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# IGSF3 tissue expression in squamous cell carcinoma of the oropharynx: a novel tool for prognosis assessment in HPV-related and HPV-unrelated disease

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Biomarkers are not broadly used in the management of head and neck cancers (HNCs). Biomarkers have been beneficial in the management of other cancers, however, not in HNCs. Therefore, we observed the immunopositivity of a novel biomarker called immunoglobulin superfamily member 3 (IGSF3) in tumor tissues in HPV-related and HPV-unrelated OPSCC. Two patient cohorts (C1 and C2) from separate time periods were available for this study (total $N = 282$). Both consisted of OPSCC patients treated at the Helsinki University Hospital (HUS, Helsinki, Finland) during 2000–2016. For HPV determination, HPV mRNA *in situ* hybridization was used. Immunohistochemistry was used to assess IGSF3 immunopositivity in cancer tissues. Overall survival (OS) was used as endpoint in the statistical analysis. In C1, stronger immunopositivity of IGSF3 in tumor-infiltrating lymphocytes (TILs) correlated with favorable OS ($p = 0.005$). Stronger IGSF3 immunopositivity in tumor cells (TCs) was associated with HPV negativity ($p = 0.017$). Stronger IGSF3 immunopositivity in TILs correlated with HPV positivity ($p < 0.001$). Elevated IGSF3 immunopositivity in TILs associates with HPV-related tumors and may signify favorable prognosis. The immunopositivity of IGSF3 differs between HPV-related and HPV-unrelated OPSCC.

Key words: OPSCC; HPV; biomarker; immunohistochemistry; prognosis.

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## BACKGROUND

Head and neck squamous cell carcinomas (HNSCCs) form a challenging set of diseases. Diagnostics can be difficult, and selecting treatment options requires multidisciplinary aspects taken into consideration [1]. Furthermore, despite successful treatment, HNSCC patients generally suffer from considerable decrease in the posttreatment quality of life [2], which often may be long-lasting [3]. Novel methods are urgently warranted to facilitate early diagnostics, to develop more efficient treatments without increasing the treatment burden, and to improve survival outcomes. Oropharyngeal squamous cell carcinoma (OPSCC) is

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a HNSCC subtype categorized into two distinct disease forms: human papillomavirus (HPV)-related and HPV-unrelated disease. Alarmingly, the incidence of HPV-related OPSCC has been rising over the recent decades [4], whereas the prognosis of HPV-unrelated OPSCC remains adverse despite the introduction of targeted treatment methods such as immunotherapy [5]. It has been estimated that more than 90,000 new OPSCC cases are diagnosed globally each year, while the mortality rate approaches 50,000 deaths per year [6].

As there are challenges involving both forms of OPSCC, and pathogenesis and treatment responses between the subtypes differ significantly, further research involving survival monitoring and HPV status differentiations is desirable. Potential lies in advancing individualized treatment methods that have previously been beneficial in OPSCC [7, 8]. As biomarkers can offer vital information about cancer pathogenesis, and this information may be used as a background in developing individualized treatments [9], biomarkers may be a promising tool in the management of OPSCC. Incorporating biomarkers in diagnostics and treatment prognostication has previously been utilized in other malignancies [10]. Notably, as the role and functions of the tumor-micro-environment (TME) has become a prominent topic in cancer research, it is possible to observe the aspects of TME in the disease with biomarker studies as well [11, 12]. Preferably, we anticipate that biomarker studies could facilitate patient selection for novel targeted treatments such as immunotherapy, as has been suggested previously [9]. Such biomarkers remain rare in the current management of HNSCCs, including OPSCCs.

The immunoglobulin superfamily (IGSF) is a vast, heterogenic set of transmembrane proteins [13], mainly involved in cell-adhesions [14]. IGSF3 is a member of IGSF, and its mRNA has earlier been shown to be expressed in several organs of the human body [15]. Although the functions of IGSF3 are not yet entirely resolved, it has been shown to be involved in neuronal morphogenesis and hyperexcitability [14, 16]. As it is a relatively novel agent in the field of oncology, only scarce data are available. However, IGSF3 has previously been linked to liver cancer [17]. Additionally, it has been shown that IGSF3 interacts with another membrane protein, tetraspanin 7 (Tspan7) [14], which has been associated with other malignancies [18]. To our knowledge, the current study is the first to estimate IGSF3 immunopositivity in HPV-related and HPV-unrelated OPSCC. Our objective was to document the immunopositivity of IGSF3 in tumor tissue and in the TME and to compare the findings to the clinical characteristics and survival data of OPSCC patients.

## MATERIALS AND METHODS

### Study population

Two existing databases of separate OPSCC patient cohorts were available, and these data have been used in previous studies [19–21]. The cohorts were analyzed separately due the difference of the used staging system, as described below. The inclusion criteria for our analysis were available, TMA slides for immunohistochemistry, and previously determined HPV status. All patients had been treated with curative intent.

The patient data were collected manually from the electronic patient records of the Helsinki University Hospital (HUS, Helsinki, Finland) and were updated during the study for assessment of prognosis. The clinical characteristics included in the current analysis were mean age at diagnosis, gender, cigarette smoking (never, previous, current), heavy use of alcohol (never, previous, current), TNM class (seventh and eighth edition of American Joint Committee on Cancer staging), stage, grade of differentiation, and tumor location.

The study design received an institutional research permission (§76/2021) and Research Ethics Board approval (Dnr: 51/13/03/02/2013) at the HUS.

#### Cohort I

Cohort I (C1) consisted of 192 patients with a newly diagnosed OPSCC during 2000–2009 at the HUS (Helsinki, Finland). The dates and causes of death were provided by the Statistics Finland. Treatment modalities were as follows: primary surgery with or without adjuvant (chemo) radiotherapy or definitive (chemo)radiotherapy. Follow-up time was a minimum of three ears or until death. The seventh edition of the American Joint Committee on Cancer staging manual was used for the TNM classification of the cases in this cohort [22]. The tumor location in C1 in this data was described as anterior wall (=base of tongue)/lateral walls (=tonsils)/posterior wall (= posterior wall of oropharynx)/superior wall (=soft palate).

#### Cohort II

Cohort II (C2) comprised of 90 patients with newly diagnosed OPSCC in 2012–2016 at the HUS (Helsinki, Finland). Treatment modalities included primary surgery with or without adjuvant (chemo)radiotherapy or definitive (chemo)radiotherapy. Follow-up time was a minimum of 3 years or until death. TNM class in this cohort was documented according to the eighth edition of the American Joint Committee on Cancer staging manual [23]. The tissue material in C2 was scarce due the earlier analyses resulting to exclusion of several patients, and hence, the sample size was smaller compared to C1.

### Immunohistochemical analysis

#### Tissue microarrays

Tissue microarray (TMA) slides had been prepared in advance with assistance from a digitalized software by

 

Auria Biobank (Turku, Finland). Representative areas were first elected from hematoxylin and eosin-stained primary tumors. Six 1-mm-diameter core samples were then extracted with a semiautomatic tissue microarrayer (Beecher Instruments, Silver Spring, MD, USA), and the cores were subsequently planted in a separate paraffin block. The process is additionally described in earlier work [19].

### HPV status determination

HPV status was defined from tumor tissue slides in advance by mRNA ISH as described in earlier work [24]. ISH to determine high-risk HPV E6/E7 mRNA from the samples was performed with the RNAscope® 2.5 HD Reagent kit (Advanced Cell Diagnostics, Inc., Hayward, CA) for high-risk genotypes 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82. As a positive control, endogenous housekeeping gene HS-PPIB (RNAscope®) probe was used. A bacterial gene DapB, diaminopimelate (RNAscope®) probe was used as a negative control. Additionally, immunohistochemistry for p16 INK4a was performed for tumor tissues. As HPV mRNA ISH provides indication of active transcription of the virus, it is considered ideal for HPV determination [25].

### IGSF3 immunohistochemistry

TMA slides were analyzed for IGSF3 immunostaining. Deparaffinization and rehydration of the samples were performed with Sakura Tissue-Tek DRS (Sakura FineTek Europe B.V., Alphen aan den Rijn, Netherlands). The heat-induced epitome retrieval and endogenous peroxidase blocking were done with the Agilent Dako Pretreatment Module (Dako Denmark Aps, Glostrup, Denmark). As a primary antibody, we used the polyclonal Anti-IGSF3 antibody produced in rabbit (Prestige Antibodies by Atlas Antibodies, Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany). As a secondary antibody, EnVision Flex/HRP SM802 DM827 (Dako) was used. As the chromogen, we used EnVision Flex DAB (Dako). Counterstaining with hematoxylin was achieved with Autostainer 480 (Thermo Fisher Scientific, Vantaa, Finland). Dehydration and mounting of the samples were performed with Pertex Histolab Mounting Medium (Histolab Products Oy, Gothenburg, Sweden). A positive control of placental tissue was used.

### Sample immunoscoring

The TMA slides were separately immunoscored for IGSF3 immunopositivity by two researchers (JH and AS). A mutual consensus was subsequently achieved at the incidence of discrepancies. To assess IGSF3 immunopositivity in tumor, the immunopositivity was scored in (OPSCC) tumor cells (TCs). IGSF3 immunopositivity was additionally scored in the tumor-infiltrating lymphocytes (TILs) which in our study represented the TME. The IGSF3 immunostaining in TCs and TILs is specified in Fig. 1, where the differences between morphology and size of the nuclei can be observed. The immunopositivity was graded by intensity of immunostaining with a scale from 0 to 3, where 0 = negative, 1 = mild positivity, 2 = moderate positivity, and 3 = strong positivity. The staining was mainly cytoplasmic, with occasional membranous staining in samples with strong positivity. In the final analysis, representative TILs were not found with one participant's samples, resulting in minor variation of *n* among C2. The grading of IGSF3 immunostaining is exemplified in Fig. 2.

### Data analysis

The statistical analyses were performed by three researchers separately (AS, LJ, and TC), and the results were later cross compared for validation. All statistics were calculated with IBM SPSS versions 27 and 28. To compare IGSF3 immunopositivity to clinical characteristics, we used the chi-squared test, independent samples *t*-test, and Mann–Whitney's test, depending on the variable distribution. For the survival analysis, the log-rank test was used. Endpoint was overall survival (OS) (defined as the time from the end of treatment to the end of the follow-up and/or death of any cause). Survival was illustrated by Kaplan–Meier curves constructed with GraphPad Prism.

To add focus on the differences of HPV-related and HPV-unrelated OPSCC, we performed all the analyses within C1 and C2 first among the entire cohorts, followed by calculations within subgroups according to the HPV status.

## RESULTS

### C1

#### Clinical characteristics

Among the C1, we observed negative/weak IGSF3 immunopositivity in TCs in 101 samples (52.6%) and moderate/strong immunopositivity in 91 samples (47.4%). Negative/weak immunopositivity of IGSF3 in TILs was seen in 104 samples (54.2%), and moderate/strong immunopositivity was found in 88 samples (45.8%). Majority of the patients (57.3%) were HPV positive. Stronger IGSF3 immunopositivity in TCs was more common (p = 0.017)

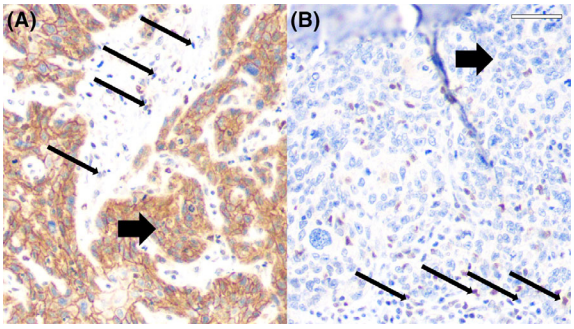


**Fig. 1.** (A) Positive immunostaining in TCs (thick arrow) and negative staining in TILs (multiple thin arrows) and (B) Negative immunostaining in TCs (thick arrow) and (mild) positive staining in TILs (multiple thin arrows). Magnification x200, scale bar length 150 μm. TCs: tumor cells; TILs: tumor-infiltrating lymphocytes.

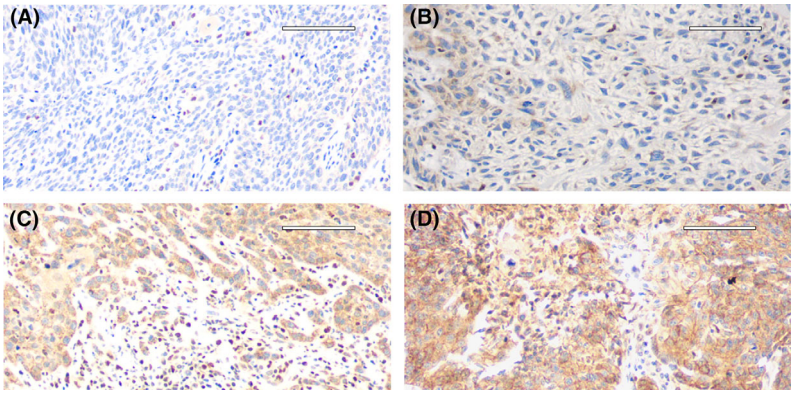


**Fig. 2.** (A) Negative, (B) Weak, (C) Moderate, and (D) Strong IGSF3 immunostaining in OPSCC TMA slides. Magnification x200, scale bar length 150 μm.

in HPV-negative samples (57.3%). In contrast, we found a significant association ($p < 0.001$) between stronger IGSF3 immunopositivity in TILs and HPV positivity.

In TCs, moderate/strong immunopositivity of IGSF3 was further linked to N0 class, whereas negative/weak immunopositivity associated with NX class ($p = 0.018$). Additionally, weaker immunopositivity of IGSF3 in TCs correlated with higher stage ($p = 0.006$), higher grade ($p = 0.009$), and with lateral wall of oropharynx as a tumor location ($p = 0.001$). IGSF3 immunopositivity in TCs did not correlate with immunopositivity in TILs.

In TILs, stronger IGSF3 immunopositivity was more common with female gender ($p = 0.008$) and stronger immunopositivity was further associated with lateral wall of oropharynx as tumor location ($p = 0.018$) and higher grade ($p = 0.012$). Weaker IGSF3 immunopositivity in TILs correlated with current smoking habit ($p = 0.036$).

Among the HPV-positive subgroup of C1 ($n = 110$), heavy alcohol use correlated with stronger IGSF3 immunopositivity in TCs ($p = 0.022$). Among the HPV-negative subgroup of C1 ($n = 82$), stronger IGSF3 immunopositivity in TCs was associated with N0 class ($p = 0.027$), lower grade of differentiation ($p = 0.026$), and tumor location ($p = 0.011$). Additionally, weaker immunopositivity of IGSF3 in TILs (among the HPV-negative subgroup) was associated with higher T class ($p = 0.038$) and tumor site ($p = 0.002$).

The results among C1 are presented in detail in Table 1. The results among the HPV subgroups are presented in Tables S1 and S2.

### Survival

The median follow-up time was 53.4 months (range 0–60 months). We detected significant correlation between stronger immunopositivity of IGSF3 in TILs and favorable OS ($p = 0.005$) among the entire cohort. Stronger TIL immunopositivity of IGSF3 additionally associated with favorable OS in HPV-positive and HPV-negative patients, but the result was not statistically significant. The survival curves within C1 are illustrated in Figs. 3 and 4.

## C2

### Clinical characteristics

In C2, we saw moderate/strong immunopositivity of IGSF3 in TCs in 48 samples (53.3%) and in TILs in 42 samples (47.2%). Majority of the patients (76.6%) were HPV positive. Among the entire cohort, stronger IGSF3 immunopositivity in TILs was more common in HPV-positive samples (51.5%), but the result was not statistically significant ($p = 0.146$). Correspondingly, stronger IGSF3 immunopositivity in TCs was more in common in HPV-negative samples (61.9%), but again, the result was not statistically significant ($p = 0.369$). No further significant associations arose from the analyses performed among the entire C2 cohort regarding IGSF3 immunopositivity in TCs or in TILs. Further, we saw no correlation between IGSF3 immunopositivity in TCs and immunopositivity in TILs.

Among the HPV-positive subgroup of C2 ($n = 69$), negative/weak IGSF3 immunopositivity in TCs was more common with patients (55.5%) who had never been heavy users of alcohol ($p = 0.042$). No significant associations were seen among the HPV-negative subgroup ($n = 21$).

The results among C2 are presented in detail in Table 2. The results among the HPV subgroups are presented in Tables S3 and S4.

 

**Table 1.** Clinicopathological characteristics and IGSF3 immunopositivity among all patients of Cohort I

| Immunostaining | IGSF3 in TCs 0–1 (%) | IGSF3 in TCs 2–3 (%) | p-value | Missing (%) | IGSF3 in TILs 0–1 (%) | IGSF3 in TILs 2–3 (%) | p-value | Missing (%) |
|---|---|---|---|---|---|---|---|---|
| Number of patients | 101 (52.6) | 91 (47.4) | | | 104 (54.2) | 88 (45.8) | | |
| Mean age at diagnosis | 57.2 | 59.5 | 0.117 | | 59.2 | 57.3 | 0.187 | |
| Gender | | | 0.122 | | | | **0.008** | |
| Male | 70 (49.3) | 72 (50.7) | | | 85 (60.0) | 57 (40.0) | | |
| Female | 31 (62.0) | 19 (38.0) | | | 19 (38.0) | 31 (62.0) | | |
| Cigarette smoking | | | 0.324 | | | | **0.036** | |
| Never | 15 (57.7) | 11 (42.3) | | | 12 (46.2) | 14 (53.8) | | |
| Previous | 20 (44.4) | 25 (55.6) | | | 20 (44.4) | 25 (55.6) | | |
| Current | 41 (44.6) | 51 (55.4) | | 29 (15.1) | 60 (65.2) | 32 (34.8) | | 29 (15.1) |
| Heavy use of alcohol | | | 0.398 | | | | 0.133 | |
| Never | 31 (52.5) | 28 (47.5) | | | 31 (44.9) | 28 (55.1) | | |
| Previous | 12 (52.2) | 11 (47.8) | | | 12 (52.2) | 11 (47.8) | | |
| Current | 14 (38.9) | 22 (61.1) | | 74 (38.5) | 26 (72.2) | 10 (27.8) | | 74 (38.5) |
| T class | | | 0.836 | | | | 0.568 | |
| T1–T2 | 57 (53.3) | 50 (46.7) | | | 56 (53.8) | 48 (46.2) | | |
| T3–T4 | 44 (51.8) | 41 (48.2) | | | 51 (58.0) | 37 (42.0) | | |
| N class | | | **0.018** | | | | 0.277 | |
| N0 | 13 (35.1) | 24 (64.9) | | | 23 (62.1) | 14 (37.9) | | |
| NX | 88 (56.8) | 67 (43.2) | | | 81 (52.2) | 74 (47.8) | | |
| Stage | | | **0.006** | | | | 0.452 | |
| I–II | 8 (7.1) | 20 (92.9) | | | 17 (60.7) | 11 (39.3) | | |
| III–IV | 93 (56.7) | 71 (43.3) | | | 87 (53.0) | 77 (47.0) | | |
| Grade of differentiation | | | **0.009** | | | | **0.012** | |
| I | 5 (33.3) | 10 (66.7) | | | 9 (60.0) | 6 (40.0) | | |
| II | 33 (49.3) | 44 (50.7) | | | 51 (66.2) | 26 (33.8) | | |
| III | 63 (63.0) | 37 (37.0) | | | 44 (44.0) | 56 (56.0) | | |
| Tumor origin | | | **0.002** | | | | **0.018** | |
| Anterior wall | 30 (52.6) | 27 (47.4) | | | 39 (68.4) | 18 (31.6) | | |
| Lateral wall | 68 (60.0) | 46 (40.0) | | | 54 (47.4) | 60 (52.6) | | |
| Superior wall | 2 (11.1) | 16 (88.9) | | | 8 (44.4) | 10 (55.6) | | |
| Posterior wall | 1 (33.3) | 2 (66.7) | | | 3 (100.0) | 0 | | |
| HPV status | | | **0.017** | | | | **<0.001** | |
| HPV+ | 66 (60.0) | 44 (40.0) | | | 45 (40.9) | 65 (59.1) | | |
| HPV− | 35 (42.7) | 47 (57.3) | | | 59 (82.0) | 23 (28.0) | | |

*Note*: 0–1: negative-weak positivity, 2–3: moderate–strong positivity. p-values <0.05 are bolded.
Abbreviations: HPV, Human papillomavirus; TCs, Tumor cells; TILs, Tumor-infiltrating lymphocytes.

Survival

The median follow-up time was 44.0 months (range 0–60 months). No significant association between OS and IGSF3 immunopositivity was found in C2; however, we detected a weak correlation between stronger IGSF3 immunopositivity in TCs and poor OS among HPV-positive patients ($p = 0.096$). The survival curves within C2 are illustrated in Figs. 5 and 6.

## DISCUSSION

According to the results of our study, immunopositivity of IGSF3 in tumor tissues associated with survival of OPSCC patients. More specifically, stronger immunopositivity in TILs was associated with favorable prognosis. Furthermore, stronger IGSF3 immunopositivity in TCs was observed to be more common in HPV-unrelated OPSCC, whereas immunopositivity in TILs was linked with HPV positivity in OPSCC. Our findings of IGSF3 in HPV-related and HPV-unrelated OPSCC are novel and they provide valuable information to supplement the knowledge on TME in OPSCC, as well as the survival assessment. Notable strengths in our research include long follow-up time, sufficient study population, and comprehensive compilation of patient history and disease characteristics. The limitations of our study include the scarce tissue material in C2. Furthermore, based on our analysis it is not possible to determine the functions and mechanism of expression of IGSF3, and further translational studies on cell level are required.

Even though diverse findings can be observed separately between the two cohorts (C1 and C2), in closer inspection few of the results were in line while comparing the cohorts to each other. In both

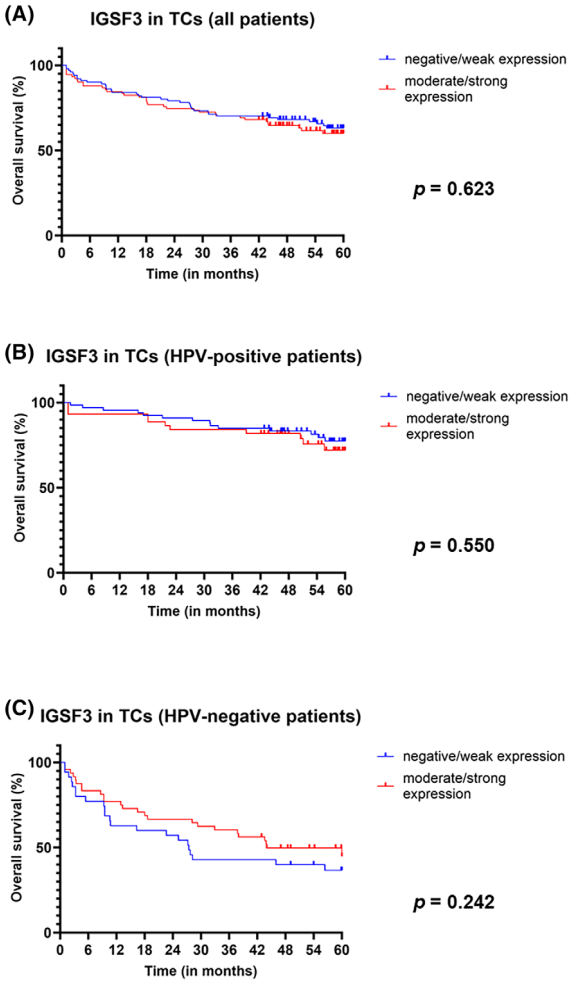


**Fig. 3.** Overall survival (OS) according to IGSF3 immunopositivity in tumor cells (TCs) in cohort I (C1).

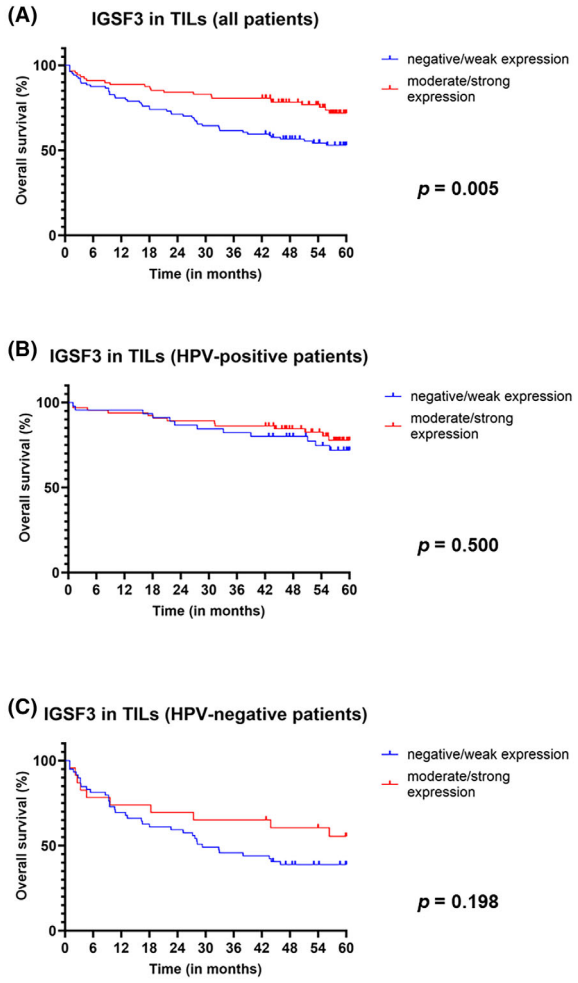


**Fig. 4.** OS according to IGSF3 immunopositivity in tumor-infiltrating lymphocytes (TILs) in C1.

cohorts, majority of the patients were HPV positive. Remarkably, the rising incidence of HPV infections in Western countries [4] can be seen in our study population, as in the more recently collected C2 the number of HPV-positive patients was larger in relation to C1. Among the HPV-positive subgroups of both C1 and C2, IGSF3 immunopositivity in TCs was associated with heavy alcohol use, although the available information about the individual use of alcohol was occasionally limited, and thus, as a variable it can be unreliable. Interestingly, the findings among the HPV-negative subgroup of C1 aligned with the results of the entire cohort regarding the significant association between stronger IGSF3 immunopositivity in TCs and N class, as well as grade of differentiation. In contrast, in C2 the marker immunopositivity was mainly divided evenly within the clinical characteristics, and no statistically significant causalities arise from the analysis. This is likely by the effect of the limited tissue material of the cohort C2.

The clinicopathological differences of HPV-related and HPV-unrelated OPSCC previously presented within our work [21] are accented in the current analysis, and these findings have been supported by previous literature [4, 5, 26]. Interestingly, as the significance of the grading of HPV-related OPSCC tumors has decreased in the past decade [23], no correlation was predictably seen between IGSF3 immunopositivity and grade among HPV-positive patients in our analysis, whereas the correlation among HPV-negative subgroup was significant. Furthermore, the association between lower N class and stronger IGSF3 immunopositivity in TCs among HPV-negative patients could be related to the lower tendency of

 

**Table 2.** Clinicopathological characteristics and IGSF3 immunopositivity among all patients of Cohort II

| Immunostaining | IGSF3 in TCs 0–1 (%) | IGSF3 in TCs 2–3 (%) | p-value | Missing (%) | IGSF3 in TILs 0–1 (%) | IGSF3 in TILs 2–3 (%) | p-value | Missing (%) |
|---|---|---|---|---|---|---|---|---|
| Number of patients | 42 (45.7) | 48 (53.3) | | | 47 (52.8) | 42 (47.2) | | |
| Mean age at diagnosis | 61.2 | 61.1 | 0.986 | | 60.8 | 61.6 | 0.680 | |
| Gender | | | 0.175 | | | | 0.464 | |
| Male | 30 (42.9) | 40 (57.1) | | | 35 (50.7) | 34 (49.3) | | |
| Female | 12 (60.0) | 8 (40.0) | | | 12 (60.0) | 8 (40.0) | | |
| Cigarette smoking | | | 0.439 | | | | 0.674 | |
| Never | 11 (47.8) | 12 (52.2) | | | 13 (56.5) | 10 (43.5) | | |
| Previous | 16 (55.2) | 13 (44.8) | | | 16 (57.1) | 12 (42.9) | | |
| Current | 15 (39.5) | 23 (60.5) | | | 18 (47.7) | 20 (52.6) | | |
| Heavy use of alcohol | | | 0.071 | | | | 0.133 | |
| Never | 23 (53.5) | 20 (46.5) | | | 21 (50.0) | 21 (50.0) | | |
| Previous | 4 (36.4) | 7 (63.6) | | | 9 (81.8) | 2 (18.3) | | |
| Current | 5 (23.8) | 16 (76.2) | | 15 (16.7) | 10 (47.6) | 11 (52.4) | | 15 (16.9) |
| T class | | | 0.208 | | | | 0.570 | |
| T1–T2 | 33 (50.8) | 32 (32) | | | 35 (54.7) | 29 (45.3) | | |
| T3–T4 | 9 (36.0) | 16 (64.0) | | | 12 (48.0) | 13 (52.0) | | |
| N class | | | 1.000 | | | | 0.081 | |
| N0 | 7 (46.7) | 8 (53.3) | | | 11 (73.3) | 4 (26.7) | | |
| NX | 35 (46.7) | 40 (53.3) | | | 36 (48.6) | 38 (51.4) | | |
| Stage | | | 0.126 | | | | 0.876 | |
| I–II | 34 (51.5) | 32 (48.5) | | | 34 (52.3) | 31 (47.7) | | |
| III–IV | 8 (33.3) | 16 (66.7) | | | 13 (54.2) | 11 (45.8) | | |
| Grade of differentiation | | | 0.765 | | | | 0.363 | |
| I | 0 | 0 | | | 0 | 0 | | |
| II | 5 (41.7) | 7 (58.3) | | | 8 (66.7) | 4 (33.3) | | |
| III | 37 (47.4) | 41 (52.6) | | | 39 (50.6) | 38 (49.4) | | |
| Tumor origin | | | 0.525 | | | | 0.679 | |
| Tonsil | 31 (51.7) | 29 (48.3) | | | 31 (51.7) | 29 (48.3) | | |
| Base of tongue | 7 (38.9) | 11 (61.1) | | | 11 (64.7) | 6 (35.3) | | |
| Soft palate | 3 (30.0) | 7 (70.0) | | | 4 (40.0) | 6 (60.0) | | |
| Posterior wall of oropharynx | 1 (50.0) | 1 (50.0) | | | 1 (50.0) | 1 (50.0) | | |
| HPV status | | | 0.369 | | | | 0.146 | |
| HPV+ | 34 (49.3) | 35 (50.7) | | | 33 (48.5) | 35 (51.5) | | |
| HPV− | 8 (38.1) | 13 (61.9) | | | 14 (66.7) | 7 (33.3) | | |

*Note*: 0–1: negative-weak positivity, 2–3: moderate–strong positivity. p-values <0.05 are bolded.
Abbreviations: HPV, Human papillomavirus; TCs, Tumor cells; TILs, Tumor-infiltrating lymphocytes.

HPV-unrelated OPSCC to form lymph node metastases compared to HPV-related disease [26]. Indeed, in both cohorts, stronger IGSF3 immunopositivity in TCs was more common among HPV-negative patients, whereas stronger IGSF3 immunopositivity in TILs was more common among HPV-positive patients. Based on our findings, we speculate that HPV has influence on the IGSF3 expression. This phenomenon requires further studies to be explained.

Upregulated IGF3 expression has previously been associated with tumorigenic potential in hepatocellular carcinoma [17], and it has been assessed as a possible biomarker in lung cancer, liver cancer, and pancreatic cancer with inconclusive results [27, 28]. IGSF3 expression has been further observed as a factor with adverse impact in drug-resistance studies concerning lung cancer [29] and metastatic osteosarcoma [30]. These results are partly in line with ours, as IGSF3 immunopositivity was seen more often in HPV-negative tumors, which tend to be more aggressive [26]. Additionally, IGSF3 has been noted in diverse studies regarding brain cancer [16], ovarian cancer [31], rectal cancer [32], and melanoma [33].

IGSF3 has known functions that may offer explanations for differentiated expression and adverse prognosis in OPSCC. For example, IGSF3 is known to interact with tetraspanin proteins, which have been associated with several malignancies and have various tumorigenic attributes [34]. Another explanation may have been provided by Curry *et al.*, as in their study IGSF3 was found to facilitate tumor-progression in glioma by enhancing cancer cell proliferation and dissemination [16] and furthermore, to activate the NF-κB signaling

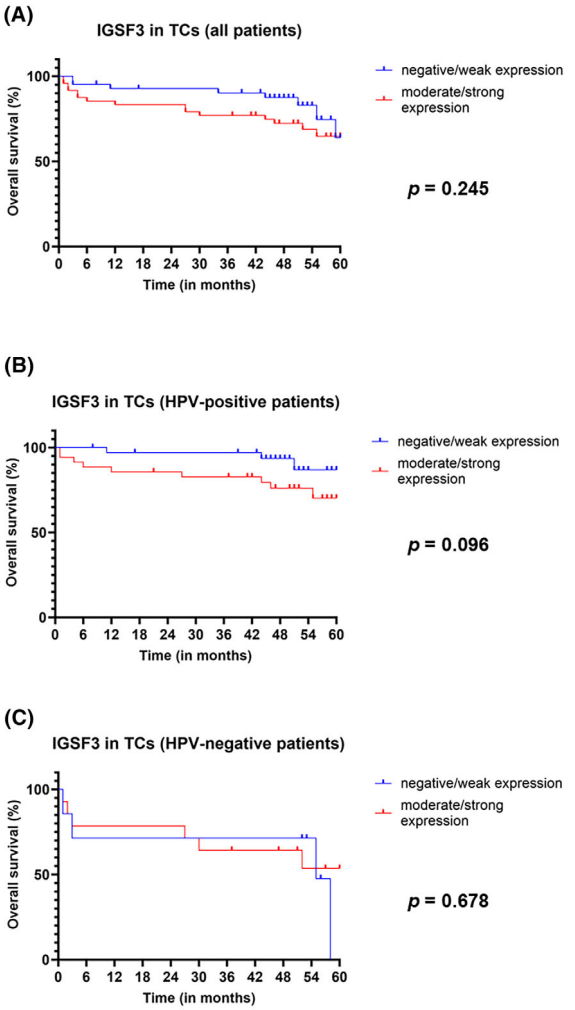


**Fig. 5.** OS according to IGSF3 immunopositivity in TCs in cohort II (C2).

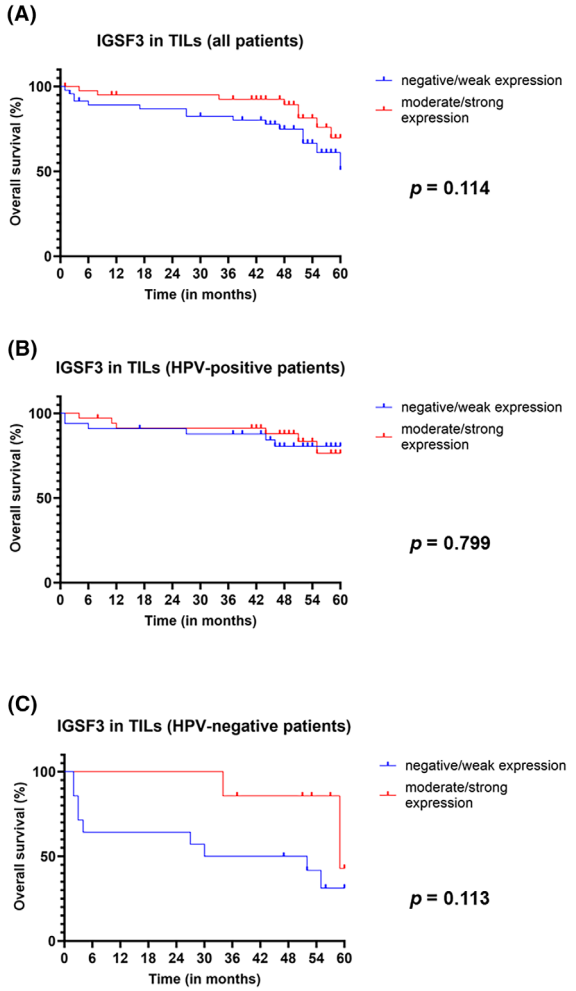


**Fig. 6.** OS according to IGSF3 immunopositivity in TILs in C2.

pathway, thus promoting tumor growth, invasion, and cell migration in hepatocellular carcinoma [17]. It is compelling to presume that corresponding phenomena account for the cancer-promoting effects of IGSF3 in OPSCC. Our results appear to challenge this speculation, as in our analysis weaker immunopositivity of IGSF3 in TCs was associated with lower stage and lower N class. However, this discrepancy may be explained by the association between HPV negativity and stronger IGSF3 immunopositivity in TCs found in our analysis. As stated before, even though HPV-negative tumors tend to have poorer outcome, they may be associated with lower N class [26], which has been taken into consideration in the new eighth TNM classification.

In contrast to earlier findings where IGSF3 has been associated with several cancer-promoting processes (e.g., facilitating invasion and metastasis), it appears to have an adverse effect in HPV-related and HPV-unrelated OPSCC based on our results. Weak IGSF3 immunopositivity in TILs associated with higher T class among HPV-negative patients in C1, and higher tumor volume is a significant predictive factor for impaired prognosis in OPSCC [4]. On the contrary, IGSF3 immunopositivity in TILs associated with favorable prognosis, and therefore, it appears that IGSF3 may have opposite impacts on tumor pathogenesis, depending on which cells it is expressed. Additionally, as stated earlier, HPV status may influence the expression and possibly even the functions of IGSF3 in OPSCC as well. We suggest that IGSF3 may boost the cytotoxic features of immune cells against tumor in the TME, particularly in HPV-related OPSCC. However, it seems more applicable that IGSF3 is more

 

commonly expressed in HPV-unrelated OPSCC and partly contributes to the aspects impairing the prognosis, whereas the improved survival associated with IGSF3 immunopositivity in TILs constitutes to the prognosis-improving influence of HPV.

Remarkably, according to earlier findings HPV positivity has been found to affect the immune environment in cancer [19], which may explain the variations of the IGSF3 immunopositivity in our analysis. The greater number of significant associations between IGSF3 immunopositivity and clinicopathological characteristics observed among the HPV-negative subgroup of C1 support this theory. However, it is important to note that in earlier findings expression of IGSF3 in lymphocytes has not been reported [15], and we suspect that in our analysis the observed IGSF3 immunopositivity may be due the cross-reaction of the used antibody and V7 protein, as structural similarities have been detected between IGSF3 and V7 [15]. V7 protein has been shown to regulate immune cell activation [35] and thus could be involved in cancer development and progression. Further research is required to validate our findings concerning IGSF3 immunopositivity in TILs.

## CONCLUSIONS

Based on our study, IGSF3 may have prognostic value in HPV-related and HPV-unrelated OPSCC, predicting poor prognosis when found in tumor, and possibly favorable prognosis when found in the TME. HPV status of the tumor may influence the expression of IGSF3 in OPSCC. In the light of these novel findings, further research can be recommended to advance the knowledge of the role of IGSF3 regulation in cancer development and progression as this knowledge may be useful considering targeted treatments.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Clinicopathological characteristics and IGSF3 expression among HPV-positive patients of Cohort 1.
**Table S2.** Clinicopathological characteristics and IGSF3 expression among HPV-negative patients of Cohort 1.
**Table S3.** Clinicopathological characteristics and IGSF3 expression among HPV-positive patients of Cohort 2.
**Table S4.** Clinicopathological characteristics and IGSF3 expression among HPV-negative patients of Cohort 2.

 