Original Research Article

P4HA1: A single-gene surrogate of hypoxia signatures in oral squamous cell carcinoma patients

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Background and purpose: Hypoxia gene expression signatures are of high prognostic value for head and neck cancer patients. Recently, the prognostic information of a multiple-gene hypoxia signature was found to be provided by the mRNA level of P4HA1 alone (Tawk et al., 2016). Therefore, we studied the prognostic value of P4HA1 in an independent cohort of oral squamous cell carcinoma (OSCC) patients.

Material and methods: Frozen tumor samples of 118 adult OSCC patients were analysed for P4HA1 mRNA level by quantitative real-time TaqMan™ PCR analysis. Kaplan-Meier analysis and Cox’s regression analysis were performed to characterize the prognostic impact of P4HA1 mRNA level in OSCC patients.

Results: The analyzed patient cohort was divided into four subgroups according to the quartiles of the P4HA1 mRNA levels. The highest intratumoral P4HA1 mRNA level was significantly correlated with a poor overall survival (RR = 2.2; P = 0.04) and an increased risk of locoregional recurrence (RR = 4.8; P = 0.02). In patients who received radiotherapy (n = 82) highest intratumoral P4HA1 mRNA level was significantly correlated with a poor overall survival (RR = 3.4; P = 0.01) and an increased risk of locoregional recurrence (RR = 10.3; P = 0.005). Moreover, significant correlations between the P4HA1 mRNA level and the mRNA level of several EMT and stem cell markers were found.

Conclusions: A high P4HA1 mRNA level, as a single-gene surrogate of hypoxia, is an independent prognostic marker for the overall survival and locoregional recurrence of OSCC patients.

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is one of the ten most common cancers worldwide with an incidence of 780,000 per year, whereby most of the HNSCC are oral squamous cell carcinomas (OSCC) [1,2]. The 5-year overall survival time has stagnated since years at about 40–50% [3]. In OSCC, tumor hypoxia is a characteristic feature as shown by the overexpression of the transcription factor hypoxia-inducible factor 1 (HIF-1) [4,5].

Efforts to characterize the extent of hypoxia within a tumor tissue and the adverse prognostic consequences in head and neck cancer have led to the evaluation of hypoxia gene signatures. Recently, the clinical relevance of three common hypoxia signature studies was established [6–8]. However, prolyl 4-hydroxylase (P4HA1), which is the only gene common to all identified hypoxia signatures, was found to provide a similar prognostic information regarding overall survival of head and neck cancer patients as the full signatures [9]. In human fibroblasts the transcriptional activity of HIF1 on P4HA1 had an impact on matrix stiffness, extra-cellular matrix production and cell-matrix interaction that affects cancer cell adhesion and invasion [3,10]. P4HA1 expression has consistently been described as stably increased under hypoxia on the mRNA as well as on the protein level [11,12].
The prolyl 4-hydroxylase (P4HA1) gene is coding for a protein involved in the hydroxylation of proline residues in post-translational collagen synthesis. The human prolyl 4-hydroxylases are tetrameric isoenzymes composed of two β subunits (encoded by P4HB) and two (catalytic) α subunits. There are three different α subunits encoded by P4HA1, P4HA2 and P4HA3 [13]. The knockout of P4HA1 in mice is lethal and is associated with overall developmental delay, due to the lack of collagen IV in the extracellular matrix and a disrupted basement membrane [14]. Recently, P4HA1 was found to be overexpressed in gliomas and the expression correlated with tumor microvesSEL density. That fact demonstrated that P4HA1 influences the neoangiogenesis in gliomas, whereas a knockdown of P4HA1 decreases the levels of collagen IV and disrupts the vascular basement membrane [15]. The authors believe P4HA1 may have a role in the transdifferentiation process of glioma stem cells into endothelial cells [15].

To evaluate the prognostic potential of P4HA1 in an independent data set, we studied its mRNA level in the tumor tissue of 118 OSCC patients and determined its association with overall survival and locoregional control as well as with mRNA levels of selected epithelial mesenchymal transition (EMT) and stem cell markers.

Material and methods

Tissue samples and histopathological data

We examined frozen primary tumor samples of 118 OSCC patients. All patients had been treated with surgery at the Department of Oral and Maxillofacial Plastic Surgery, Martin Luther University Halle Wittenberg, Germany. The tissue samples were cut by a cryocut microtome and the first and the last histologic sections were stained with hematoxylin and eosin. Experienced pathologists (UB, DB) verified the sections. We defined samples as tumor tissue when >70% of the first and the last histologic sections were tumor tissue. All patients gave written informed consent. The study was carried out in compliance with the Helsinki Declaration, and it was approved by the Ethics Committee of the Medical Faculty of Martin Luther University Halle-Wittenberg.

51 patients were alive after a median observation time of 31 months (mean 33 months), whereas 67 patients died after a median time of 13 months (mean 16 months) after diagnosis. The histopathological and clinical data have been summarized in Tables 1 and 2.

Cell culture

To study the hypoxic P4HA1 mRNA level in relevant in vitro models, we analyzed the human cell lines CAL-33 (derived from a primary tumor of the tongue; Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany), SAS (derived from a primary tumor of the tongue; Deutsches Krebsforschungszentrum, Heidelberg, Germany) and XF354 (derived from a primary SCC of the floor of the mouth; Deutsches Krebsforschungszentrum, Heidelberg, Germany). The cells were cultured as monolayers in RPMI 1640 medium (Lonza, Walkersville, MD, USA) containing 10% fetal calf serum, 1% sodium pyruvate, 180 μg/ml penicillin and 180 μg/ml streptomycin. The cultures were maintained at 37 °C in a humidified atmosphere supplemented with 5% CO₂.

Cells were cultured in RPMI medium containing glutamine and 10% fetal calf serum overnight under normoxic (21% oxygen) or hypoxic conditions (<1% oxygen) which was achieved using a gas generator system as described previously [16]. Cells were then harvested by treatment with trypsin and RNA was isolated.

RNA-Isolation

Snap-frozen tumor samples were cut into 20 μm tissue sections and RNA was isolated by Trizol reagent according to the manufacturer’s protocol (Invitrogen, Karlsruhe, Germany). The RNA of treated cell lines was isolated equally. DNA contaminations were removed by DNase I digestion (Qiagen, Hilden, Germany). The RNA concentration was determined using a Nanodrop spectrophotometer (Thermo Scientific, Karlsruhe, Germany).

Quantitative RT-PCR

6 μg of total RNA was used for cDNA synthesis (tissue samples) and 1 μg for cell line samples according to standard protocols (Fermentas, St. Leon-Rot, Germany) as previously described [17]. The cDNA was amplified by automated real-time quantitative TaqMan™ assays for P4HA1 and RPII as a housekeeping gene using kits from Thermo Fisher Scientific (Darmstadt, Germany). The P4HA1 transcript amounts were normalized to RPII transcript amounts using the ΔΔCt method [18].

Moreover, the same cDNA was used to analyze the normalized mRNA levels of the EMT-markers ZEB2, Twist, TGFβ, MMP7, CTGF, the stem cell markers Oct3/4, Snai1, Snai2, LGR4, LGR5FL, the structure proteins CDH1, vimentin, KRT13 the hypoxic markers CA9, VEGF, Glut1, HIF1α and mRNA of MDM2, survivin, HER2, EGFR, PDL-1 and Osteopontin using TaqMan™ assays (Thermo Fisher Scientific (Darmstadt, Germany) or as previously described [19,20]. The primers for the analysis of CTGF were: fw: 5’ gag cag cgg cca gta cca gt, rw: 5’ gtc ctc cag tgc gta cag gc. The mRNA-level of P4HA1 was correlated with those markers via Spearman’s rank correlation (see Table 3).

Statistical analysis

Cox’s regression hazard model and Kaplan-Meier analysis were used to estimate a correlation of P4HA1 mRNA with overall survival of OSCC patients. Cox’s regression hazard model for analysis of overall survival and locoregional control was adjusted for the prognostic effect of covariates (T-stage and N-stage and grading), and the relative risk (RR) was calculated. Survival times were calculated from the day of tumor diagnosis. The end point for the overall survival analysis was the time of death of the patient. The end point for the locoregional control analysis was the first recurrence. The interrelationships between the different mRNA levels were tested with the Spearman’s rank correlation (r, correlation coefficient). The correlation of the P4HA1 mRNA level, T-stage, N-stage, grading and gender of the patients were tested with the Kruskal Wallis test. A probability (P) of <0.05 was defined as significant. Statistical analyses were carried out using SPSS software version 20.0 (SPSS Inc., Chicago, USA).

Results

Correlation of the P4HA1 mRNA level with the survival of OSCC patients

The P4HA1 mRNA level of 118 OSCC samples was normalized to the RPII mRNA level. The OSCC cohort was then divided into quartiles (low P4HA1 mRNA level, <75.7; moderate P4HA1 mRNA level, 75.71–131.9; high P4HA1 mRNA level, 131.91–174.1 and very high P4HA1 mRNA level, >174.1). The transcript ratios of 118 OSCC samples ranged from 13 to 565. (mean 162; median 127.5).

The 3-year overall survival rate was 70% for patients with a low, 45% with a moderate, 45% with a high and 33% with a very high intratumoral P4HA1 mRNA level. OSCC patients with a very high,...
intratumoral P4HA1 mRNA level died in median 20 months earlier compared to patients with lower intratumoral P4HA1 expression. Multivariate Cox's regression hazard analysis revealed an increased risk of earlier death (RR = 2.2; \( P = 0.039 \)) (see Table 2, see Fig. 1) for patients with a very high intratumoral P4HA1 mRNA level compared to patients with a low intratumoral P4HA1 mRNA level (see Table 2, see Fig. 1, Supplemental Fig. 1).

Cox's regression analysis also identified a higher intratumoral P4HA1 mRNA level as associated with a higher risk of locoregional recurrence. The risk of recurrence was calculated as RR = 2.6 (\( P = 0.16 \)) for a moderate, RR = 5.8 (\( P = 0.008 \)) for a high and RR = 4.8 (\( P = 0.023 \)) for a very high intratumoral P4HA1 mRNA expression compared to the control group (low level). (see Fig. 2, Table 2, Supplemental Fig. 3).

Moreover, multivariate Cox's regression hazard analysis of those OSCC patients of the same cohort received a radiotherapy after surgery (\( n = 82; \) see Table 1) showed an increased risk of earlier death (RR = 3.4; \( P = 0.009 \)) (see Supplemental Fig. 2) for patients with a very high intratumoral P4HA1 mRNA level compared to patients with a low intratumoral P4HA1 mRNA level.

**Table 1**
Clinicopathological data of OSCC patients.

| Category           | Number of cases | P4HA1 mRNA level | Low | Moderate | High | Very high |
|--------------------|-----------------|------------------|-----|----------|------|-----------|
| Total              | 118             |                  | 30  | 29       | 29   | 30        |
| Gender             |                 |                  |     |          |      |           |
| Men                | 94              |                  | 19  | 23 (79%) | 24   | 28 (93%)  |
| Women              | 24              |                  | 11  | 6 (21%)  | 5    | 2 (7%)    |
| Age (years)        |                 |                  |     |          |      |           |
| <60                | 67              |                  | 17  | 18 (62%) | 17   | 15 (50%)  |
| >60                | 51              |                  | 13  | 11 (38%) | 12   | 15 (50%)  |
| T-stage            |                 |                  |     |          |      |           |
| I                  | 19              |                  | 6   | 5 (17%)  | 4    | 4 (13%)   |
| II                 | 35              |                  | 8   | 10 (35%) | 12   | 5 (17%)   |
| III                | 21              |                  | 7   | 7 (24%)  | 3    | 4 (13%)   |
| IV                 | 43              |                  | 9   | 7 (24%)  | 10   | 17 (57%)  |
| N-stage            |                 |                  |     |          |      |           |
| N0                 | 44              |                  | 11  | 14 (48%) | 10   | 9 (30%)   |
| N1-3               | 74              |                  | 19  | 15 (52%) | 19   | 21 (70%)  |
| Grading            |                 |                  |     |          |      |           |
| 1                  | 12              |                  | 5   | 2 (7%)   | 2    | 3 (10%)   |
| 2                  | 82              |                  | 16  | 21 (81%) | 25   | 20 (77%)  |
| 3                  | 22              |                  | 5   | 0       | 0    | 1 (3%)    |
| X                  | 2               |                  | 1   | 0       | 0    | 1 (3%)    |
| Radiation therapy  | 106             |                  |     |          |      |           |
| Yes                | 82              |                  | 16  | 21 (81%) | 25   | 20 (77%)  |
| No                 | 24              |                  | 9   | 5 (19%)  | 4    | 6 (23%)   |
| Median dose (Gy)   | 54              |                  |     |          |      |           |
| Range (Gy)         | 14–72           |                  |     |          |      |           |
| Recurrence until 3 years after diagnosis | | | | | |
| Yes                | 3 (10%)         |                  | 7   | 12 (41%) |
| No                 | 27 (90%)        |                  | 22  | 17 (59%) |

**Table 2**
Survival analysis of OSCC patients.

| Category            | No. of pts | Overall survival (OS) | Locoregional control (LRC) |
|---------------------|------------|-----------------------|----------------------------|
|                     |            | Univariate analyses   | Multivariate analyses      | Univariate analyses | Multivariate analyses |
|                     |            | RR (95% CI) p-value   | RR (95% CI) p-value        | RR (95% CI) p-value | RR (95% CI) p-value   |
| T-stage             |            |                       |                            |                          |                          |
| I + II              | 54         | 1.0 (reference)       | 1.0 (reference)            | 1.0 (reference)         | 1.0 (reference)         |
| II + IV             | 64         | 2.5 (1.5–4.2) \( p = 0.001 \) | 2.4 (1.3–4.4) \( p = 0.003 \) | 1.5 (0.8–3.1) \( p = 0.24 \) | 1.9 (0.8–4.1) \( p = 0.13 \) |
| N-stage             |            |                       |                            |                          |                          |
| N0                  | 44         | 1.0 (reference)       | 1.0 (reference)            | 1.0 (reference)         | 1.0 (reference)         |
| N1-3                | 74         | 1.9 (1.1–3.3) \( p = 0.014 \) | 1.3 (0.7–2.4) \( p = 0.37 \) | 1.3 (0.6–2.7) \( p = 0.43 \) | 1.0 (0.4–2.2) \( p = 0.99 \) |
| Grading (b)         |            |                       |                            |                          |                          |
| 1                   | 12         | 1.0 (reference)       | 1.0 (reference)            | 1.0 (reference)         | 1.0 (reference)         |
| 2                   | 82         | 0.7 (0.3–1.5) \( p = 0.36 \) | 0.6 (0.3–1.1) \( p = 0.13 \) | 1.0 (0.3–3.2) \( p = 0.93 \) | 0.5 (0.2–1.9) \( p = 0.33 \) |
| 3                   | 22         | 0.7 (0.3–1.6) \( p = 0.37 \) | 0.5 (0.2–1.4) \( p = 0.19 \) | 1.3 (0.3–4.9) \( p = 0.73 \) | 0.7 (0.2–2.9) \( p = 0.61 \) |
| P4HA1 mRNA level    |            |                       |                            |                          |                          |
| Low                 | 30         | 1.0 (reference)       | 1.0 (reference)            | 1.0 (reference)         | 1.0 (reference)         |
| Moderate            | 29         | 1.5 (0.7–3.3) \( p = 0.27 \) | 1.8 (0.9–4.0) \( p = 0.12 \) | 2.6 (0.7–10.0) \( p = 0.17 \) | 2.6 (0.7–10.3) \( p = 0.16 \) |
| High                | 29         | 1.6 (0.7–3.3) \( p = 0.24 \) | 2.0 (0.9–4.3) \( p = 0.07 \) | 4.9 (1.4–17.4) \( p = 0.014 \) | 5.8 (1.5–21.3) \( p = 0.008 \) |
| Very high           | 30         | 1.9 (0.9–4.0) \( p = 0.08 \) | 2.2 (1.0–4.8) \( p = 0.039 \) | 4.4 (1.2–16.0) \( p = 0.025 \) | 4.8 (1.2–18.7) \( p = 0.023 \) |

\( b \)- information not available for 2 patients.
The patients received a radiotherapy after surgery had a risk to have a recurrence calculated as RR = 2.8 (P = 0.22) for a moderate, RR = 5.4 (P = 0.032) for a high and RR = 10.3 (P = 0.005) for a very high intratumoral P4HA1 mRNA expression compared to the control group (low level) (Supplemental Fig. 2).

Correlation of P4HA1 mRNA level with tumor–specific parameters and other molecular markers of OSCC patients

The P4HA1 mRNA level narrowly missed the significance threshold when correlated with the T-stage (P = 0.081) but correlated with the gender of the patients (P = 0.005), whereas no correlation was found between the P4HA1 mRNA level and the grade of the tumor, or the nodal status using Kruskal-Wallis test (see Table 1).

Using Spearman’s rank correlation as a bivariate correlation we found significant associations between the intratumoral P4HA1 mRNA level and the intratumoral mRNA level of EMT markers like ZEB2, TWIST, TGFβ or CTGF or stem cell markers like Oct3/4, Snai1 and 2 and LGR5FL. However, no correlations to the intratumoral mRNA level of hypoxic marker like CA9, VEGFα and Glut1 were found (see Table 3). Moreover, Spearman’s rank analyses showed significant correlations between the intratumoral P4HA1 mRNA level and the mRNA level of vimentin, KRT13, MDM2, survivin, HER2, PDL-1 and osteopontin (see Table 3).

mRNA level of P4HA1 in OSCC cell lines cultivated under different oxygen conditions

To validate the hypoxic increase of the P4HA1 mRNA level in vitro, we determined the level of P4HA1 in three different OSCC cell lines cultivated 24 h under (I) normoxic (21% oxygen) and (II) hypoxic conditions (0.1% oxygen). As expected, the mRNA level of P4HA1 was massively increased in those cell lines cultivated under

| Table 3 |
| Bivariate correlations between P4HA1 level in tumor tissues and clinicopathological parameters of OSCC patients (Kruskal Wallis test) or mRNA level of different biomarkers (Spearman’s Rho test) (r_s-correlation coefficient). |
| --- |
| **Clinicopathological parameters** |
| T-stage | 0.081 | 118 |
| Gender | 0.005 | 118 |
| N-stage | 0.599 | 118 |
| Grading | 0.486 | 118 |
| **EMT-markers** |
| ZEB2 | 0.250 | 0.009 | 108 |
| Twist | 0.406 | 0.008 | 108 |
| TGFβ | 0.548 | <0.001 | 108 |
| MMP7 | 0.216 | 0.070 | 71 |
| CTGF | 0.341 | 0.004 | 69 |
| **Stem cell marker** |
| Oct3/4 | 0.253 | 0.033 | 71 |
| Snai1 | 0.360 | 0.002 | 71 |
| Snai2 | 0.292 | 0.014 | 71 |
| LRGR4 | 0.171 | 0.077 | 108 |
| LRGR5FL | 0.308 | 0.010 | 69 |
| **Structure proteins** |
| CDH | 0.111 | 0.912 | 108 |
| Vimentin | 0.356 | 0.002 | 71 |
| KRT13 | −0.251 | 0.039 | 71 |
| **Hypoxic markers** |
| CA9 | 0.063 | 0.604 | 71 |
| VEGFα | 0.118 | 0.329 | 71 |
| Glut1 | 0.084 | 0.488 | 71 |
| HIF1α | −0.037 | 0.765 | 69 |
| **Others** |
| MDM2 | 0.391 | 0.001 | 71 |
| Survivin | 0.322 | 0.006 | 71 |
| HER2 | −0.420 | <0.001 | 69 |
| EGFR | −0.112 | 0.36 | 69 |
| PDL-1 | 0.427 | <0.001 | 105 |
| Osteopontin | 0.461 | <0.001 | 69 |

Fig. 1. Multivariate Cox’s hazard regression model: association of P4HA1 mRNA expression level and survival of OSCC patients. The model was adjusted to patients tumor stage, lymph status (N-stage) and grading of the tumor. The OSCC cohort was divided into four groups (quartiles) according to the intratumoral P4HA1 mRNA level (low, moderate, high, very high). The patients risk of death was calculated as RR = 1.8 (P = 0.12) for a moderate, RR = 2.0 (P = 0.075) for a high and RR = 2.2 (P = 0.039) for a very high intratumoral P4HA1 mRNA expression compared to the control group (low level).
hypoxic conditions. In the cell lines CAL-33, XF354 and SAS the P4HA1 mRNA level was increased due to oxygen deprivation by a factor of 10.9, 9.8 and 4.8, respectively (see Fig. 3).

Discussion

A negative prognostic impact of tumor hypoxia has been described for different tumor entities [21,22], including tumors of the head and neck region. Furthermore, we demonstrated a negative prognostic impact of a higher intratumoral P4HA1 mRNA level for the overall survival and locoregional control in a cohort of 118 OSCC patients (see Tables 1 and 2; see Figs. 1 and 2). In bivariate correlation analyses the P4HA1 mRNA level of OSCC samples correlated significantly with the mRNA level of EMT or stem cell markers like ZEB2, Twist, TGFβ, Oct3/4, Snai1, Snai2 and LGR5FL. Our findings indicate an association of P4HA1 with different EMT and stem cell markers. That is remarkable, because other authors believe that P4HA1 may have a role in the transdifferentiation process of e.g. glioma stem cells into endothelial cells [15].

However, no correlation of the P4HA1 mRNA level with the mRNA level of hypoxic induced markers like CA9, VEGFA and Glut1 was found (see Table 3). Hence, we analyzed the mRNA level of the hypoxia-associated marker P4HA1 in three OSCC cell lines and compared its expression level under hypoxic and normoxic culture conditions (see Fig. 3). This in vitro analysis showed an increased mRNA level of P4HA1 under hypoxic conditions (see Fig. 3). Moreover, we found a hypoxia specific increase of the mRNA level of CA9, VEGFA and Glut1 (data not shown). These data demonstrate that correlations of biomarkers in in vivo samples do not necessarily reflect the in vitro situation, as it was found for the mRNA level of P4HA1 and CA9, VEGFA, Glut1 (HIF1-target genes) in the three analysed OSCC cell lines.

In the literature, P4HA1 has previously been shown to be transcriptionally regulated by HIF1. [10,23,24]. Prolyl 4-hydroxylases are associated with hypoxic processes during osteoarthritis [25], Moreover, the collagen prolyl hydroxylase P4HA1 was found to be associated with lymphatic and lung metastasis in breast cancer patients [26]. In addition, P4HA1 is transcriptionally regulated by E2F, which was described for an E2F knock-out mouse model in which knock-out of E2F decreased the level of P4HA1 and led to remodelling of the extracellular matrix and supported the process of metastasis. [27]. In our analysis, P4HA1 mRNA was not significantly increased in tumor samples of patients with nodal metastasis. The observation that P4HA1 mRNA and protein levels are stably upregulated via hypoxia suggests P4HA1 as a clinically useful and prognostic relevant marker to identify hypoxic tumors [12].

A meta-analysis of genome–wide RNA sequencing data identified this hypoxic marker as a unique prognostic marker for hypoxic tumors [9]. Tawk et al. analysed three different hypoxia gene
signatures studies in a set of 302 head and neck tumor patients [6–9]. Our data confirmed the prognostic power of P4HA1 in an independent cohort of 118 OSCC patients.

The possible cause for that finding might be the transcriptional impact of HIF1 on the level of hypoxic P4HA1 [10,24]. However, due to the short half-life time of HIF1, indirect measurements of its transcriptional activity such as P4HA1 may be the ideal surrogate indicators of hypoxic gene expression. Tumor hypoxia is associated with HIF1 activation, metastasis, and resistance to chemotherapy and radiotherapy, as well as poor patient survival [28]. Rankin and Giaccia concluded that HIF1 promotes each step during metastasis including the remodelling of the ECM, which implies the activity of P4HA1 [28]. HIF1 promotes signalling in the primary tumor which contributes to the expression of secreted factors that are involved in formation of the premetastatic niche [28] which then not only modifies the ECM but also affects the activity of the immune system [3]. These findings are supported by the correlation of the P4HA1 mRNA level with the miRNA level of stem cell markers or genes associated with EMT, like ZEB2, Twist, LGR4, LGR5 and TGFβ1 (see Table 3). These results are in accordance with the regulation of multiple steps within the metastatic cascade by HIF1. Although, ZEB2, Twist, LGR4, LGR5 and TGFβ1 are not HIF1-regulated they are also strongly associated with epithelial mesenchymal transition (EMT), a mechanism essential for metastasis [29–33]. In addition the correlation of the P4HA1 mRNA level with the transcript levels of HER2 and PDL1 could help to stratify OSCC patients for future targeting and/or immunotherapies.

Conclusion

In this study, we showed for the first time that P4HA1 is an independent prognostic factor for overall survival of OSCC patients and is associated with a higher risk of locoregional recurrence. The data confirm the potential use of P4HA1 as a single-gene surrogate of multiple-gene hypoxia signatures in head-and-neck cancer, whereby different tumor-specific and hypoxic independent processes like EMT may influence the expression of P4HA1, too.

Conflict of interest statement

On behalf of all co-authors, the corresponding author declare, that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ctro.2017.05.002.

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