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**UTICAJ PATOGENIH TP53 MUTACIJA NA PREZIVLJAVANJE I ODGOVR NA HEMIOTERAPIJU KOD HPV-NEGATIVNIH ORALNIH KARCINOMA**

**Authors** Sasa Jovic1,2, Ruzica Kozomara1,2, Srboljub Stosic1,2, Stevo Jovandic3, Katarina Zeljic4, Gordana Supic2,3*, Vojnosanitetski pregled (2021); Online First June, 2021.

UDC:

DOI: https://doi.org/10.2298/VSP200525068J

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
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UTICAJ PATOGENIH TP53 MUTACIJA NA PREZIVLJAVANJE I ODGOVOR NA HEMIOTERAPIJU KOD HPV-NEGATIVNIH ORALNIH KARCINOMA

Pathogenic TP53 Mutations Influence Chemotherapy Response and Survival Rate of HPV-Negative Oral Squamous Cell Carcinomas

Sasa Jovic1,2, Ruzica Kozomara1,2, Srboljub Stosic1,2, Stevo Jovandic3, Katarina Zeljic4, Gordana Supic2,3*

1Clinic for Maxillofacial Surgery, Military Medical Academy, Belgrade, Serbia
2Medical Faculty of Military Medical Academy, University of Defence, Belgrade, Serbia
3Institute of Medical Research, Military Medical Academy, Belgrade, Serbia
4Faculty of Biology, University of Belgrade, Belgrade, Serbia

*Corresponding author: Military Medical Academy, Crnotravska 17, 11000 Belgrade, Serbia

Funding: Medical Faculty of Military Medical Academy, University of Defense, Serbia [Grant #MFVMA/02/20-22];

Sasa Jovic sasajovic71@icloud.com,
Ruzica Kozomara ruzicakozomara@yahoo.com,
Srboljub Stosic srbastosic@gmail.com,
Stevo Jovandic stevo.jovandic@gmail.com,
Katarina Zeljic katjaze@yahoo.com,
prof. dr Gordana Šupić gogasupic@gmail.com
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 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 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 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 mutation, and 26 patients (31.7%) carried pathogenic TP53 mutations. Patients with pathogenic TP53 mutations had significantly reduced overall survival, p=0.009. Recurrences status, but not TP53 mutations, was an independent marker of poor survival in our cohort, HR=4.733, [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.

Conclusions. 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.

Key words:

Abstract

Uvod/Ciljevi. Oralni planocelularni karcinom (OPCC) je najčešći tip karcinoma glave i vrata, koji se odlikuje visokom stopom recidiva i lošim preživljavanjem. Dalje razjašnjenje uloge i regulacije TP53, ključnog tumor supresorskog gena, omogućilo bi napredak u predviđanju toka, prognoze i hemoterapijskog odgovora pacijenata sa OPCC. Usled toga, istražili smo povezanost mutacija gena TP53 sa preživljavanjem i odgovorom na hemoterapiju cisplatinom kod pacijenata sa HPV-negativnim oralnim karcinomom. Metode. Potencijalni klinički značaj mutacija TP53 analiziran je kod 82 pacijenta sa HPV-negativnim OPCC. Svi pacijenti su podvrgnuti radioterapiji, a 25 pacijenata je primilo hemoterapiju cisplatinom. Negativni HPV
status utvrđen je PCR specifičnim za tip, za visoko rizične HPV 16, 18, 31 i 33. Ciljno sekvenciranje TP53 eksona 4-8 rađeno je Sanger kapilarnim sekvenciranjem. Rezultati. Od 82 HPV-negativnih OPCC pacijenata, 49 (59,79%) je imalo TP53 mutaciju, a 26 pacijenata (31,7%) je imalo patogene TP53 mutacije. Pacijenti 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 u našoj studiji, HR = 4,733, [2,027-11,053], p = 0,0001. U podgrupi pacijenata 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 OPCC tumorima mogle biti prognostički marker smanjenog ukupnog preživljavanja pacijenata. Pored toga, patogene TP53 mutacije u HPV-negativnom OPCC mogu biti marker lošeg odgovora na hemoterapiju.

Ključne reči: 

Introduction

Oral squamous cell carcinoma (OSCC) is the most common tumor type of head and neck carcinomas, characterized by a high recurrence rate and poor survival. This malignancy is the sixth most common cancer worldwide in men and eighth in women in developed countries, and third most common cancer in men and fourth in women in developing countries (1), which affects approximately 600000 new patients every year worldwide. Oral carcinogenesis is a multi-step process, which encompasses an accumulation of genetic and epigenetic changes that lead to the disruption of the various signalling pathways controlling the cell cycle, proliferation, apoptosis, senescence, and DNA (2). Genetic changes are progressively accumulated and 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% 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 evidence of an association of oral cancer and some types of Human Papilloma Virus (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). p53 is a key factor in a signaling pathway that helps the cell to recover from DNA damage (6). Upon a DNA damage, the wild type p53 arrests cell cycle in the G1 phase prohibiting transition to 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, adrenal gland carcinoma (8).

TP53 mutations are early alterations during oral carcinogenesis and more than 25000 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 therefore could 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 wild type p53 can also be achieved throughout human papillomavirus (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 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 the 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.

Materials and Methods
The current study was approved by the Ethics Committee of the Military Medical Academy, according to the Helsinki Declaration (2008). The study group included 82 OSCC patients, Caucasians of the same ethnicity. All patients were diagnosed and subsequently operated at the Clinic for Maxillofacial Surgery, Military Medical Academy, Belgrade, Serbia, between
2015 and 2018. All of them were operated and received radiotherapy (60 Gy in 2 Gy per day dose), and 25 of the patients received cisplatin therapy in a dosage of 100 mg/m² 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 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 PCR was assessed for high-risk HPV types 16, 18, 31, 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/genscreen/) 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) (https://www.ncbi.nlm.nih.gov/clinvar/) and a web server application Simple ClinVar (http://simple-clinvar.broadinstitute.org/) (18).

TP53 mutations were classified as pathogenic, and non-pathogenic mutations, according to ClinVar database, Simple ClinVar (24) 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 $\chi^2$-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 95% confidence interval (95% CI). Variables found significant in the univariate analysis, including those with significance level below 20%, were subsequently analyzed in multivariate Cox’s regression. The Cox model was performed using the forward stepwise method, that 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 patients (59.8%). TP53 mutations were classified as pathogenic and non-pathogenic, as previously suggested (19) (20). The list of detected TP53 mutations and their classification according to clinical significance, assessed by ClinVar database and Simple ClinVar web server, is given in Supplement Table 1. Pathogenic p53 mutations were detected in 26 of 82 (31.7%) of OSCC patients.

The association of TP53 gene mutations, pathogenic TP53 mutations and polymorphism p72 with demographic and clinicopathological features 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, $p=0.009$, Table 1. 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 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, $p=0.017$. 

Table 1. Association of p53 gene mutations and polymorphism p72 with demographic and clinicopathological features of OSCC patients.

| Variables                   | Total N | All TP53 mutation s | 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 (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** |
| **Histol. grade**           |         |         |       |     |     |     |     |     |     |     |     |     |   |   |
| I                           | 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**           |         |         |       |     |     |     |     |     |     |     |     |     |   |   |
| I                           | 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** |
Association of TP53 gene mutations, pathogenic TP53 mutations and polymorphism p72 with overall survival

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 overall survival, 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 the OS between OSCC patients with different genotypes of p72 polymorphism (p=0.905, log-rank test).

Figure 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 and wild type (wt) TP53; B. Comparison of pathogenic TP53 mutations and wt p53.

| 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 |

N total number of patients; E – exon
Significant values, $p <0.05$, are bolded
In the subgroup of 25 patients who received chemotherapy in our cohort, when all p53 mutations were taken into account, p53 mutation status was not associated with chemotherapy response, \( p=0.641 \), Figure 2A. However, overall survival in patients who had received cisplatin chemotherapy was significantly shorter for those with pathogenic p53 mutations compared to patients with wild type 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 (\( p=0.453 \), 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 p53 mutations were resistant to platinum-based chemotherapy, as opposed to the patients with wild type p53.

**Figure 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 and wild type (wt) \( TP53 \); **B.** Comparison of pathogenic \( TP53 \) mutations and wt \( TP53 \).
The Cox Regression analysis demonstrated that the high alcohol intake, stage, tumor size, nodal status and recurrences are highly associated with hazard risk, Table 2. While patients with p53 mutations had increased, but insignificant hazard risk HR=1.747, [0.907-3.366], p=0.096, patients with pathogenic p53 mutations had significantly increased risk of poor survival, HR=2.230, [1.186-4.194], p=0.013. Variables found to be statistically significant, according to the univariate analysis, including the variables with significance level below the 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, [2.027-11.053], p=0.0001), Table 2.

Table 2. Cox Proportional Hazards Regression Analysis, according to overall survival (OS) 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   |
| Hystological 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 | Hazard Ratio | 95% CI       | p       |
|----------------------|-------------|--------------|--------------|---------|
| All TP53 mutations   | 1.747       | [0.907-3.366]| 0.096       |
| Pathogenic TP53mutations | 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 |

HR indicates a hazard ratio; CI, confidence interval.

SNP – Single Nucleotide Polymorphism

Significant values, p <0.05, are bolded

**Discussion**

Oral squamous cell carcinoma is the most common tumor type of head and neck carcinomas, characterized by a high recurrence rate and poor survival. While oropharyngeal carcinomas are predominantly HPV-positive, oral cancers are mostly HPV-negative, basal type (21). HPV-negative oral cancer patients have a significantly reduced overall survival (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 p53 mutations had a significantly lower survival rate. In the subcohort of patients who underwent cisplatin-based chemotherapy, overall survival was significantly shorter for those with pathogenic p53 mutations than those with wild type TP53. In contrast, when all TP53 mutations were taken into account, TP53 mutation status was not associated with overall survival. These findings indicate that the overall survival 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 overall survival 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 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. The multiple studies are demonstrating a divergent prognosis based on HPV status in OSCC patients. 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 therefore are characterized as tumors with poorer prognosis (26). 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 overall survival compared to the HPV-positive cancers (24). HPV-positive HNSCCs are commonly associated with a favorable prognosis in a number of studies (24) (25) (26) (27). 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 wild type, 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 the greater sensitivity to radiotherapy, and radiation-induced apoptosis (29) (30). Clinical studies have demonstrated that HPV-negative tumors have decreased survival as opposed to HPV-positive OSCC (31).

In line with our results, p53 mutation status associates with resistance to chemotherapy in HNSCC (32) (33) (34). The loss of function due to p53 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 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, which are or HPV-negative but do not fulfill other criteria (37). The commonly recommended treatment regimen for postoperative high-risk OSCC include administration of cisplatin in a dosage of 100 mg/m2. 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 overall survival 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, which received cisplatin chemotherapy, patients carrying the high-risk p53 mutations had reduced cisplatin sensitivity and 10 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 mono-therapy or in 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-Fu based therapy resistance, compared to patients with functional p53 (33). In addition, a strong connection between nonfunctional p53 and 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 wild type 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).

In 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, 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.

Acknowledgements:
Funding: Medical Faculty of Military Medical Academy, University of Defense, Serbia [Grant #MFVMA/02/20-22].

Conflict of interest: None to declare.

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**Supplement Table 1.** Data on TP53 mutations, assessed by ClinVar database of the NCBI ([https://www.ncbi.nlm.nih.gov/clinvar/](https://www.ncbi.nlm.nih.gov/clinvar/)) and a web server application Simple ClinVar ([http://simple-clinvar.broadinstitute.org/](http://simple-clinvar.broadinstitute.org/)).

| 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 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 | Likely benign | Hereditary cancer-predisposing syndrome |
| Gene       | Chromosome/Exon | Mutation Type | Phenotype | Hereditary Cancer-Predisposing Syndrome |
|------------|-----------------|---------------|-----------|----------------------------------------|
| NM_000546.5(TP53):c.389T>C | (NM_000546.5(TP53):c.389T>C) | pm128= | Likely pathogenic | Hereditary cancer-predisposing syndrome |
| NM_000546.5(TP53):c.401T>G | (NM_000546.5(TP53):c.401T>G) | Leu130Pro | Likely pathogenic | Hereditary cancer-predisposing syndrome |
| NM_000546.5(TP53):c.411G>C | (NM_000546.5(TP53):c.411G>C) | Leu137= | Likely benign | Hereditary cancer-predisposing syndrome |
| NM_000546.5(TP53):c.421T>A | (NM_000546.5(TP53):c.421T>A) | 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, 1|Neoplasm of brain|Neoplasm of the breast|Pancreatic adenocarcinoma|Neoplasm of the large intestine|Colorectal Neoplasms|Malignant neoplasm of body of uterus|Adenocarcinoma of prostate |
| NM_000546.5(TP53):c.428T>G | (NM_000546.5(TP53):c.428T>G) | Val143Gly | Uncertain significance | Hereditary cancer-predisposing syndrome |
| NM_000546.5(TP53):c.517G>T | (NM_000546.5(TP53):c.517G>T) | 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 brain|Neoplasm of the breast|Hepatocellular carcinoma|Pancreatic adenocarcinoma|Brainstem glioma|Neoplasm of the large intestine|Carcinoma of esophagus|Colorectal Neoplasms|Adrenocortical carcinoma|Adenocarcinoma of stomach|Ovarian Serous Cystadenocarcinoma|Malignant neoplasm of body of uterus |
| NM_000546.5(TP53):c.560-15A>C | (NM_000546.5(TP53):c.560-15A>C) | | Likely benign | Hereditary cancer-predisposing syndrome |
| NM_000546.5(TP53):c.578A>G | (NM_000546.5(TP53):c.578A>G) | | Likely pathogenic | Liver cancer|Chronic lymphocytic leukemia|Squamous cell carcinoma of the
| Accession | Mutation | Effect | Tumor Type |
|-----------|----------|--------|------------|
| 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 |
| Gene | Mutation | Pathogenicity | Associated Conditions |
|------|----------|--------------|-----------------------|
| 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 esophagus, Colorectal Neoplasms, Adenocarcinoma of stomach, Ovarian Serous Cystadenocarcinoma, Adenocarcinoma of prostate, Li-Fraumeni syndrome |
| 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 brain, Neoplasm of the breast, Pancreatic adenocarcinoma, Brainstem glioma, Neoplasm of the large intestine, Carcinoma of esophagus, Colorectal Neoplasms, Adenocarcinoma of stomach, Ovarian Serous Cystadenocarcinoma, Malignant neoplasm of body of 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 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 esophagus, Colorectal Neoplasms, Uterine cervical neoplasms, Adenocarcinoma of 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 | Likely pathogenic | not provided |
| Variant                           | Pathogenicity          | Associated Conditions                                                                 |
|----------------------------------|------------------------|---------------------------------------------------------------------------------------|
| p.Cys242Arg                      |                        |                                                                                       |
| 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 brain|Glioblastoma|Hepatocellular carcinoma|Hereditary cancer-predisposing syndrome|Neoplasm of the large intestine|Carcinoma of esophagus|Colorectal Neoplasms|Adenocarcinoma of 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 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 esophagus|Colorectal Neoplasms|Adenocarcinoma of stomach|Ovarian Serous Cystadenocarcinoma|Adenocarcinoma of prostate|Uterine Carcinosarcoma |
| NM_000546.5(TP53):c.737T>G (p.Met246Arg) | Pathogenic | not provided                                                                 |
| NM_000546.5(TP53):c.770T>A | Uncertain | Li-Fraumeni-like syndrome|Li-Fraumeni syndrome |
| Genomic Position | Significance       | Conditions                                                                 |
|------------------|--------------------|----------------------------------------------------------------------------|
| (p.Leu257Gln)    |                   |                                                                             |
| 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   | Pathogenic         | not provided|Hereditary cancer-predisposing syndrome                                      |
| (p.Gly262Valfs)  |                   |                                                                             |
| 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 1|Lung adenocarcinoma|Renal cell carcinoma, papillary, 1|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 stomach|Ovarian Serous Cystadenocarcinoma|Malignant neoplasm of body of uterus |
| Genomic Location                                      | Allele Description | Classification       | Clinical Significance                                    |
|-------------------------------------------------------|--------------------|----------------------|----------------------------------------------------------|
| 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                   |

Received on May 25, 2020.
Accepted June 15, 2021.
Online First June, 2021.