV16 status in duplicates for quality control. The results of these samples were 100% concordant with the original ones.

Plasmid constructs, transfection, and luciferase assays

The *MDM4* rs10900598 (both T and G alleles) and rs11801299 (both A and G alleles) allelic reporter constructs were generated by amplifying a 633-bp and 675 fragment of the *MDM4* 3′-UTR region from subjects homozygous for the rs10900598 TT or rs10900598 GG genotype as well as rs11801299AA or rs11801299GG genotype. The PCR products were separated, digested with *Xba* I (Promega, Madison, WI), purified, and cloned, respectively, into an appropriately digested pGL3-control vector (Promega, Madison, WI) for generation of constructs including pGL3-rs10900598T and pGL3-rs10900598G, pGL3-11801299A, and pGL3-11801299G, respectively. The DNA sequencing was used to confirm the inserts. The two head and neck cancer cell lines [UMSCC4 (HPV16-negative) and UMSCC47 (HPV16-positive)] were seeded at 0.5×10^5 cells/per well in 24-well plates for transfection after 24h. A firefly luciferase reporter plasmid (pGL3-control, pGL3-rs10900598T and pGL3-rs10900598G, pGL3-11801299A, and pGL3-11801299G) plus 50 ng pRL-TK plasmid (Promega; Madison, WI) were co-transfected as a transfection internal control into the two cells. The relative luciferase units (RLU) were calculated after transfection using the dual-luciferase reporter assay system (Promega; Madison, WI) according to the manufacturer's instructions (BD Monolight™ 3010 Luminometer, Becton, Dickinson Company, Mississauga, ON, Canada). The independent experiments were performed in triplicate for all samples. Differences were determined by Student *t* test, and $P < 0.05$ was considered significant.

Statistical analysis

Mean age at diagnosis and follow-up time between recurrence and recurrence-free SCCOP patients were compared using Student's *t* test. Differences of categorical variables including ethnicity, sex, smoking status, alcohol drinking, stage, comorbidity, treatment method, *MDM4* allele and genotype frequencies between patients with and without recurrence were assessed with χ^2 test. The primary end-point of this study was tumor recurrence. Time to event was defined as date from diagnosis of the index of SCCOP to the first development of detectable recurrent disease. Patients who had no event at their last contact time and were lost to follow-up or died of other cause were considered to be censored. The associations between demographic/epidemiologic risk factors, clinical characteristics, and time to recurrence were analyzed using univariate and multivariate Cox proportional hazards regression models. Moreover, the relationship between variables and disease-free survival (DFS) was examined by using the log-rank test. The estimates of the associations between *MDM4* SNPs and SCCOP recurrence risk were presented as hazard ratios (HRs) and 95%

confidence intervals (CIs). The multivariate Cox models included adjustment for potential prognostic confounders. The level of significance was set at P <0.05, all statistical tests were 2-sided, and performed using the SAS statistical software version 9.3 (SAS Institute, Inc.).

Results

Demographic, epidemiological and clinical characteristics and 5-year recurrence rates of all patients with SCCOP were summarized in Table 1. A total of 1008 patients were followed up from May 1995 to October 2013 in this study. The median follow-up time was 44.7 months (range from 1.7 to 171.0 months) for all patients, with 50.9 and 11.6 months for the patients of non-recurrence group and recurrence group, respectively. The overall incidence of SCCOP recurrence was approximately 20%. Of the 181 patients with SCCOP recurrence, local recurrence was observed in 49 patients (27.1 %) and regional/distant recurrence occurred in 20 patients (11.0 %)/70 patients (38.7%), respectively. A total of 42 patients (23.2%) experienced recurrence of more than one type. Additionally, among a subgroup of 432 SCCOP patients whose tumor specimens were available for tumor HPV status determination, 324 cases were found to have HPV16-positive tumors.

The univariate Kaplan-Meier analysis showed that patients, who were older than 57 years old (P < .0001), other ethnicity (P < .0001), ever smokers (P = 0.0004) and alcohol drinkers (P = 0.0005), had moderate to severe comorbidity (P = 0.0370), and received combined treatment of surgery with chemoradiotherapy (P = 0.0030), respectively, were significantly associated with DFS. However, no significant associations were observed between patients with and without recurrence according to other factors, including sex (P = 0.3110), index tumor stage (P = 0.5280).

Table 2 showed that the genotypes distribution of three selected *MDM4* polymorphisms, 5-year recurrence rates by genotype, and the associations between SNPs and recurrence risk in patients with SCCOP. Patients with the *MDM4*-rs10900598 GT/TT, *MDM4*-rs11801299 GG genotypes had significantly worse DFS compared with their corresponding GG and AG/AA genotypes (log-rank, P = 0.0002 and P < 0.001, respectively) (Figure 1). The similar findings were found among 324 patients with HPV16-positive SCCOP (log-rank, P = 0.051 for *MDM4*-rs10900598 and P = 0.029 for *MDM4*-rs11801299; Figure 1). In multivariable Cox proportional hazards regression analyses, the risk of disease recurrence differed significantly in patients carrying the *MDM4*-rs10900598 GT/TT and *MDM4*-rs11801299 GG genotypes compared with their corresponding GG and AG/AA genotypes (HR, 2.0, 95% CI, 1.4-2.9 and HR, 0.4, 95% CI, 0.3-0.6) after adjusted for some possible confounders (Table 2).

Given the roles of both MDM4 and HPV involving in the p53 pathway, we further evaluated the associations between genotypes of the three *MDM4* polymorphisms and recurrence risk among 324 HPV16-positive SCCOP cases. Table 3 showed that patients with *MDM4*-rs10900598 GT/TT variant genotypes had a significantly higher recurrence risk than those with GG common homozygous genotype (aHR, 1.8; 95% CI, 1.0-3.9), while patients with *MDM4*-rs11801299 AG/AA had a significantly lower risk of recurrence than those carrying *MDM4*-rs11801299 GG common homozygous genotype (aHR, 0.4; 95% CI, 0.2-0.9).

However, no significant association between *MDM4*-rs1380576 polymorphism and recurrence risk was observed among both overall and HPV16-positive SCCOP patients (aHR, 0.8; 95% CI, 0.6-1.1 for overall SCCOP and aHR, 0.8; 95% CI, 0.2-1.7 for HPV16-positive SCCOP).

We further evaluated the combined effect of the 3 polymorphisms on recurrence risk among SCCOP patients. The SCCOP patients were categorized into 2 different risk groups based on the number of the combined risk genotypes of the 3 polymorphisms. Compared with patents in high-risk group, patients in the low risk group had significantly lower DFS among both overall and HPV16-positive SCCOP patients (both log-rank: $P < 0.001$) (Figure 2). Moreover, significant associations were observed between the combined risk genotypes of the 3 polymorphisms and risk of recurrence among both overall and HPV16-positive SCCOP patients (aHR, 0.2; 95% CI, 0.1-0.3 for overall SCCOP and aHR, 0.1; 95% CI, 0.1-0.3 for HPV16-positive SCCOP) as shown in Table 4. Besides, we did not analyze the associations between the 3 *MDM4* polymorphisms and recurrence risk among the patients with HPV16-negative SCCOP because there were limited sample size and few outcome events for recurrence in this subgroup.

To further support the 3-UTR binding site SNP (*MDM4*-rs10900598 and *MDM4*-rs11801299) of *MDM4* as a risk factor for SCCOP recurrence, we replaced the 3′-UTR of a luciferase reporter gene with the 633-bp or 675-bp *MDM4* 3′-UTR containing either rs10900598 T or rs10900598 G and rs11801299A or rs11801299G, respectively (Fig. 3). As shown in Fig. 3, significantly lower levels of luciferase expression were observed when UMSCC4 and UMSCC47 cells were cotransfected with *MDM4* 3′UTR luciferase reporter plasmids carrying the rs11801299A allele than with those plasmids carrying the G allele in both cell lines ($P = 0.032$ in UMSCC4 and $P = 0.019$ in UMSCC47 cells), While borderline significantly higher levels of luciferase expression were observed when UMSCC4 and UMSCC47 cells were cotransfected with *MDM4* 3′UTR luciferase reporter plasmids carrying the rs10900598 T allele than with those plasmids carrying the G allele in both cell lines ($P = 0.058$ in UMSCC4 and $P = 0.053$ in UMSCC47 cells).

Discussion

In the present study, we found 3 SNPs within the *MDM4* genes that may play a critical role in predicting tumor recurrence of SCCOP patients. *MDM4*-rs10900598 and *MDM4*-rs11801299 variants may individually, or more likely jointly, significantly modulate the risk of SCCOP recurrence after adjusting for other important confounders, particularly in HPV16-positive tumors of SCCOP. To our knowledge, this study is the first to investigate the associations between *MDM4* polymorphisms and increased risk of recurrence in SCCOP patients.

Our previous study [7] reported that combined effect of 3 *MDM4* variants may be linked to the risk of SCCOP, particularly for HPV16-positive SCCOP. In the present study, we further provided evidence for a significant association between *MDM4* variants and a modified risk of SCCOP recurrence. If our results are further validated, improved strategies based on these *MDM4* genetic variants could be used to identify patients with higher recurrence risk.

Therefore, there may be a better-tailored therapy and prevention regimen for SCCOP patients, which may contribute to better outcomes and lower adverse events.

The molecular mechanisms underlying the effects of *MDM4*-rs10900598 and *MDM4*-rs11801299 genetic variations on cancer recurrence are still not well understood. However, there are some plausible explanations. Recent study [33] has suggested that *MDM2* might play a role in determining the risk of recurrence of SCCOP. As *MDM4* has a strong similarity in gene sequence and structure to *MDM2*, it is conceivable that *MDM4* may have the similar mechanism in modifying the recurrence risk of SCCOP.

Previous studies have shown that other genetic changes, such as amplification or overexpression, of MDM4 was associated with tumor progression and poor prognosis [21,30,34,35]. Therefore, it is our speculation that these three SNPs in *MDM4* may affect expression of MDM4, result in different efficacy for binding to p53 or MDM2, and consequently attenuate the p53-mediated tumor-suppressing activities involving in regulation of several cellular activities, such as cell cycle control, DNA repair and apoptosis, eventually contributing to recurrence of SCCOP. Our observed associations were supported by additional experiments for their possible underlying molecular mechanism. For example, in the luciferase reporter determination, the rs11801299 variant A allele was found to be associated with significantly lower luciferase activity compared with the G allele, indicating the A variant allele might be biologically functional to reduce expression of MDM4. Such downexpression of MDM4 may subsequently attenuate the binding to p53 and result in increased p53-mediated apoptotic capacity, subsequently leading to lower risk of SCCOP recurrence. However, this hypothesis needs to be further validated in future studies.

It has been known that HPV is an independent prognostic factor associated with prognosis of SCCOP. Our previous study indicated that both HPV16 E6 and MDM4 oncoproteins may function synergistically in the development and progression of HPV-positive SCCOP through the common pathways that cause p53 degradation due to HPV16 E6 binding to p53 and targets it for proteasomal degradation[36]. In this study, we found that the association were statistically significant between *MDM4* variants and risk of recurrence among HPV-positive SCCOP. Thus, these *MDM4* polymorphisms may modify the susceptibility to radiotherapy through interaction with HPV16 in p53 pathway, affect the risk of recurrence. However, our current findings may be confounded by other important prognostic factors or may be a chance finding due to small sample size in the subgroup. Therefore, further studies with larger sample sizes are needed to validate our findings, particularly in HPV16-positive subgroup. In addition, there was a significant combined effect of the 3 *MDM4* variants on risk of recurrence among SCCOP patients in the present study. The effect was even more pronounced in HPV-positive SCCOP patients. Thus, our findings in combined analyses further supported that risk genotypes of these functional polymorphisms of *MDM4* may be involved in the recurrence of SCCOP and suggested that combination of *MDM4* SNPs could be clinically more valuable recurrence biomarkers.

Our findings are strengthened by some points. First, our study patient population was a well-defined cohort of SCCOP patients with the largest sample size and the careful quality control in genotyping. Second, this is the first study to date to examine the effects of *MDM4*

polymorphisms on SCCOP recurrence risk based on HPV16 tumor status instead of serology. Finally, our analysis was focused on only one homogeneous tumor site (SCCOP) to avoid the bias within different types of head and neck cancers. However, several limitations of the present study should be acknowledged. First, since our study included patients who were predominant non-Hispanic white at a single cancer center, these results may not be generalizable to other ethnic populations and some significant findings could be due to chance. Second, our study lacked detailed information on the exact radiotherapy dosage and duration for each SCCOP patient. Although the treatment of radiotherapy or chemotherapy was associated 5-year recurrence of SCCOP patients, while we found that there were no significant associations between treatment and these three *MDM4* genetic variants. In addition, in this study treatment was also included as a covariate in the Cox regression model for *MDM4* SNPs. Therefore, treatment is unlikely to be a confounder; and *MDM4* variants appear to be independent prognostic factors for recurrence of SCCOP. Furthermore, as our study had a relatively small number of SCCOP patients with HPV16-positive tumors and event outcome of recurrence, the finding could be due to chance. Finally, besides these three polymorphisms, some other possible genetic variants might be also evaluated in a large-scale and comprehensive study.

Conclusions

In conclusion, our report provides the first evidence that the *MDM4* genetic variants may individually, and more like jointly contribute to susceptibility to SCCOP recurrence risk, particularly in HPV16-positive tumors of SCCOP. However, further studies are needed to explore underlying mechanisms and further validate the clinical significance of these polymorphisms.

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.

Funding support: 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.).

References

1. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. The New England journal of medicine. 2010; 363:24–35. [PubMed: 20530316]
2. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2013; 31:4550–4559. [PubMed: 24248688]
3. Bhatia A, Burtness B. Human Papillomavirus-Associated Oropharyngeal Cancer: Defining Risk Groups and Clinical Trials. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2015; 33:3243–3250. [PubMed: 26351343]

4. Dahlstrom KR, Anderson KS, Sturgis EM. Human Papillomavirus-Associated Oropharyngeal Cancer: Not Just White Men Anymore. JAMA oncology. 2016
5. Dahlstrom KR, Garden AS, William WN Jr, Lim MY, Sturgis EM. Proposed Staging System for Patients With HPV-Related Oropharyngeal Cancer Based on Nasopharyngeal Cancer N Categories. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2016; 34:1848–1854. [PubMed: 26884553]
6. Stracquadanio G, Wang X, Wallace MD, et al. The importance of p53 pathway genetics in inherited and somatic cancer genomes. Nature reviews Cancer. 2016; 16:251–265. [PubMed: 27009395]
7. Yu H, Wang LE, Liu Z, et al. Polymorphisms of MDM4 and risk of squamous cell carcinoma of the head and neck. Pharmacogenetics and genomics. 2011; 21:388–396. [PubMed: 21540763]
8. Yen CY, Liu SY, Chen CH, et al. Combinational polymorphisms of four DNA repair genes XRCC1, XRCC2, XRCC3, and XRCC4 and their association with oral cancer in Taiwan. Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology. 2008; 37:271–277.
9. Canova C, Hashibe M, Simonato L, et al. Genetic associations of 115 polymorphisms with cancers of the upper aerodigestive tract across 10 European countries: the ARCAGE project. Cancer research. 2009; 69:2956–2965. [PubMed: 19339270]
10. Lane DP. Cancer. p53, guardian of the genome. Nature. 1992; 358:15–16. [PubMed: 1614522]
11. Burgess A, Chia KM, Haupt S, Thomas D, Haupt Y, Lim E. Clinical Overview of MDM2/X-Targeted Therapies. Frontiers in oncology. 2016; 6:7. [PubMed: 26858935]
12. Zhou G, Liu Z, Myers JN. TP53 Mutations in Head and Neck Squamous Cell Carcinoma and Their Impact on Disease Progression and Treatment Response. Journal of cellular biochemistry. 2016; 117:2682–2692. [PubMed: 27166782]
13. Wasylishen AR, Lozano G. Attenuating the p53 Pathway in Human Cancers: Many Means to the Same End. Cold Spring Harbor perspectives in medicine. 2016; 6
14. Wade M, Wang YV, Wahl GM. The p53 orchestra: Mdm2 and Mdmx set the tone. Trends in cell biology. 2010; 20:299–309. [PubMed: 20172729]
15. Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nature reviews Cancer. 2013; 13:83–96. [PubMed: 23303139]
16. Marine JC, Jochemsen AG. Mdmx as an essential regulator of p53 activity. Biochemical and biophysical research communications. 2005; 331:750–760. [PubMed: 15865931]
17. Toledo F, Wahl GM. Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. Nature reviews Cancer. 2006; 6:909–923. [PubMed: 17128209]
18. Sharp DA, Kratowicz SA, Sank MJ, George DL. Stabilization of the MDM2 oncoprotein by interaction with the structurally related MDMX protein. The Journal of biological chemistry. 1999; 274:38189–38196. [PubMed: 10608892]
19. Tanimura S, Ohtsuka S, Mitsui K, Shirouzu K, Yoshimura A, Ohtsubo M. MDM2 interacts with MDMX through their RING finger domains. FEBS letters. 1999; 447:5–9. [PubMed: 10218570]
20. Marine JC, Francoz S, Maetens M, Wahl G, Toledo F, Lozano G. Keeping p53 in check: essential and synergistic functions of Mdm2 and Mdm4. Cell death and differentiation. 2006; 13:927–934. [PubMed: 16543935]
21. Bao J, Nanding A, Song H, Xu R, Qu G, Xue Y. The overexpression of MDM4: an effective and novel predictor of gastric adenocarcinoma lymph node metastasis. Oncotarget. 2016; 7:67212–67222. [PubMed: 27626496]
22. Salvi S, Calistri D, Gurioli G, et al. Copy number analysis of 24 oncogenes: MDM4 identified as a putative marker for low recurrence risk in non muscle invasive bladder cancer. International journal of molecular sciences. 2014; 15:12458–12468. [PubMed: 25026175]
23. Wang MY, Jia M, He J, et al. MDM4 genetic variants and risk of gastric cancer in an eastern chinese population. Oncotarget. 2017
24. Sun T, Lee GS, Oh WK, et al. Single-nucleotide polymorphisms in p53 pathway and aggressiveness of prostate cancer in a Caucasian population. Clinical cancer research: an official journal of the American Association for Cancer Research. 2010; 16:5244–5251. [PubMed: 20855462]

25. Yu H, Sturgis EM, Liu Z, Wang LE, Wei Q, Li G. Modifying effect of MDM4 variants on risk of HPV16-associated squamous cell carcinoma of oropharynx. Cancer. 2012; 118:1684–1692. [PubMed: 21823114]
26. Pesole G, Mignone F, Gissi C, Grillo G, Licciulli F, Liuni S. Structural and functional features of eukaryotic mRNA untranslated regions. Gene. 2001; 276:73–81. [PubMed: 11591473]
27. Jupe ER, Badgett AA, Neas BR, et al. Single nucleotide polymorphism in prohibitin 3′ untranslated region and breast-cancer susceptibility. Lancet (London, England). 2001; 357:1588–1589.
28. Wong PM, Yuan Q, Chen H, Sultzer BM, Chung SW. A single point mutation at the 3′-untranslated region of Ran mRNA leads to profound changes in lipopolysaccharide endotoxin-mediated responses. The Journal of biological chemistry. 2001; 276:33129–33138. [PubMed: 11429415]
29. Fang Y, van Meurs JB, d'Alesio A, et al. Promoter and 3′-untranslated-region haplotypes in the vitamin d receptor gene predispose to osteoporotic fracture: the rotterdam study. American journal of human genetics. 2005; 77:807–823. [PubMed: 16252240]
30. Yang Y, Gao W, Ding X, et al. Variations within 3′-UTR of MDM4 gene contribute to clinical outcomes of advanced non-small cell lung cancer patients following platinum-based chemotherapy. Oncotarget. 2016
31. Zhang F, Sturgis EM, Sun Y, et al. Apoptotic variants as predictors of risk of oropharyngeal cancer recurrence after definitive radiotherapy. International journal of cancer. 2015; 137:2454–2461. [PubMed: 25976983]
32. Ji X, Sturgis EM, Zhao C, Etzel CJ, Wei Q, Li G. Association of p73 G4C14-to-A4T14 polymorphism with human papillomavirus type 16 status in squamous cell carcinoma of the head and neck in non-Hispanic whites. Cancer. 2009; 115:1660–1668. [PubMed: 19197996]
33. Zhang Y, Sturgis EM, Li Y, Wei Q, Huang Z, Li G. Modifying effect of mouse double minute-2 promoter variants on risk of recurrence for patients with squamous cell carcinoma of oropharynx. Scientific reports. 2017; 7:39765. [PubMed: 28045062]
34. Bartel F, Schulz J, Bohnke A, et al. Significance of HDMX-S (or MDM4) mRNA splice variant overexpression and HDMX gene amplification on primary soft tissue sarcoma prognosis. International journal of cancer. 2005; 117:469–475. [PubMed: 15906355]
35. Laurie NA, Donovan SL, Shih CS, et al. Inactivation of the p53 pathway in retinoblastoma. Nature. 2006; 444:61–66. [PubMed: 17080083]
36. Thomas M, Pim D, Banks L. The role of the E6-p53 interaction in the molecular pathogenesis of HPV. Oncogene. 1999; 18:7690–7700. [PubMed: 10618709]

Abbreviations

MDM4	Mouse double minute 4
CI	confidence interval
HPV	human papillomavirus
HRs	hazard ratios
SCCOP	squamous cell carcinoma of oropharynx
SCCHN	squamous cell carcinoma of head and neck
SNP	single nucleotide polymorphisms
DFS	disease-free survival

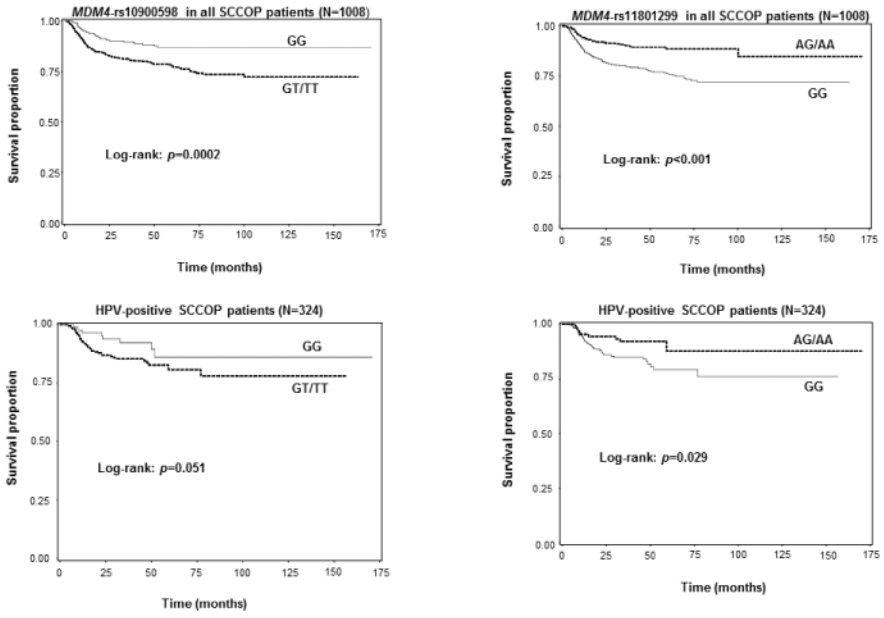

Figure 1.

Kaplan–Meier estimates for DFS of patients according to the *MDM4*-rs10900598 and *MDM4*-rs11801299 genotypes among all patients and those with HPV16-positive SCCOP.

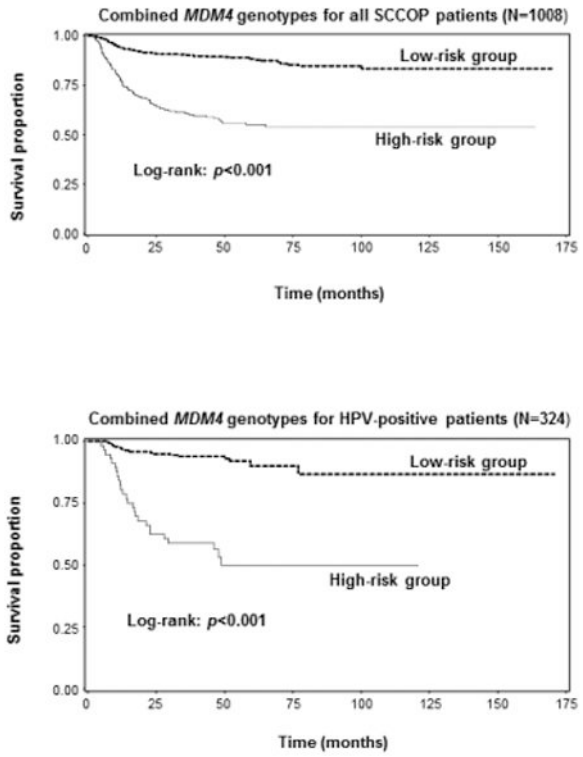

Figure 2.

Kaplan–Meier estimates for the DFS of patients according to the combined risk genotypes of the three polymorphisms of *MDM4* among all patients and those with HPV16-positive SCCOP (the high-risk group included patients with 1-3 risk genotypes, and the low-risk group included patients with 0 risk genotype).

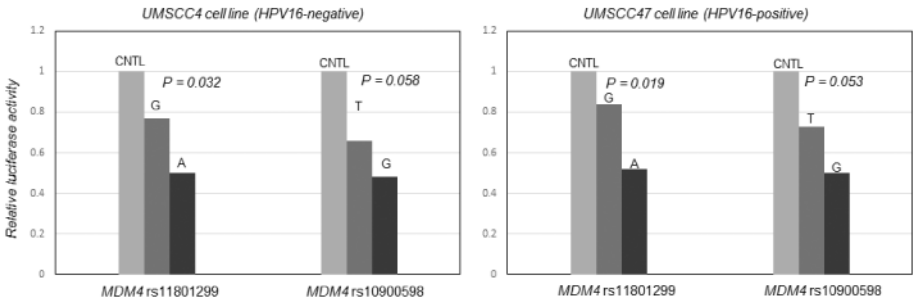

Figure 3.

Luciferase activity to determine the A/G and T/G allele difference for MDM4rs11801299 and *MDM4*rs10900598 in both head and neck cell lines:UMSCC4 (HPV16-negative) and UMSCC47 (HPV16-positive).

Table 1

Characteristics of patients with SCCOP (N =1008)

Characteristics	No. (%) of patients	No. of patients with recurrence	5-year recurrence rate (%)	*P* value[a]	cHR (95% CI)
No. of patients	1008 (100)	181	20		
Age, years					
≤ 57	621 (61.6)	85	15	< 0.0001	1.0
> 57	387 (38.4)	96	27		2.0 (1.5-2.7)
Sex					
Male	872 (86.5)	161	20	0.3110	1.0
Female	136 (13.5)	20	19		0.8 (0.5-1.3)
Ethnicity					
Non-Hispanic white	913 (90.6)	146	17	< 0.0001	1.0
Other	95 (9.4)	35	41		2.8 (1.9-4.0)
Smoking					
Never	388 (38.5)	51	14	0.0004	1.0
Ever	620 (61.5)	130	23		1.7 (1.2-2.5)
Alcohol use					
Never	247 (24.5)	26	10	0.0005	1.0
Ever	761 (75.5)	155	23		2.0 (1.4-3.1)
Comorbidity					
None or mild	913 (90.6)	157	19	0.0370	1.0
Moderate to severe	95 (9.4)	24	27		1.6 (1.0-2.4)
Index cancer stage					
1 or 2	72 (7.1)	11	19	0.5280	1.0
3 or 4	936 (92.9)	170	20		1.2 (0.7-2.3)
Treatment					
X	947 (93.9)	166	19	0.0030	1.0
XC	61 (6.1)	15	32		2.2 (1.3-3.7)

[a] *P*: Log-rank test for DFS between the two groups.

X, radiotherapy; C, chemotherapy.

cHR, crude hazard ratio (univariate cox model).

Table 2

Associations between polymorphisms in *MDM4* genes and recurrence risk of patients with SCCOP (N = 1008)

Genotype	No. of recurrences/no. of patients	5-year recurrence rate	Log-rank *P* value	Adjusted HR* (95% CI)
rs10900598			0.0002	
GG†	34/304	0.13		1.0
GT + TT	147/704	0.23		2.0(1.4-2.9)
rs1380576			0.079	
CC†	92/459	0.22		1.0
CG + GG	89/549	0.17		0.8(0.6-1.1)
rs11801299			<0.001	
GG†	146/656	0.24		1.0
AG + AA	35/352	0.11		0.4(0.3-0.6)

HR, hazard ratio.

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

†Reference group.

Table 3

Associations between polymorphisms in *MDM4* genes and recurrence risk of patients with HPV16-positive SCCOP (N = 324)

Genotype	No. of recurrences/no. of patients	5-year recurrence rate	Log-rank *P* value	Adjusted HR* (95% CI)
rs10900598			0.051	
GG†	8/91	0.14		1.0
GT + TT	37/233	0.20		1.8(1.0-3.9)
rs1380576			0.308	
CC†	18/149	0.16		1.0
CG + GG	27/175	0.18		0.8(0.2-1.7)
rs11801299			0.029	
GG†	36/210	0.22		1.0
AG + AA	9/114	0.12		0.4(0.2-0.9)

HR, hazard ratio.

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

†Reference group.

Table 4

Association between combined *MDM4* genotypes and recurrence risk in overall and tumor HPV16-positive patients with SCCOP.

***Combined* genotypes**	**No. of recurrence/no. of patients**	**5-year recurrence rate**	**Log-rank *p* values**	**aHR[1], 95% CI**
All patients (*N*= 1008)			<0.001	
High risk group [*](Ref.)	95/224	0.45		1.0
Low risk group	86/784	0.12		0.2 (0.1-0.3)
HPV-positive SCCOP patients (*N*= 324)			<0.001	
High risk group(Ref.)	35/99	0.49		1.0
Low risk group	10/225	0.13		0.1 (0.1-0.3)

[*] high-risk group: individuals carrying 1-3 risk genotypes; Low-risk group: individuals carrying 0 risk genotype.

[1] aHR, adjusted hazard ratio for age, sex, ethnicity, smoking status, alcohol use status, stage, comorbidity, and treatment.

Ref: Reference group.

Mol Carcinog. Author manuscript; available in PMC 2019 March 01.