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ORIGINAL ARTICLE – CANCER RESEARCH

Prevalence of fibroblast growth factor receptor 1 (FGFR1) amplification in squamous cell carcinomas of the head and neck

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Abstract
Background FGFR1 is a receptor tyrosine kinases involved in tumor growth signaling, survival, and differentiation in many solid cancer types. There is growing evidence that FGFR1 amplification might predict therapy response to FGFR1 inhibitors in squamous cell lung cancers. To estimate the potential applicability of anti FGFR1 therapies in squamous cell carcinomas of the head and neck, we studied patterns of FGFR1 amplification using fluorescence in situ hybridization (FISH).
Materials and methods A tissue microarray was constructed from 453 primary treatment-naive squamous cell carcinomas of the head and neck regions with histopathological and clinical follow-up data [including oral cavity ($n = 222$), oropharynx ($n = 126$), and larynx ($n = 105$)]. *FGFR1 and centromere 8* copy numbers were assessed by dual-color FISH. FGFR1 amplification was defined as a copy number ratio FGFR1: centromere 8 ≥ 2.0. HPV sequencing and p16 immunohistochemistry (IHC) were applied to FGFR1-amplified cancers.
Results FISH analysis was successful in 297 (66%) of the 453 cancers. FGFR1 amplification was found in 6% of analyzable tumors, and was more frequent in tumors of the oral cavity (13/133 amplified, 10%), than cancers of other localizations (1/79 oropharynx, 4/85 larynx; $p = 0.007$ and 0.159, respectively). One out of 18 FGFR1 amplified cancers was HPV positive. No associations were found between FGFR1 amplification and tumor phenotype or p16 IHC.
Conclusions Head and neck cancers are recurrently affected by FGFR1 amplification, with a predominance in cancers of the oral cavity. Finding only one HPV positive and FGFR1 amplified cancer argues against a causal relationship between HPV and FGFR1 amplifications.

Keywords Fibroblastic growth factor receptor 1 (FGFR1) · Head and neck squamous cell carcinoma (HNSCC) · Oral squamous cell carcinoma (OSCC) · Larynx squamous cell carcinoma (LSCC) · Targeted therapy

Introduction

Head and neck squamous cell carcinoma (HNSCC) is worldwide the sixth leading cancer by incidence and more than 650,000 cases are diagnosed annually (Jemal et al. 2009; Kamangar et al. 2006; Lim et al. 2016). Beside alcohol and tobacco, human papilloma virus (HPV) infection is one of the main risk factors in carcinogenesis of HNSCC and seems to have better therapy response in case of positivity (Castellsague et al. 2004; Fakhry et al. 2008; Gillison and

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Lowy 2004; Herrero et al. 2003; Macfarlane et al. 2010). To date, the anti-EGF receptor (EGFR) monoclonal antibody, cetuximab, is the first and only molecularly targeted therapy to demonstrate a survival benefit for patients with recurrent or metastatic HNSCC (Bonner et al. 2006; Burtness et al. 2005; Markovic and Chung 2012).

A potential new molecular target in squamous cell carcinoma is fibroblast growth factor receptor 1 (FGFR1). FGFR1 is a member of the FGFR family of receptor tyrosine kinases which are involved in biological functions such as cellular proliferation, survival and differentiation (Turner and Grose 2010). Amplification of a genomic region on 8p12 which includes the FGFR1 gene locus is frequently observed in several tumor entities including squamous cell cancer of the lung and oesophagus as well as ovarian, bladder, and prostate cancer (Edwards et al. 2003; Gorringe et al. 2007; Ishizuka et al. 2002; Simon et al. 2001). However, only recently, with the experimental introduction of FGFR1-inhibitors, it was shown that amplification of FGFR1 within the 8p12 genomic region is indeed a driver mutation. FGFR1 amplification induces FGFR1 dependency in lung cancer cell lines via the mitogen-activated protein kinase (MAPK) pathway. Thus, it is suggested that targeting FGFR1 by small molecule inhibitors might become a viable therapeutic option in squamous cell carcinoma of the lung (Dutt et al. 2011; Weiss et al. 2010).

Only limited data are available on amplification of the FGFR1 gene locus in squamous cell carcinoma of head and neck. Freier et al. (2007) reported amplification of 8p12 genomic region by fluorescence in situ hybridization (FISH) in 17.4% (16/92) oral squamous cell carcinomas. Marshall et al. (2011) recently showed that a subset of HNSCC cell lines is dependent on autocrine signaling by fibroblastic growth factors (FGF) and that these cell lines are sensitive to FGFR inhibition. Recently, two other groups reported of amplification of the FGFR1 gene locus in 9.3% (10/107) analyzed squamous cell carcinomas of the tongue and 20% (9/45) analyzed sinonasal squamous cell carcinoma (Schrock et al. 2013; Young et al. 2013).

As FGFR1 amplification might represent an opportunity for targeted therapy in HNSCC, we performed an extensive analysis for FGFR1 gene copy gain in squamous cell carcinomas of the head and neck.

Materials and methods

Specimen collection and TMA construction

A tissue microarray was constructed from a total of 453 primary surgical HNSCC specimens from formalin-fixed, paraffin-embedded archived tissue samples of the Institute of Pathology at the University Medical Center Hamburg-Eppendorf as described (Kononen et al. 1998). The usage of tissue microarrays for research purposes has been approved by the local ethics committee. Only surgical specimens of tumorectomy without the previous therapy were used for tissue microarray construction. All cases included were reviewed by two pathologists (TSC and WW). The pathologic stage was obtained from the primary report of the Institute of Pathology. UICC stage was determined regarding the 7th edition of the UICC TNM classification of malignant tumors (Sobin et al. 2009). Raw survival data were available from 441 patients. The median follow-up period was 24.1 months for oral cavity, 36 months for hypo-/oropharynx, and 54 months for laryngeal carcinomas. Recurrence was defined as tumor relapse after operation with or without adjuvant therapy. Data on therapy, adjuvant therapy setting as well as smoking and drinking were not available for the cohort. An overview of clinical and pathological data of the whole cohort is shown in Table 1. Consecutive, freshly cut sections of the tissue micro arrays were used for FISH, immunohistochemical analysis, and H&E stained reference.

FGFR1 fluorescence in situ hybridization

For analysis of FGFR1 gene copy gain, a dual-color FISH probe set was used. The set consisted of a self-constructed spectrum green-labeled bacterial artificial chromosome clone (RP11-350N15; Source Bioscience, Nottingham, UK; Abbott KIT) and a spectrum red labeled commercial centromere 8 probe (Zytovision, Bremerhaven, Germany) as a reference. Freshly cut sections (4 µm) were deparaffinized and proteolytically pretreated using a commercial kit (paraffin pretreatment reagent kit; Abbott Molecular), followed by dehydration, air drying, and denaturation for 10 min at 72 °C. Hybridization was performed overnight at 37 °C in a humidified chamber. Slides were then washed and counterstained with 0.2 µmol/L of DAPI.

Screening of TMAs was performed by evaluating 20 unequivocal tumor cells. Cases with < 20 analyzable tumor cells were considered not evaluable. Gene amplification was defined as ratio FGFR1/CEP8 ≥ 2 and average FGFR1 signals > 4. All cases showing gene amplification or any ambiguity (blurred signals, high polysomy) were subjected to validation by FGFR1 FISH on sections of the original paraffin block. In addition to the above-mentioned dual-color FISH probe set, the ZytoLight SPEC FGFR1/CEN 8 dual-color FISH probe (Zytovision, Bremerhaven, Germany) was performed on all sections of original paraffin blocks as recommended by the manufacturer.

Table 1 Clinical and pathological characteristics of 453 patients

Characteristics	Study cohort on TMA number of patients (%)
Localization	
Oral cavity	222 (49)
Hypo-/oropharynx	94 (20.8)
Larynx	137 (30.2)
Median follow-up (months)	
Oral cavity	24.1
Hypo-/oropharynx	36.0
Larynx	54.0
Age (years)	
< 40	18 (3.98)
40–49	65 (14.48)
50–59	158 (34.95)
60–69	134 (29.64)
> 70	77 (17.03)
pT category (WHO 2009)	
pT1	103 (23.14)
pT2	133 (29.88)
pT3	79 (17.75)
pT4	130 (29.21)
pN category (WHO 2009)	
pN0	219 (59.21)
pN1	68 (15.28)
pN2	134 (30.11)
pN3	24 (5.39)
pM/cM category	
cM0	409 (93.16)
pM1	30 (6.83)
UICC category (WHO 2009)	
I	73 (16.7)
II	72 (16.4)
III	83 (18.9)
IV	209 (47.8)

Numbers do not add up to 452 in the different categories because of cases with lack of data

HPV detection by PCR

Detection of HPV-DNA was performed on formalin-fixed, paraffin-embedded tumor specimens. 4 µm sections were used for DNA extraction with the QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA integrity was evaluated through amplification of a beta-globin sequence with primers generating an amplicon of 127 or 111 bp, respectively (forward 5′-GCCATC ACTAAAGGCACCGAG-3′ and reverse 5′-TTCCCACCC TTAGGCTGCTG-3′). Detection of HPV was performed using specific primers in the L7 region of HPV16 (forward 5′-ACAAGCAGAACCGGACAGAG -3′ and reverse 5′-GCC CATTAACAGGTCTTCCA-3′; amplicon size = 127 bp) and HPV18 (forward 5′-AAGCTCAGCAGACGACCTTC-3′ and reverse 5′-CCTTCTGGATCAGCCATTGT-3′; amplicon size = 111 bp). The three reactions were performed under identical conditions: 100 ng of DNA were subjected to PCR using the AmpliTaq Gold PCR mastermix (Applied Biosystems, Darmstadt, Germany) as recommended by the manufacturer (43 cycles, annealing temperature 55 °C). In all cases with a positive result for HPV, PCR products were sequenced for confirmation of HPV type.

Immunohistochemistry

Immunohistochemical analyses were performed on 4 µm-thick TMA sections. Staining for p16[ink4a] (BD Pharmingen™; BD bioscience USA, dilution scale 1:25) was carried out after heat pretreatment in Bond Epitope Retrieval Solution 2 (pH 9; Leica Microsystems) in a bond-automated system (Leica Microsystems). Nuclear as well as cytoplasmic p16 expression was evaluated semiquantitatively and classified as previously described (Klaes et al. 2001): negative (< 1% of cells positive), sporadic (isolated cells positive, but < 5%), focal (small cell clusters, but < 25% of the cells positive), and diffuse (> 25% of the cells stained).

Statistical analysis

To study an association between FGFR1 gene copy gain to clinical–pathological parameters, contingency table analysis and Chi-square test (likelihood) were used. Analysis on recurrence free and overall survival was done using the Kaplan–Meier method and has been compared via Logrank test. To compare the follow-up time between tumors with and without successful FISH analysis, analysis of variance (ANOVA) was performed. All p values were two-sided and p values < 0.05 were considered as significant. For statistical analysis the JMP 11.0 software (SAS institute Inc., Cary, NC, USA) was used.

Results

FGFR1 gene copy numbers were interpretable in 297 arrayed tumor samples. Analysis failed in 156 samples, because hybridization quality was too low, not enough tumor cells were analyzable or the entire tissue spot was missing on the tissue microarray slide. 31 cases were reevaluated on large sections of the original paraffin block by two different FGFR1 FISH probe sets. FGFR1 gene amplification defined by an FGFR1/CEP8 ratio ≥ 2 and average FGFR1 signals ≥ 4 was confirmed in 16 cases. The positive cases showed FGFR1 gene amplification with an FGFR1/CEN8 ratio > 2 and an average FGFR1 signal count ranging from

Table 2 FGFR1 FISH results of self labeled and commercial probes

Locus	Case	Selflab FGFR1-Gen	Selflab FGFR1-Cen	Selflab FGFR1-Ratio	Selflab FGFR1-Result	ZytoVi FGFR1-Gen	ZytoVi FGFR1-Cen	ZytoVi FGFR1-Ratio	ZytoVi FGFR1-Result	HPV pos./neg	HPV type
Oral cavity	#3	9.4	2.45	3.84	Amplified	9.7	2.7	3.6	Amplified	Positive	16
Oral cavity	#15	7.70	3.35	2.3	Amplified	9.7	4.45	2.93	Amplified	Negative	–
Oral cavity	#52	10.9	5.35	2.04	Amplified	8.75	3.8	2.3	Amplified	Negative	–
Oral cavity	#65	6.3	2.2	2.86	Amplified	5.8	2.05	2.83	Amplified	Negative	–
Oral cavity	#113	10.15	4.65	2.18	Amplified	10	2.75	3.64	Amplified	Negative	–
Oral cavity	#120	13.45	5.45	2.47	Amplified	13.95	4.05	3.44	Amplified	Negative	–
Oral cavity	#133	9.3	2.45	3.8	Amplified	10.65	2.55	4.18	Amplified	Negative	–
Oral cavity	#144	16.5	3.0	5.5	Amplified	16.75	2.65	6.32	Amplified	Negative	–
Oral cavity	#158	5.85	2.7	2.17	Amplified	5.6	2.65	2.11	Amplified	Negative	–
Oral cavity	#163	10.25	2.3	4.46	Amplified	12.55	2.1	5.98	Amplified	Negative	–
Oral cavity	#199	7.4	3.1	2.39	Amplified	14.75	3.65	4.04	Amplified	Negative	–
Oral cavity	#207	4.25	1.95	2.18	Amplified	6.7	2.3	2.91	Amplified	Negative	–
Oral cavity	#219	12.5	3.35	3.74	Amplified	13.9	3.5	3.97	Amplified	Negative	–
Oropharynx	#319	15	3.95	3.8	Amplified	13.5	3	4.5	Amplified	Negative	–
Larynx	#357	19.25	2.35	8.19	Amplified	20.5	2.45	8.37	Amplified	Negative	–
Larynx	#378	7.4	1.9	3.9	Amplified	8.4	1.85	4.54	Amplified	Negative	–
Larynx	#399	17.5	2.4	7.29	Amplified	16.5	2.3	7.17	Amplified	Negative	–
Larynx	#417	16.75	2.95	5.68	Amplified	23.75	2.55	9.31	Amplified	Negative	–

4.35 to 20.50 per tumor cell. The two probe sets used for FGFR1 FISH showed high concordance. The interpretation of the FGFR1 gene copy gain was identical with both sets in all cases. The individual results of the positive cases are shown in Table 2. Analysis on non-malignant squamous tissues (18 probes) did not show any genomic aberration in FGFR1 FISH by both probe sets. FGFR1 amplification was found in 13/133 (9.8%) tumors of the oral cavity, 1/79 (1.3%) tumors of the oropharynx, and 4/85 (4.7%) tumors of the hypopharynx/larynx. Examples of a positive and a negative case are shown in Fig. 1. FGFR1 gene amplification was found to be homogeneous in all tumor cells on the sections of the original tumor block. For this purpose, an H&E stained reference was carefully compared with the FISH slides.

FGFR1 FISH results were analyzed according to tumor localization, pathologic classification, and clinical stage. FGFR1 amplification was found in all pT stages, all pN stages, all UICC stages, and all grades of tumor differentiation. Analysis between the tumor subgroups (oral cavity, oropharynx, and hypopharynx/larynx) showed a predominance of amplified cases in the oral cavity. Detailed information can be found in Table 3. No significant correlation between FGFR1 amplification and any of the clinicopathological parameters was found in the whole HNSCC cohort or in the tumor localization subsets ($p > 0.1$). Survival analysis of the whole cohort and the tumor subsets of HNSCCs did not show a significant association of FGFR1 gene amplification regarding recurrent free and overall survival ($p \geq 0.38$ and $p \geq 0.12$, respectively). The suitability of the data for survival analysis was demonstrated by the finding of the known prognostic value of pT ($p < 0.0001$), pN ($p = 0.0011$), and UICC stage ($p = 0.0001$). Detailed results regarding FGFR1 gene amplification and clinico-pathologic data are summarized in Table 3.

The evaluation of p16 expression of the whole cohort did not show a significant association with FGFR1 gene amplification ($p = 0.58$; Table 4). All cases showing FGFR1 gene amplification were tested for HPV-DNA by polymerase chain reaction and subsequent sequencing. In one of the 18 FGFR1 amplified tumors (oral carcinoma; case #3), HPV-DNA type 16 was detected. This tumor showed diffuse expression of p16. In all other carcinomas with FGFR1 amplification, no HPV-DNA was detected.

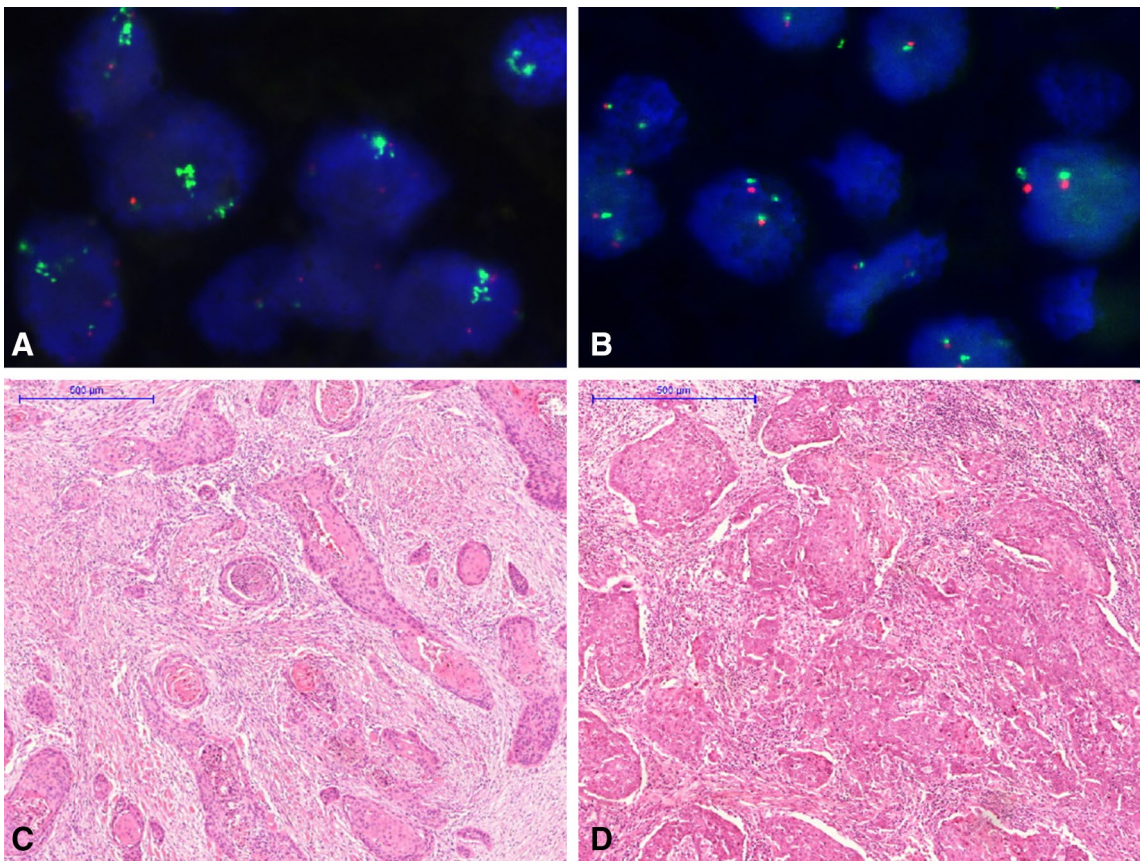

Fig. 1 *FGFR1* FISH analysis (**a**) shows 10–20 *FGFR1* gene copies per tumor cell nucleus in clusters (green signals) and 2 CEN8 signals (red) and non-amplified FISH analysis (**b**). H&E stained detail of amplified and non-amplified cases are shown in (**c**, **d**; scale bars 500 µm)

Table 3 Correlation between FGFR1 gene amplification and clinicopathologic characteristics in patients with HNSCC

Category	HNSCC			Oral cavity			Oropharynx			Larynx		
	FGFR1 gene			FGFR1 gene			FGFR1 gene			FGFR1 gene		
	Non-amp. *n* (%)	Amp. *n* (%)	*p* value	Non-amp. *n* (%)	Amp. *n* (%)	*p* value	Non-amp. *n* (%)	Amp. *n* (%)	*p* value	Non-amp. *n* (%)	Amp. *n* (%)	*p* value
Primary tumor site	279 (93.94)	18 (6.06)		120 (90.23)	13 (9.77)		78 (98.73)	1 (1.27)	0.007*	81 (95.29)	4 (4.71)	0.159*
Gender			0.350			0.242			0.114			0.206
Male	206 (93.21)	15 (6.79)		84 (88.4)	11 (11.6)		56 (100.00)	0 (0.00)		66 (94.29)	4 (5.71)	
Female	73 (96.05)	3 (3.95)		36 (94.7)	2 (6.3)		22 (95.65)	1 (4.35)		15 (100.00)	0 (0.00)	
Age			0.546			0.7962			0.800			0.177
< 40	11 (100.00)	0 (0.00)		8 (100)	0		1 (100.00)	0 (0.00)		2 (100.00)	0 (0.00)	
40–49	42 (91.30)	4 (8.70)		12 (75)	4 (25)		14 (100.00)	0 (0.00)		16 (100.00)	0 (0.00)	
50–59	97 (96.04)	4 (3.96)		36 (92.3)	3 (7.7)		34 (97.14)	1 (2.86)		27 (100.00)	0 (0.00)	
60–70	80 (93.02)	6 (6.98)		34 (91.9)	3 (8.1)		21 (100.00)	0 (0.00)		25 (89.29)	3 (10.71)	
> 70	49 (92.45)	4 (7.55)		30 (90.9)	3 (9.1)		8 (100.00)	0 (0.00)		11 (91.67)	1 (8.33)	
pT stage			0.567			0.222			0.373			0.172
T1	60 (96.77)	2 (3.23)		32 (94.1)	2 (5.9)		15 (100.00)	0 (0.00)		13 (100.00)	0 (0.00)	
T2	79 (92.94)	6 (7.06)		41 (91.1)	4 (8.9)		22 (100.00)	0 (0.00)		16 (88.89)	2 (11.11)	
T3	50 (90.91)	5 (9.09)		11 (73.3)	4 (26.7)		16 (94.12)	1 (5.88)		23 (100.00)	0 (0.00)	
T4	87 (94.57)	5 (5.43)		36 (92.3)	3 (7.7)		25 (100.00)	0 (0.00)		26 (92.86)	2 (7.14)	
pN stage			0.349			0.577			0.491			0.910
N0	126 (93.33)	9 (6.67)		62 (91.2)	6 (8.8)		23 (95.83)	1 (4.17)		41 (95.35)	2 (4.65)	
N1	39 (90.70)	4 (9.30)		16 (84.2)	3 (15.8)		10 (100.00)	0 (0.00)		13 (92.86)	1 (7.14)	
N2	92 (94.85)	5 (5.15)		36 (90)	4 (10)		36 (100.00)	0 (0.00)		20 (95.24)	1 (4.76)	
N3	19 (100.00)	0 (0.00)		6 (100)	0		9 (100.00)	0 (0.00)		4 (100.00)	0 (0.00)	
Distant metastasis			0.609			0.141			0.741			0.530
M0	247 (93.56)	17 (6.44)		109 (89.3)	13 (10.7)		71 (98.61)	1 (1.39)		67 (95.71)	3 (4.29)	
M1	24 (96.00)	1 (4.00)		10 (100)	0		4 (100.00)	0 (0.00)		10 (90.91)	1 (9.09)	
UICC			0.535			0.922			0.374			0.339
I	44 (95.65)	2 (4.35)		24 (92.31)	2 (7.69)		6 (100.00)	0 (0.00)		14 (100.00)	0 (0.00)	
II	41 (91.11)	4 (8.89)		19 (90.48)	2 (9.52)		8 (100.00)	0 (0.00)		14 (87.50)	2 (12.50)	
III	49 (90.74)	5 (9.26)		19 (86.36)	3 (13.64)		16 (94.12)	1 (5.88)		14 (93.33)	1 (6.67)	
IV	142 (95.30)	7 (4.70)		58 (90.63)	6 (9.38)		48 (100.00)	0 (0.00)		36 (97.30)	1 (2.70)	

* *p* values subsite analyzes (oral cavity vs. oropharynx/larynx)

Table 4 Correlation between FGFR1 gene amplification and p16 immunohistochemistry results

Category	HNSCC			Oral cavity			Oropharynx			Larynx		
	FGFR1 gene			FGFR1 gene			FGFR1 gene			FGFR1 gene		
	Non-amp. *n* (%)	Amp. *n* (%)	*p* value	Non-amp. *n* (%)	Amp. *n* (%)	*p* value	Non-amp. *n* (%)	Amp. *n* (%)	*p* value	Non-amp. *n* (%)	Amp. *n* (%)	*p* value
P16[ink4a]			0.355			0.989			0.435			0.258
Negative	70 (97.22)	2 (2.78)		15 (88.24)	2 (11.76)		36 (100)	0		19 (100)	0	
Sporadic	13 (86.67)	2 (13.33)		8 (88.89)	1 (11.11)		1 (100)	0		4 (80)	1 (20)	
Fokal	32 (91.43)	3 (8.57)		21 (91.30)	2 (8.70)		0	0		11 (91.67)	1 (8.33)	
Diffuse	133 (93.01)	10 (6.99)		66 (89.19)	8 (10.81)		28 (96.55)	1 (3.45)		39 (97.50)	1 (2.50)	

Discussion

Our analysis of head and neck squamous cell carcinomas shows FGFR1 amplification in 18/297 tumors with a clear predominance of carcinomas of the oral cavity (13/133 tumors; $p = 0.026$). In total 10% of the OSCCs, 5% of the larynx and 1% of the oropharynx carcinomas showed an FGFR1 gene amplification. Regarding the available clinicopathologic data (gender, age, pT, pN, cM, Grade, UICC, radiation therapy, tumor recurrence, and overall survival), no significances were found, which is in concordance with the literature (Freier et al. 2007; Kohler et al. 2012; Reis-Filho et al. 2006; Schrock et al. 2013; Weiss et al. 2010; Young et al. 2013).

Especially, our results for the oral cavity are in line with the previous studies, where the authors reported amplification of the 8p12 locus in 16 of 92 oral squamous cell carcinomas or 10/107 squamous cell carcinomas of the tongue by FISH (Freier et al. 2007; Young et al. 2013).

An amplification of the FGFR1 gene locus (8p12) was reported for several other malignancies (squamous cell carcinoma of the lung, breast carcinoma, prostate, sinonasal undifferentiated, and ovary carcinoma) (Edwards et al. 2003; Gorringe et al. 2007; Schrock et al. 2013; Weiss et al. 2010). For squamous cell carcinoma of the lung, Weiss et al. (2010) recently proposed an FGFR1 driver mutation being regulated by transformation in the MAP kinase pathways. In addition, they reported that treatment with FGFR1- inhibitors leads to downstream inhibition and induction of apoptosis in FGFR1 amplified tumor cells using a xenograft mouse model (Weiss et al. 2010).

To date, different FGFR1 inhibitors are being tested to expand the possibilities of tumor treatment. Several studies reported of different anti-FGFR therapeutics showing an effect on different malignancies (myeloproliferative disorders, NSCLC, HNSCC, breast, prostate, and ovarian cancer) (Andre et al. 2013; Bousquet et al. 2011; Cheng et al. 2012; Dutt et al. 2011; Gozgit et al. 2012; Ledermann et al. 2011; Marshall et al. 2011; Sweeny et al. 2012; Weiss et al. 2010).

For HNSCC, Marshall et al. (2011) reported of high FGFR1 RNA levels in a cell line experiment and found out that several FGFR-specific tyrosine kinase inhibitors (TKI) led to a reduction of cell growth, suggesting that FGFR1 might be a serious therapy target for HNSCC. Experiments on HNSCC xenografts showed that treatment with inhibitors affecting FGFR1 (dovitinib, BIBF1120) led to reduced regional lymph node metastasis or inhibition of tumor growth (Hilberg et al. 2008; Sweeny et al. 2012).

Recently, the inhibitor BIBF1120 was tested in a phase I trial on prostate cancer as well as a phase II trials on ovarian cancer and squamous cell cancer of the lung, which showed therapy response (Bousquet et al. 2011; Ledermann et al. 2011).

Regarding our results of FGFR1 gene amplification in HNSCC, especially of oral squamous cell carcinoma (10% of cases), it might be very useful evaluating FGFR inhibitors on HNSCC patients to possibly improve the survival of these patients.

Contrary to squamous cell carcinomas of the lung, human papilloma virus (HPV) has been reported to be involved in tumor development of carcinomas of the head and neck regions (Castellsague et al. 2004; Gillison and Lowy 2004). Therefore, we evaluated the HPV infection and p16 expression status of all FGFR1 amplified cases, to find out if there might be crosslink of infection and gene amplification. In our analysis, only one of the 17 cases (5.6%) showed detectable HPV-DNA type 16 and was diffusely p16 positive. In addition, the comparison of p16 expression according to the FGFR1 gene status (non-/amplified) and available clinicopathologic data showed no significances for the whole cohort and tumor subsets. Due to the low amount of cases in this analysis, a precise statement on influence of HPV on FGFR1 gene amplification cannot be made.

The literature of HPV infection and its effects on FGFR1 is sparse. One study of cervical squamous cell cancer reported of increased FGFR1 gene expression levels in HPV (type 16)-transfected mice (Cheng et al. 2012). Another study analyzed a possible association of HPV infection

and amplification of the FGFR1 gene locus using p16 as a surrogate marker for HPV infection and did not find any significance between p16 and FGFR1 gene status (Schrock et al. 2013).

In summary, FGFR1 gene amplification can be found in up to 10% of HNSCCs depending on the tumor site (oral cavity, oropharynx, and hypopharynx/larynx). To find out if HPV infections have any influence on the expression of FGFR1 in HNSCC, further studies have to be carried out.

The most promising fact of this study is, regarding the findings of several groups that anti-FGFR1 therapeutics had influence on tumor growth and even led to tumor reduction and lymph node metastasis, that FGFR1 might become a serious therapy target for HNSCC therapy in the future.

Compliance with ethical standards

Conflict of interest All authors of this manuscript declare that they have no conflict of interest.

Human and animal rights statement The usage of archived diagnostic left-over tissues for manufacturing of tissue microarrays and their analysis for research purposes as well as patient data analysis has been approved by local laws (HmbKHG, §12,1) and by the local ethics committee (Ethics commission Hamburg, WF-049/09 and PV3652). All works have been carried out in compliance with the Helsinki Declaration. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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