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Pathogenic *TP53* mutations influence chemotherapy response and survival rate of patients with HPV-negative oral carcinomas

Uticaj patogenih *TP53* mutacija na preživljavanje i odgovor na hemioterapiju bolesnika sa HPV-negativnim oralnim karcinomima

Saša Jović*†, Ružica Kozomara*†, Srboljub Stošić*†, Stevo Jovandić‡, Katarina Zeljić§, Gordana Šupić†‡

Military Medical Academy, *Clinic for Maxillofacial Surgery, ‡Institute for Medical Research, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; §University of Belgrade, Faculty of Biology, Belgrade, Serbia

Abstract

Background/Aim. Oral squamous cell carcinoma (OSCC) is the most common tumor type of head and neck carcinomas, characterized by a high recurrence rate and patients' poor survival. Further elucidation of the function and regulation of the *TP53*, a pivotal tumor suppressor gene, would provide advances in predicting the clinical behavior, prognosis, and chemotherapy response of OSCC patients. Thus, we investigated the association of *TP53* gene mutations with survival and response to cisplatin chemotherapy in human papilloma virus (HPV)-negative OSCC patients. **Methods.** The potential clinical relevance of *TP53* mutations was analyzed in 82 patients with HPV-negative OSCC. All patients underwent radiotherapy, and 25 patients received cisplatin chemotherapy. A negative HPV status was determined by type-specific polymerase chain reaction (PCR) for high-risk HPV 16, 18, 31, and 33. Targeted sequencing of *TP53* exons 4–8 was assessed by Sanger sequencing. **Results.** Of 82 HPV-negative OSCC patients, 49 (59.79%) had *TP53* mutations, and 26 patients (31.7%) carried pathogenic *TP53* mutations. Patients with pathogenic *TP53* mutations had significantly reduced overall survival ($p = 0.009$). Recurrence status, but not *TP53* mutations, was an independent marker of poor survival in our cohort [hazard ratio (HR) = 4.733, 95% confidence interval (95% CI): 2.027–11.053, $p = 0.0001$]. In the subcohort of patients who underwent cisplatin-based chemotherapy, pathogenic *TP53* mutations were predictors of poor response to chemotherapy ($p = 0.026$). **Conclusion.** Our findings indicate that pathogenic *TP53* mutations in HPV-negative OSCC tumors could be a prognostic marker of patients' reduced overall survival. In addition, pathogenic *TP53* mutations in HPV-negative OSCC could be a marker of poor chemotherapy response of OSCC patients.

Key words:
carcinoma, squamous cell; drug therapy; genes, tumor suppressor; head and neck neoplasms; mutation; prognosis; radiotherapy; survival rate.

Apstrakt

Uvod/Cilj. Oralni planocelularni karcinom (OPCK) je najčešći tip karcinoma glave i vrata, koji se odlikuje visokom stopom recidiva i lošim preživljavanjem bolesnika. Dalje razjašnjenje uloge i regulacije *TP53*, ključnog tumor supresorskog gena, omogućilo bi napredak u predviđanju toka, prognoze i hemioterapijskog odgovora obolelih od OPCK. Zbog toga smo istražili povezanost mutacija gena *TP53* sa preživljavanjem i odgovorom na hemioterapiju cisplatinom bolesnika sa HPV-negativnim OPCK. **Metode.** Potencijalni klinički značaj mutacija *TP53* analiziran je kod 82 bolesnika sa HPV-negativnim OPCK. Svi bolesnici su bili podvrgnuti radioterapiji, a 25 bolesnika primilo je hemioterapiju cisplatinom. Negativni HPV status utvrđen je tip-specifičnom metodom *polymerase chain reaction* (PCR), za visoko rizične HPV 16, 18, 31 i 33. Ciljno sekvenciranje *TP53* egzona 4–8 rađeno je Sanger kapilarnim sekvenciranjem. **Rezultati.** Od 82 HPV-negativnih OPCK bolesnika, njih 49 (59,79%) imalo je *TP53* mutaciju, a 26 (31,7%) bolesnika je imalo patogene *TP53* mutacije. Bolesnici sa patogenim mutacijama *TP53* imali su značajno smanjeno celokupno preživljavanje ($p = 0,009$). Status recidiva, ali ne i *TP53* mutacije, bio je nezavisni marker lošeg preživljavanja bolesnika u našoj studiji [*hazard ratio* (HR) = 4,733, 95% *confidence interval* (95% CI): 2,027–11,053; $p = 0,0001$]. U

Correspondence to: Gordana Šupić, Military Medical Academy, Institute for Medical Research, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: gogasupic@gmail.com

podgrupi bolesnika koji su bili podvrgnuti hemioterapiji cisplatinom, patogene *TP53* mutacije bile su prediktori slabog odgovora na hemioterapiju (p = 0,026). **Zaključak.** Naši nalazi ukazuju da bi patogene *TP53* mutacije u HPV-negativnim OPCK tumorima mogle biti prognostički marker smanjenog ukupnog preživljavanja bolesnika. Pored toga, patogene *TP53* mutacije u HPV-negativnom OPCK mogu biti marker lošeg odgovora tih bolesnika na hemioterapiju.

Ključne reči:
karcinom, planocelularni; lečenje lekovima; geni, tumor-supresori; glava i vrat, neoplazme; mutacija; prognoza; radioterapija; preživljavanje, stepen.

Introduction

Oral squamous cell carcinoma (OSCC) is the most common tumor type of head and neck carcinomas, characterized by a high recurrence rate and patients'poor survival. This malignancy is the sixth most common cancer worldwide in men and eighth in women in developed countries, while in developing countries, it is the third most common cancer in men and fourth in women, which affects approximately 600,000 new patients every year worldwide [1]. Oral carcinogenesis is a multi-step process that encompasses an accumulation of genetic and epigenetic changes which lead to the disruption of the various signaling pathways controlling the cell cycle, proliferation, apoptosis, senescence, and DNA [2]. Genetic changes are progressively accumulated, and the inactivation of tumor suppressor genes by point mutations, deletions, and gene rearrangement is one of the key changes for malignant transformation. Known etiological factors for developing OSCC are predominantly smoking, alcohol intake, and poor oral hygiene. Approximately 20–30% of OSCC cases can be associated with tobacco smoking and 7–19% with heavy alcohol drinking, which increases the risk of oral cavity cancer 2–3 times [3]. One of the most important advances in oral carcinogenesis in recent decades is the evidence of an association between oral cancer and some types of human papillomavirus (HPV) infection, predominantly HPV16 [4].

Gene coding for protein p53 (*TP53*) is one of the most prominent tumor suppressor genes located on the short arm of chromosome 17 (17p13.1) [5]. Protein p53 is a key factor in a signaling pathway that helps the cell to recover from DNA damage [6]. Upon DNA damage, the wild type (WT) p53 arrests the cell cycle in the G1 phase prohibiting transition to the S phase until the damage is repaired. Additionally, throughout the retinoblastoma tumor suppressor pathway, p53 can direct cells to a state of permanent cell cycle arrest or induce pro-apoptotic genes cellular senescence [7]. *TP53* is one of the most frequently mutated human genes in more than 50% of cancers. Germline *TP53* mutations cause Li-Fraumeni syndrome, a rare autosomal, hereditary disorder predisposing to sarcoma, breast cancer, leukemia, and adrenal gland carcinoma [8].

TP53 mutations are early alterations during oral carcinogenesis, and more than 25,000 mutations have been discovered so far [9]. Most of them (70%) are missense mutations in the coding regions [10], where approximately 30% of mutations occur in exon 7 and exon 8, known as mutation hot spots. These exons code for the DNA binding domain, preventing the p53 binding to the promoter of target genes [11]. Common *TP53* codon 72 gene polymorphism in this domain produces two functional variants of p53 − p53P (proline) and p53A (arginine), which could reduce its ability to mediate apoptosis and cell cycle arrest and could, therefore, affect the survival and chemotherapy response [12]. A number of studies reported inconsistent findings regarding whether the *TP53* mutations and codon 72 polymorphism influence survival and chemotherapy response in OSCC patients [13].

Inactivation of WT p53 can also be achieved throughout HPV E6 protein [11]. HPV-positive oropharyngeal cancer cells have a different molecular profile from HPV-negative oropharyngeal cancer cells, where HPV-negative oropharyngeal cancers have a more frequent loss of heterozygosity of 3p, 9p, or 17p chromosomal regions [14]. HPV-negative oropharyngeal cancers have at least two times more mutations compared to HPV-positive tumors [15, 16], and worse outcome [17], indicating the necessity of molecular characterization of p53 in HPV-negative OSCC.

Further elucidation of the function and regulation of p53 in HPV-negative OSCC would provide advances in predicting the clinical behavior, prognosis, and patients' chemotherapy response. Finding the potential markers that could predict tumor response to chemotherapy, developing new strategies with therapeutics targeting different pathways that will override the resistance, and tumor molecular profiling would provide an individualized approach to the treatment modalities of OSCC patients.

Methods

The current study was approved by the Ethics Committee of the Military Medical Academy (No 162/2019, from December 26, 2019), according to the Helsinki Declaration (1964). The study group included 82 OSCC patients, Caucasians of the same ethnicity. All patients were diagnosed and subsequently operated on at the Clinic for Maxillofacial Surgery, Military Medical Academy, Belgrade, Serbia, between 2012 and 2020. All of them were operated on and received radiotherapy (60 Gy in 2 Gy dose per day), and 25 of the patients received cisplatin therapy in a dosage of 100 mg/m^2 of body surface area in one-week cycles. The face-to-face interviews were conducted to obtain demographic data. The evaluation of lymph node status and the tumor, node, metastasis (TMN) classification were determined by an experienced pathologist in accordance with the classification of the American Head and Neck Society and American Joint Committee on Cancer (AJCC Cancer Staging Manual 8th Edition, 2018). Of 82 OSCC patients, 35 (42.7%) were under 58 years of age, 58 (70.7%) were male,

57 (69.5%) had a history of alcohol abuse, 19 (23.2%) had stage II OSCC while 63 (76.8%) had advanced-stage tumors.

DNA isolation and HPV analysis

OSCC tissue samples were stored at -20 ˚C until DNA extraction. Genomic DNA was isolated by the TRI Reagent® (Sigma-Aldrich, USA) according to the manufacturer's protocol. DNA samples were stored at -20 ˚C until further analysis. Type-specific polymerase chain reaction (PCR) was assessed for high-risk HPV types 16, 18, 31, and 33.

TP53 Sanger sequencing

Targeted sequencing of p53 exons 4-8 was assessed by Sanger sequencing on ABI 3130 automated sequencer (Applied Biosystems, USA). The primers flanking exons 4-8 were retrieved from the IARC *TP53* database. PCR reactions were performed using the Platinum Taq DNA Polymerase PCR kit (Life Technologies). Amplicons were sequenced using the BigDye terminator cycle sequencing kit. Sequencing traces were analyzed with GeneScreen (http://dna.leeds.ac.uk/genescreen/) followed by visual inspection, with reference to the human genome reference sequence build hg19/GRCh37 (http://genome.ucsc.edu).

TP53 mutation classification according to its clinical significance

To provide information on pathogenic *TP53* mutations, genetic variants with clinical significance, we assessed the ClinVar database of the NCBI (National Center for Biotechnology Information) and a web server application Simple ClinVar [18].

TP53 mutations were classified as pathogenic and non-pathogenic mutations according to the ClinVar database, Simple ClinVar [18], and previous studies on head and neck carcinoma [19, 20]. Missense, stop-gain, in-frame insertions/deletions, frameshift, and splice site *TP53* mutations with pathogenic and likely pathogenic significance, and criteria provided by multiple or single submitters, reviewed by expert panels or given in practice guidelines, were classified as pathogenic mutations. On the other hand, likely benign, protective, or with uncertain significance were classified as non-pathogenic mutations.

Statistical analysis

Obtained data were analyzed by SPSS 20.0 software (IBM Inc., Chicago, IL, USA). Contingency tables were assessed by χ^2-test or Fisher's exact test. Overall survival was calculated from the date of diagnosis until death from any cause. Kaplan-Meier survival curves were compared using the log-rank test. Cox proportional hazard regression analysis was performed to estimate the hazard ratios (HR) with a 95% confidence interval (95% CI). Variables found significant in the univariate analysis, including those with a significance level below 20%, were subsequently analyzed in multivariate Cox's regression. The Cox model was performed using the forward stepwise method, which removed variables with $p < 0.1$. The associations were considered as significant when *p*-values were less than 0.05.

Results

Association of p53 gene mutations, pathogenic p53 mutations, and polymorphism p72 with demographic and clinicopathological features of HPV-negative OSCC patients

Eighty-two HPV-negative OSCC samples were screened for *TP53* mutations in exons 4-8, and mutations were found in a total of 49 (59.8%) patients. *TP53* mutations were classified as pathogenic and non-pathogenic, as previously suggested [19, 20]. Pathogenic *TP53* mutations were detected in 26 of 82 (31.7%) OSCC patients.

The association of *TP53* gene mutations, pathogenic *TP53* mutations, and polymorphism p72 with demographic and clinicopathological features of OSCC patients are presented in Table 1. No association was found between *TP53* mutations or pathogenic *TP53* mutations and sex or smoking. Pathogenic *TP53* mutations were significantly associated with age ($p = 0.005$) and high alcohol intake ($p = 0.009$). Locally advanced tumors did not have a statistically higher *TP53* mutation rate or pathogenic *TP53* mutations compared to early-stage OSCC. Mutations in exon 4 of the p53 gene were significantly associated with histological and nuclear grade ($p = 0.012$ and $p = 0.032$, respectively), while mutations in exon 7 were associated with smoking status ($p = 0.017$).

Association of TP53 gene mutations, pathogenic TP53 mutations, and polymorphism p72 with overall survival of OSCC patients

Overall survival (OS) curves were assessed by the Kaplan-Meier analysis and compared by the log-rank test**.** HPV-negative OSCC patients with mutated *TP53* tended to have worse survival ($p = 0.085$) as opposed to patients without *TP53* mutations. However, OSCC patients with pathogenic mutations in *TP53* had significantly reduced OS ($p = 0.009$, Figure 1). Non-pathogenic *TP53* mutations were not associated with OS of OSCC patients ($p = 0.785$, log-rank test). No significant difference was observed in OS between OSCC patients with different genotypes of p72 polymorphism ($p = 0.905$, log-rank test).

In the subgroup of 25 patients who received chemotherapy in our cohort, when all *TP53* mutations were taken into account, *TP53* mutation status was not associated with chemotherapy response ($p = 0.641$, Figure 2A). However, OS in patients who had received cisplatin chemotherapy was significantly shorter for those with pathogenic p53 mutations compared to patients with WT *P53* ($p = 0.026$, Figure 2B). Non-pathogenic *TP53* mutations in patients who had received cisplatin chemotherapy were not related to OS in our cohort

Table 1

Association of p53 gene mutations and polymorphism p72 with demographic and clinicopathological features of OSCC patients

Variables	Total (n)	All *TP53* Mutations		E4		E5		E6		E7		E8		p72 rs1042522			Pathogenic *TP53* mutations	
		+	-	+	-	+	-	+	-	+	-	+	-	WT	het	mut	+	-
Sex																		
male	58	35	23	30	28	6	52	16	42	2	56	16	42	35	22	1	16	42
female	24	14	10	13	11	1	23	10	14	0	24	4	20	11	11	2	10	14
p		0.866		0.840		0.362		0.357		0.295		0.479			0.231		0.231	
Age (years), median																		
< 58	35	23	12	17	18	4	31	17	18	1	34	9	26	17	15	3	17	18
≥ 58	47	21	26	26	21	3	44	9	38	1	46	11	36	29	18	0	9	36
p		0.342		0.545		0.419		0.832		0.810		0.563			0.093		**0.005**	
Smoking																		
never	25	15	10	15	10	1	24	7	18	0	25	7	18	13	12	0	7	18
ever	57	34	23	28	29	6	51	19	38	2	55	13	44	25	32	0	19	38
p		0.976		0.364		0.330		0.343		0.614		**0.017**			0.375		0.798	
High alcohol intake																		
no	57	33	24	30	27	5	52	13	44	1	56	14	43	33	23	1	13	44
yes	25	16	9	13	12	2	23	13	12	1	24	6	19	13	10	2	13	12
p		0.604		0.958		0.908		0.544		0.957		0.419			0.375		**0.009**	
Histological grade																		
1	61	34	27	27	34	6	55	16	45	1	60	15	46	39	19	3	16	45
2/3	21	15	6	16	5	1	20	10	11	1	20	5	16	7	14	0	10	11
p		0.206		**0.012**		0.473		0.424		0.943		0.342			**0.014**		0.069	
Nucleus grade																		
1	58	32	26	26	32	6	52	17	41	1	57	15	43	36	19	3	17	41
2/3	24	17	7	17	7	1	23	9	15	1	23	5	19	10	14	0	9	15
p		0.188		**0.032**		0.362		0.514		0.629		0.220			0.072		0.468	
Nodal status																		
-	19	14	5	10	9	2	17	7	12	0	19	5	14	9	10	0	7	12
+	63	35	28	29	34	5	58	19	44	2	61	15	48	37	23	3	19	44
p		0.158		0.614		0.723		0.432		0.824		0.865			0.336		0.583	
Tumor size																		
T1/2	60	37	23	29	31	6	54	21	39	2	58	15	45	31	26	3	21	39
T3/4	22	12	10	14	8	1	21	5	17	0	22	5	17	19	7	0	5	17
p		0.560		0.219		0.434		0.386		0.832		0.137			0.299		0.290	
Tumor stage																		
II	19	13	6	8	11	3	16	5	14	1	18	4	15	8	10	1	5	14
III	63	36	27	35	28	4	59	21	42	1	62	16	47	38	23	2	21	42
p		0.380		0.303		0.197		0.363		0.699		0.387			0.372		0.564	

OSCC – oral squamous cell carcinoma; n – total number of patients; E – exon; WT – wild type; het – heterozygosity; mut – mutation.
Statistically significant values are bolded.

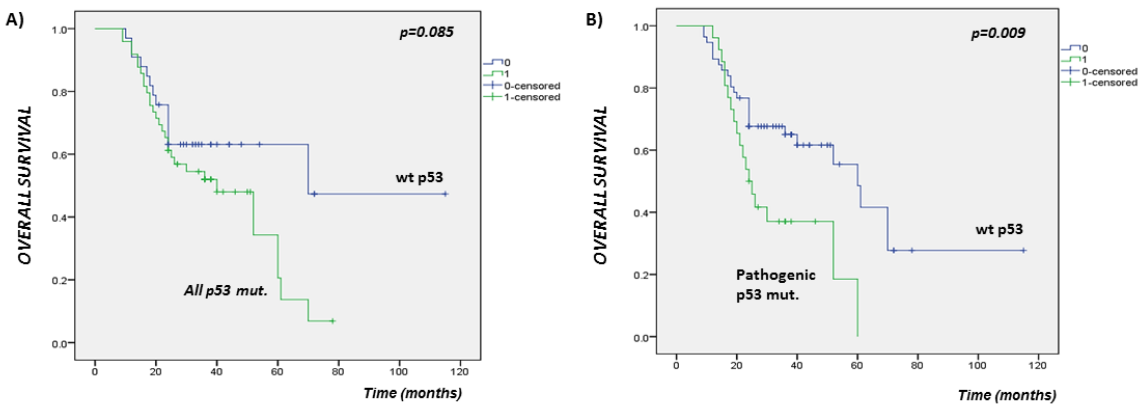

Fig. 1 – Kaplan-Meier curves for overall survival based on the *TP53* mutation status in a total cohort of 82 HPV-negative OSCC patients: A) Survival comparison between all *TP53* mutations (mut.) and wild type (WT) *TP53*; B) Comparison of pathogenic *TP53* mutations and WT *TP53*.
OSCC – oral squamous cell carcinoma.

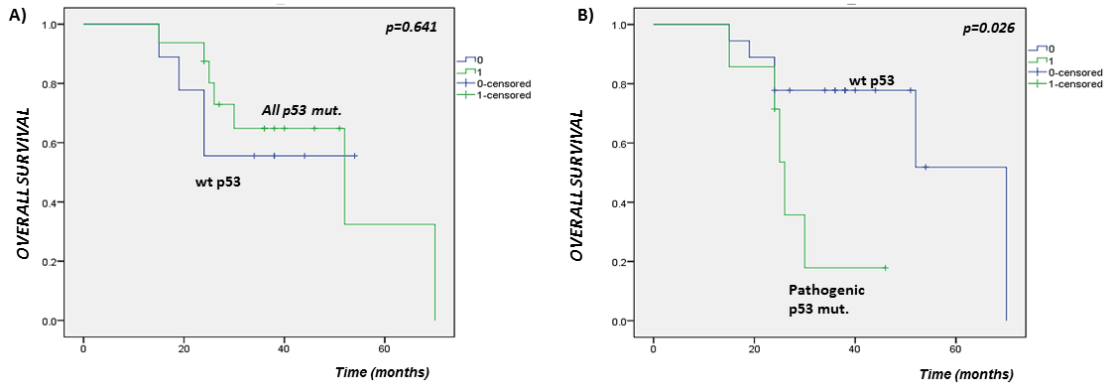

Fig. 2 – Associations between *TP53* mutations and survival outcome of the subgroup of 25 OSCC patients who received platinum-based chemotherapy: A) Survival comparison between all *TP53* mutations (mut.) and wild type (WT) *TP53*; B) Comparison of pathogenic *TP53* mutations and WT *TP53*.
OSCC – oral squamous cell carcinoma.

(p = 0.453, log-rank test). These findings indicate that the response to chemotherapy was associated with the type of p53 mutation and that the patients with pathogenic *TP53* mutations were resistant to platinum-based chemotherapy, as opposed to the patients with WT *TP53*.

The Cox regression analysis demonstrated that high alcohol intake, stage, tumor size, nodal status, and recurrences are highly associated with hazard risk (Table 2). While patients with *TP53* mutations had increased but insignificant hazard risk [HR=1.747, 95% CI (0.907–3.366), p = 0.096], patients with pathogenic *TP53* mutations had significantly increased risk of poor survival [HR = 2.230, 95% CI (1.186–4.194), p = 0.013]. Variables found to be statistically significant, according to the univariate analysis, including the variables with a significance level below 20%, were subsequently analyzed in multivariate Cox hazards regression analysis. The multivariate analysis revealed that the recurrences persisted as an independent prognostic factor in our cohort [HR = 4.733, 95% CI (2.027–11.053), p = 0.0001] (Table 2). The list of detected *TP53* mutations and their classification according to clinical significance, assessed by the ClinVar database and Simple ClinVar web server, is given in Table 3.

Table 2
Cox proportional hazards regression analysis, according to overall survival of OSCC patients

Cox regression analysis	Demographic or pathological features	Overall survival	
		HR (95% CI)	*p*
Univariate analysis	Sex	0.660 (0.335–1.300)	0.230
	Age	0.605 (0.332–1.102)	0.100
	Smoking	1.682 (0.804–3.519)	0.167
	High alcohol intake	2.938 (1.610–5.360)	**0.0001**
	Nuclear grade	1.245 (0.900–1.721)	0.186
	Histological grade	1.270 (0.914–1.764)	0.155
	Stage	3.898 (1.388–10.947)	**0.010**
	Tumor size	1.654 (1.189–2.302)	**0.003**
	Nodal status	3.055 (1.199–7.786)	**0.019**
	Recurrences	4.727 (2.108–10.597)	**0.0001**
	All *TP53* mutations	1.747 (0.907–3.366)	0.096
	Pathogenic *TP53* mutations	2.230 (1.186–4.194)	**0.013**
	p72 SNP	1.065 (0.645–1.759)	0.806
Multivariate analysis	Recurrences	4.733 (2.027–11.053)	**0.0001**

OSCC – oral squamous cell carcinoma; HR – hazard ratio; CI – confidence interval; SNP – single nucleotide polymorphism.
Statistically significant values are bolded.

Table 3

Data on *TP53* mutations, assessed by the ClinVar database of the NCBI and a web server application Simple ClinVar[18]

TP53 mutation name	Clinical significance (Last reviewed)	Condition(s)
NM_000546.5(TP53):c.215= (p.Pro72=)	Benign	Not specified
NM_000546.5(TP53):c.273G>A (p.Trp91Ter)	Pathogenic	Hereditary cancer-predisposing syndrome; Neoplasm of the ovary; Li-Fraumeni syndrome; Li-Fraumeni syndrome 1
NM_001126114.2(TP53):c.560-11_560-8dup	Likely benign	Li-Fraumeni syndrome
NM_000546.5(TP53):c.820_821delGT (p.Val274Leufs)	Pathogenic	Li-Fraumeni syndrome
NM_000546.5(TP53):c.896_909del (p.Leu299Hisfs)	Pathogenic	Not provided
NM_000546.5(TP53):c.273G>A (p.Trp91Ter)	Pathogenic	Li-Fraumeni syndrome; Not provided; Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.380C>G (p.Ser127Cys)	Uncertain significance	Li-Fraumeni syndrome
NM_000546.5(TP53):c.384T>C (p.Pro128=)	Likely benign	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.389T>C (p.Leu130Pro)	Likely pathogenic	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.401T>G (p.Phe134Cys)	Likely pathogenic	Not provided; Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.411G>C (p.Leu137=)	Likely benign	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.421T>A (p.Cys141Ser)	Likely pathogenic	Multiple myeloma; Squamous cell carcinoma of the head and neck; Lung adenocarcinoma; Squamous cell lung carcinoma; Acute myeloid leukemia; Renal cell carcinoma, papillary; Neoplasm of the brain; Neoplasm of the breast; Pancreatic adenocarcinoma; Neoplasm of the large intestine; Colorectal neoplasms; Malignant neoplasm of body of uterus; Adenocarcinoma of the prostate
NM_000546.5(TP53):c.428T>G (p.Val143Gly)	Uncertain significance	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.517G>T (p.Val173Leu)	Pathogenic	Liver cancer; Malignant melanoma of skin; Squamous cell carcinoma of the head and neck; Small cell lung cancer; Lung adenocarcinoma; Li-Fraumeni syndrome; Neoplasm of the brain; Neoplasm of the breast; Hepatocellular carcinoma; Pancreatic adenocarcinoma; Brainstem glioma; Neoplasm of the large intestine; Carcinoma of the esophagus; Colorectal neoplasms; Adrenocortical carcinoma; Adenocarcinoma of the stomach; Ovarian serous cystadenocarcinoma; Malignant neoplasm of body of the uterus
NM_000546.5(TP53):c.560-15A>C	Likely benign	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.578A>G (p.His193Arg)	Likely pathogenic	Liver cancer; Chronic lymphocytic leukemia; Squamous cell carcinoma of the head and neck; Small cell lung cancer; Lung adenocarcinoma; Li-Fraumeni syndrome; Squamous cell lung carcinoma; Acute myeloid leukemia; Not provided; Neoplasm of the brain, Neoplasm of the breast; Hepatocellular carcinoma; Hereditary cancer-predisposing syndrome; Pancreatic adenocarcinoma; Transitional cell carcinoma of the bladder; Brainstem glioma; Neoplasm of the large intestine, Carcinoma of the esophagus; Colorectal neoplasms; Papillary renal cell carcinoma, sporadic; Adenocarcinoma of the stomach; Ovarian serous cystadenocarcinoma; Malignant neoplasm of body of uterus; Adenocarcinoma of the prostate; Uterine carcinosarcoma
NM_000546.5(TP53):c.591G>A (p.Val197=)	Uncertain significance	Li-Fraumeni syndrome
NM_000546.5(TP53):c.592delG (p.Glu198Lysfs)	Pathogenic	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.599delA (p.Asn200Ilefs)	Pathogenic	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.642T>C (p.His214=)	Likely benign	Li-Fraumeni syndrome
NM_000546.5(TP53):c.678C>A (p.Gly226=)	Likely benign	Not specified
NM_000546.5(TP53):c.698A>G (p.His233Arg)	Uncertain significance	Not specified; Hereditary cancer-predisposing syndrome

Table 3 (continued)

NM_000546.5(TP53):c.700T>C (p.Tyr234His)	Pathogenic	Liver cancer; Squamous cell carcinoma of the head and neck; Small cell lung cancer; Li-Fraumeni syndrome; Squamous cell lung carcinoma; Neoplasm of the breast, Glioblastoma; Hepatocellular carcinoma; Hereditary cancer-predisposing syndrome; Pancreatic adenocarcinoma; Transitional cell carcinoma of the bladder; Neoplasm of the large intestine; Carcinoma of the esophagus; Colorectal neoplasms; Adrenocortical carcinoma; Adenocarcinoma of the stomach; Ovarian serous cystadenocarcinoma; Adenocarcinoma of the prostate
NM_001126113.2(TP53):c.710T>A (p.Met237Lys)	Likely pathogenic	Squamous cell carcinoma of the head and neck; Lung adenocarcinoma; Squamous cell lung carcinoma; Neoplasm of the brain; Neoplasm of the breast; Pancreatic adenocarcinoma; Brainstem glioma; Neoplasm of the large intestine; Carcinoma of the esophagus; Colorectal neoplasms; Adenocarcinoma of the stomach, Ovarian serous cystadenocarcinoma; Malignant neoplasm of body of the uterus
NM_000546.5(TP53):c.712T>C (p.Cys238Arg)	Pathogenic	Liver cancer; Chronic lymphocytic leukemia; Multiple myeloma; Squamous cell carcinoma of the head and neck; Lung adenocarcinoma; Neoplasm of the brain; Neoplasm of the breast; Glioblastoma; Hepatocellular carcinoma; Hereditary cancer-predisposing syndrome; Pancreatic adenocarcinoma; Transitional cell carcinoma of the bladder; Neoplasm of the large intestine; Carcinoma of the esophagus; Colorectal Neoplasms; Uterine cervical neoplasms; Adenocarcinoma of the stomach; Ovarian serous cystadenocarcinoma; Malignant neoplasm of body of uterus; Uterine carcinosarcoma
NM_000546.5(TP53):c.718A>G (p.Ser240Gly)	Likely pathogenic	Li-Fraumeni syndrome
NM_000546.5(TP53):c.724T>C (p.Cys242Arg)	Likely pathogenic	Not provided
NM_000546.5(TP53):c.727A>C (p.Met243Leu)	Uncertain significance	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.728T>C (p.Met243Thr)	Conflicting interpretations of pathogenicity	Li-Fraumeni syndrome; Not specified; Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.730G>A (p.Gly244Ser)	Pathogenic/Likely pathogenic	Liver cancer; Squamous cell carcinoma of the head and neck; Small cell lung cancer; Lung adenocarcinoma; Li-Fraumeni syndrome; Squamous cell lung carcinoma; Neoplasm of the brain; Glioblastoma; Hepatocellular carcinoma; Hereditary cancer-predisposing syndrome; Neoplasm of the large intestine; Carcinoma of the esophagus; Colorectal Neoplasms; Adenocarcinoma of the stomach; Ovarian serous cystadenocarcinoma, Malignant neoplasm of body of uterus; Uterine carcinosarcoma
NM_000546.5(TP53):c.734G>C (p.Gly245Ala)	Likely pathogenic	Liver cancer; Squamous cell carcinoma of the head and neck; Lung adenocarcinoma; Squamous cell lung carcinoma; Not provided; Neoplasm of the brain; Neoplasm of the breast; Glioblastoma; Hepatocellular carcinoma; Pancreatic adenocarcinoma; Transitional cell carcinoma of the bladder; Brainstem glioma; Neoplasm of the large intestine; Carcinoma of the esophagus; Colorectal neoplasms; Adenocarcinoma of the stomach; Ovarian serous cystadenocarcinoma; Adenocarcinoma of the prostate; Uterine carcinosarcoma
NM_000546.5(TP53):c.737T>G (p.Met246Arg)	Pathogenic	Not provided
NM_000546.5(TP53):c.770T>A (p.Leu257Gln)	Uncertain significance	Li-Fraumeni-like syndrome; Li-Fraumeni syndrome
NM_000546.5(TP53):c.782+12C>T	Benign/Likely benign	Li-Fraumeni syndrome 1; Not specified; Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.782+14T>G	Likely benign	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.783-1G>A	Pathogenic/Likely pathogenic	Li-Fraumeni syndrome; Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.785delG (p.Gly262Valfs)	Pathogenic	Not provided; Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.789T>C (p.Asn263=)	Likely benign	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.794T>C (p.Leu265Pro)	Pathogenic/Likely pathogenic	Li-Fraumeni syndrome 1; Li-Fraumeni syndrome; Not provided; Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.401T>G (p.Phe134Cys)	Likely pathogenic	Hereditary cancer-predisposing syndrome; Not provided
NM_000546.5(TP53):c.798A>T (p.Gly266=)	Likely benign	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.814G>T (p.Val272Leu)	Likely pathogenic	Medulloblastoma; Multiple myeloma; Squamous cell carcinoma of the head and neck; Li-Fraumeni syndrome; Lung adenocarcinoma; Renal cell carcinoma; papillary; Neoplasm of the breast; Hereditary cancer-predisposing syndrome; Pancreatic adenocarcinoma; Squamous cell carcinoma of the skin; Transitional cell carcinoma of the bladder; Neoplasm of the large intestine; Colorectal neoplasms; Adenocarcinoma of the stomach; Ovarian serous cystadenocarcinoma; Malignant neoplasm of body of the uterus
NM_000546.5(TP53):c.829T>C (p.Cys277Arg)	Likely pathogenic	Not provided
NM_000546.5(TP53):c.869G>A (p.Arg290His)	Uncertain significance	Li-Fraumeni syndrome; Li-Fraumeni syndrome not specified; Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.890A>G (p.His297Arg)	Uncertain significance	Hereditary cancer-predisposing syndrome
NM_000546.5(TP53):c.904G>C (p.Gly302Arg)	Uncertain significance	Li-Fraumeni syndrome
NM_000546.5(TP53):c.907A>G (p.Ser303Gly)	Uncertain significance	Li-Fraumeni syndrome; Hereditary cancer-predisposing syndrome

Discussion

As already stated, OSCC is the most common tumor type of head and neck carcinomas, characterized by a high recurrence rate and poor survival of those patients. While oropharyngeal carcinomas are predominantly HPV-positive, basal-type oral cancers are mostly HPV-negative [21]. HPV-negative oral cancer patients have a significantly reduced OS [17], as opposed to patients with HPV-positive cancer [22]. Further elucidation of the function and regulation of the *TP53*, a pivotal tumor suppressor gene, would provide advances in predicting the clinical behavior, prognosis, and chemotherapy response of HPV-negative oral cancers.

Our findings indicate that HPV-negative OSCC patients with pathogenic *TP53* mutations had a significantly lower survival rate. In the subcohort of patients who underwent cisplatin-based chemotherapy, OS was significantly shorter for those with pathogenic *TP53* mutations than those with WT *TP53*. In contrast, when all *TP53* mutations were taken into account, *TP53* mutation status was not associated with OS. These findings indicate that the OS and the resistance to platinum-based chemotherapy in OSCC could be associated with the type of *TP53* mutation and that pathogenic *TP53* mutations are a significant predictor of poor OS as opposed to benign or likely-benign mutations.

Based on the p53 mechanism of action, as one of the key cell cycle regulators after DNA damage, a number of trials investigate the association between *TP53* mutation and survival, as well as radio and chemotherapy response. Our findings of the high incidence of *TP53* mutations in HPV-negative OSCC are in accordance with previous studies, where *TP53* is mutated in approximately 50% of head and neck squamous cell carcinoma (HNSCC) cases [1]. Mutations in the DNA-binding domain of *TP53* may influence individual responsiveness to chemotherapy via its ability to mediate apoptosis and cell cycle arrest [12]. The most frequent genetic change in our study was *TP53* codon 72 polymorphism, but it was not associated with prognosis or chemotherapy response.

Multiple studies are demonstrating a divergent prognosis based on HPV status in OSCC patients. HPV-negative OSCCs have diverse pathological and clinical features compared to HPV-positive tumors [23]. HPV-negative oral cancers are poorly differentiated tumors, and these patients had worse rates of OS compared to the HPV-positive cancers [24]. HPV-positive HNSCCs are commonly associated with a favorable prognosis in a number of studies [24–27]. HPV-positive head and neck tumors are predominantly driven by HPV infection, as opposed to HPV-negative tumors, which are driven by genetic mutations in *TP53* or other tumor suppressor genes, and are, therefore, characterized as tumors with poorer prognosis [26]. The key transcriptional factors that differentiate HPV-positive and HPV-negative oral cancers are p53, AP-1, NF-kappaB, and STAT3 [23]. In HPV-positive HNSCC, p53 protein is generally WT, but its low levels are attributed to the HPV E6 protein activity, which targets p53 and induces its ubiquitination and degradation [28]. This feature of HPV-positive tumors could lead to greater sensitivity to radiotherapy and radiation-induced apoptosis [29, 30]. Clinical studies have demonstrated that patients with HPV-negative tumors have decreased survival as opposed to HPV-positive OSCC patients [31].

In line with our results, *TP53* mutation status associates with resistance to chemotherapy in HNSCC patients [32–34]. The loss of function due to *TP53* mutation was associated with a low remission rate and suboptimal response to cisplatin-based neoadjuvant chemotherapy in patients with OSCC [34]. Although HPV infection is not a predictor for surgery or the response to radiotherapy of oropharyngeal cancers [35], cisplatin, a standard chemotherapy regimen in head and neck cancers, is more effective in HPV-negative cells [36]. Results of the TAX 324 (WU) trial for locally-advanced oropharyngeal cancer suggested that high-risk OSCC patients are HPV-negative and show elevated expression of βT- II or at least 2 out of 3 of the other adverse markers: GST-π, p53, and low Bcl-2. These patients have significantly decreased survival time compared to moderate-risk HPV-positive patients, who are HPV-negative but do not fulfill other criteria [37]. The commonly recommended treatment regimen for postoperative high-risk OSCC includes the administration of cisplatin in a dosage of 100 mg/m^2. Cisplatin induces DNA damage and those cells should be directed to apoptosis, and p53 proapoptotic pathway is carried out through flice-like inhibitory protein (FLIP), direct binding, and inhibition of the antiapoptotic function of Bcl–xL, enhanced expression of PTEN and AMPK inhibition [38].

Mutated *TP53* was previously associated with shorter OS and poor radio and chemotherapy response, which indicates its potential as a marker for a clinical course in OSCC patients. In the study of locally advanced oral cancer patients, who received cisplatin chemotherapy, patients carrying the high-risk *TP53* mutations had reduced cisplatin sensitivity and a ten times greater risk for residual disease compared to patients with low-risk mutations [39].

Lower response to cisplatin-based chemotherapy in patients with *TP53*-mutated tumors [32] suggested the potential clinical use of p53-based therapeutics in restoring the p53 function. As a result of p53 adenoviral monotherapy or the combination with radio and chemotherapy, tumor regression was observed [40]. OSCC patients carrying *TP53* mutations had a 2.7 times higher risk for cisplatin and 5-fluorouracil (5-FU)-based therapy resistance compared to patients with functional p53 [33]. In addition, a strong connection between nonfunctional p53 and a low response rate to cisplatin-based neoadjuvant chemotherapy was demonstrated in OSCC patients [34]. Another potentially promising approach is treatment with small molecules that reactivate mutated p53, using PRIMA-1 (p53 Reactivation and Induction of Massive Apoptosis) as a single agent and in combination with standard chemotherapy [41]. PRIMA-1 therapy is more active in cell lines containing mutant p53 than WT p53 cells and results in the increased expression of p53-target genes *p21, Bax, Puma,* and *Noxa* [41]. Another p53 reactivating molecule RITA (Reactivation of the p53 and Induction of Tumor cell Apoptosis) induces p53 accumulation and reactivation,

promotes apoptosis via p21, BAX, and caspase-3 upregulation, and induces growth inhibition in OSCC cells *in vitro* and *in vivo* [42].

Conclusion

Our findings indicate that pathogenic *TP53* mutations in HPV-negative OSCC tumors could be a prognostic marker of patients' reduced OS. In addition, HPV-negative OSCC patients with the pathogenic *TP53* mutation who received cisplatin chemotherapy have a significantly lower survival rate, indicating that the pathogenic *TP53* mutations might be a marker of chemotherapy resistance in those patients. Further elucidation of the function and regulation of *TP53* and novel therapeutic approach with small molecules that reactivate mutated *TP53* would significantly advance oral cancer therapy.

Conflict of interest

The authors declare no conflict of interest.

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