Aberrations of MET are associated with copy number gain of EGFR and loss of PTEN and predict poor outcome in patients with salivary gland cancer

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Abstract Hepatocyte growth factor receptor (MET) is a key driver of oncogenic transformation. Copy number gain and amplification of MET positively enhance tumour growth, invasiveness and metastasis in different cancer types. In the present study, 266 carcinomas of the major and minor salivary glands were investigated for genomic MET status by fluorescence in situ hybridization and for protein expression by immunohistochemistry. Results were matched with clinicopathological parameters, long-term survival and the status of epidermal growth factor receptor (EGFR) and phosphatase and tensin homologue (PTEN). Low polysomy (n=42), high polysomy (n=27), amplification (n=2) and deletion (n=18) were found as aberrations of genomic MET in certain subtypes. MET aberrations were associated with increased patient age (>70 years, p=0.003), male gender (p=0.01), increased tumour size (p=0.002), lymph node metastases (p<0.001), high-grade malignancy (p<0.001) and unfavourable overall survival (p<0.001). Both copy number gain (p<0.001) and deletion (p=0.031) of MET correlated with copy number gain of EGFR. Tumours with genomic loss of PTEN (n=48) concurrently presented aberration of genomic MET (p<0.001). MET gene status significantly correlated with protein status (p=0.038). In conclusion, gain but also loss of genomic MET activity correlates with aggressive tumour growth, nodal metastasis and worse overall survival in salivary gland cancer. Moreover, aberrations of MET are associated with EGFR and PTEN signalling and might possess relevance for targeted therapies of salivary gland carcinomas in the future.

Keywords Salivary gland cancer · MET · EGFR · PTEN · Prognosis

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Introduction

Salivary gland carcinomas are rare tumours comprising about 5 % of cancers of the head and neck region [1]. Due to their histomorphological diversity, dealing with these tumours poses an enormous challenge for pathologists and clinicians. MET, also known as hepatocyte growth factor

receptor, is frequently overexpressed in different cancer subtypes like non-small cell lung cancer, gastric cancer or oesophageal cancer [2–4]. Copy number gain or amplification of MET enhances tumour growth, invasiveness and metastasis [5]. Moreover, increased MET gene copy number is reported to go along with resistance against epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), among others by activating the PI3K-AKT-mammalian target of rapamycin (mTOR) pathway [6]. The present study investigates genomic aberrations and immunohistochemical MET expression in a representative cohort of salivary gland cancer and correlates the results to genomic EGFR and phosphatase and tensin homologue (PTEN) status, as PTEN is a negative regulator of the PI3K-AKT-mTOR signalling axis.

Patients and methods

Patients and therapeutic procedures

The study collective consists of 266 patients (135 females, 50.8 %) with a mean age of 60.2 years (range, 11–98) diagnosed with a carcinoma of the major or minor salivary glands. All patients were treated at the University Hospital Regensburg, the University Hospital Erlangen-Nuremberg and at the Hospital Clinic of Nuremberg between 1984 and 2008. Of the tumours, 188 (70.3 %) were localised in the parotid gland; 36 (13.5 %), in the submandibular gland; and 2 (0.8 %), in the sublingual gland. A minor salivary gland was the origin of 41 tumours (14.1 %). All patients underwent primary surgery. In 188 cases (70.7 %), conservative or radical neck dissection was performed. A total of 156 patients (58.6 %) were treated by adjuvant radio- or radiochemotherapy because of N + stage, high-grade malignancy, perineural invasion or positive resection margins. Pathological examination revealed that 82 patients (31.7 %) had cervical lymph node metastases. Of the patients, 29 (11.0 %) developed distant metastases. Altogether, 55.3 % of the cases were classified as in an advanced tumour stage (UICC III, IV).

Follow-up data were obtained from the clinical tumour registries of Regensburg and Erlangen-Nuremberg in agreement with the respective Research Ethic Guidelines of the medical faculties. The follow-up time ranges from 0.1 to 21.2 years (mean 4.85). Recurrence developed in 68 patients (26.6 %). The disease-related death rate was 24.8 % (66 patients) with a 5- and 10-year disease-specific survival rate of 74.6 and 69.3 %, respectively.

Histological classification

All tumours had been paraffin wax embedded, and haematoxylin–eosin-stained sections were examined by two independent experienced pathologists. The (sub)classification of the tumours was performed according to the current WHO classification of salivary gland tumours [7], resulting in 40 acinic cell carcinomas (ACCC), 45 adenoid cystic carcinomas (ADCC), 45 mucoepidermoid carcinomas (MEC), 26 salivary duct carcinomas (SDC), 26 adenocarcinomas NOS (ACNOS), 26 squamous cell carcinomas (SQCC), 21 myoepithelial carcinomas (MYEC), 8 polymorphous low-grade adenocarcinomas (PLGA), 7 basal cell adenocarcinomas (BCAC), 8 oncocytic carcinomas (OCC), 5 epithelial-myoepithelial carcinomas (EMC), 3 primary malignant mixed tumours (MMT), 2 undifferentiated carcinomas (UC), 2 large cell carcinomas (LCC) and 2 cystadenocarcinoma (CAC). In 20 cases, a diagnosis of carcinoma ex pleomorphic adenoma was made with as malignant component of the tumour: two ACCC, four SDC, eight ACNOS, one SQCC, two MYEC, two BCAC and one EMC. Grading was based on a three-tiered grading system [8, 9], with ACCC, BCAC, EMC, CAC and PLGA being evaluated as low grade (G1). Dedifferentiated tumours and SDC, SQCC, MMT, OCC, UC and LCC were evaluated as high grade (G3). Following the Elston and Ellis grading of breast cancer, all ACNOS and MYEC were classified in consideration of nuclear pleomorphism and mitotic activity [10]. The grading of mucoepidermoid carcinomas was performed according to the current WHO classification [7]. The mainly tubulo-cribriform adenoid cystic carcinomas were categorised as intermediate grade, and the mainly solid adenoid cystic carcinomas, as high grade. Carcinomas ex pleomorphic adenoma were graded on the basis of the malignant part of the tumour.

In the group of SQCC, intensive diagnostic procedures had been performed (CT/MRI of the head and neck, panendoscopy, X-ray/CT of the chest, ultrasonography of the abdomen) to exclude metastases to the salivary gland. Squamoid variants of mucoepidermoid carcinoma were ruled out as described before [11]. This allowed us to classify all SQCC as primary salivary gland tumours.

Immunohistochemistry

Immunohistochemistry was performed on tissues microarrays (TMA) with 2.0-mm punch cores from formalin fixed and paraffin-embedded tissue samples of all patients. Immunostaining of MET (detection EnVision Dual Link System, Dako, Glostrup, Denmark) was accomplished on 3-μm sections of the TMA. After preprocessing by dewaxing, washing and rehydration through xylene and graded alcohol, antigen retrieval was performed by microwave heating in Tris/EDTA buffer. The next steps were blocking of endogenous peroxidase by ChemMate peroxidase-blocking solution (Dako) and incubation with a primary antibody (D1C2, no. 8198, monoclonal rabbit, dilution 1:200, Cell Signaling Technology, Inc., Danvers, MA, USA). TMA sections were

also stained by haematoxylin–eosin for reference histological examination.

Immunohistochemical staining of MET was found in the cell membrane and cytoplasm. For semiquantitative analysis, the percentage of stained cells (0–100) and staining intensity (0, 1+, 2+, 3+) were multiplied to obtain an immunoreactivity score (IRS) ranging from 0 to 300 points. For dichotomisation, cases with an IRS 0–10 were considered MET-negative, whereas an IRS of >10 was considered MET positive (see Fig. 1). Samples of non-neoplastic salivary glands served as negative controls.

Fluorescence in situ hybridisation

TMA sections were fixed on charged slides (SuperFrost Plus; Menzel GmbH, Braunschweig, Germany) and directly labelled. ZytoLight SPEC EGFR/CEN7, SPEC PTEN/CEN10 and SPEC MET/CEN7 dual colour probes (ZytoVision, Ltd., Bremerhaven, Germany) were used to accomplish fluorescence in situ hybridisation (FISH). Afterwards, the labelled hybridisation nuclei were counterstained with anti-fading 4,6-diamidino-2-phenylindole VectaShield (Vector Laboratories, Burlingame, CA, USA). Haematoxylin–eosin-stained TMA sections were used for reference histology.

By epifluorescence microscopy (AxioImager-Z1, Zeiss, Göttingen, Germany), 50 non-overlapped single cell nuclei were detected by two independent examiners to assess the hybridisation signals. Samples of non-neoplastic salivary glands served as negative control.

The FISH ratio was assessed as the number of genes proportional to the number of centromeres. EGFR and MET were evaluated according to the principles of Cappuzzo et al. [12, 13]. Disomy was called when 2 gene/2 centromere signals were observed in >50 % of nuclei; low polysomy/trisomy, when 3 gene/3 centromere signals in >40 % were observed; and high polysomy, in case of ≥4 gene/≥4 centromere signals in >40 %. Amplification was defined by a ratio of gene/centromere ≥2 or gene signals ≥10 or gene clusters. Deletion of MET was defined as a ratio of gene/centromere <0.5 in more than 30 % of nuclei (Fig. 1). Low polysomy, high polysomy and amplification were categorised as copy number gain of MET. MET aberrations included deletion, low polysomy, high polysomy and amplification. For EGFR, only high polysomy and amplification were defined as copy number gain, according to previous studies [14].

PTEN was evaluated as described before [15]. Homozygous deletion of PTEN was defined by the simultaneous lack of both PTEN locus signals and by the presence of centromere signals in >20 % of nuclei. Hemizygous deletion of PTEN was defined as more than 30 % of tumour nuclei containing either one PTEN locus signal and ≥2 centromere signals or 2 PTEN locus signals and ≥4 centromere signals (relative deletions).

Statistical analysis

Data were analysed with IBM SPSS Statistics 19 (Ehningen, Germany). The Kaplan–Meier method was used to create the univariate survival curves, and distributions were compared by the log-rank test. Disease-specific overall survival was defined as the time lapse between the date of diagnosis and the disease-caused death or the end of follow-up. Cox proportional hazards model was used in multivariate analyses.

Results

MET FISH analysis was performed in 233 salivary gland carcinomas (Table 1). Physiological disomy was found in 144 (61.8 %) cases, 42 (18.0 %) cases presented low polysomy, 27 (11.6 %) high polysomy and 2 (0.9 %) true amplifications. Of the tumours, 18 (7.7 %) showed a deletion of MET. Disomy was found most frequently in ACCC (86.5 %), ADCC (88.6 %) and MEC (68.3 %). Low polysomy was frequently detected in SDC (41.7 %). SQCC (34.6 %), ACNOS (20.0 %) and SDC (16.7 %) often presented high polysomy. Among all 233 salivary gland carcinomas, only one ACNOS and one MYEC presented an amplification of MET. The MET deletion rate was highest in SDC (25.0 %), SQCC (11.5 %) and MYEC (10.0 %), but deletions were never found in ACCC or ADCC. Overall, 83.3 % (29/24) of salivary duct carcinomas showed MET aberrations.

Aberrations of MET (low and high polysomy, amplification and deletion) were clearly associated with increased patient age (>70 years, $p=0.003$), male gender ($p=0.01$), increased tumour size ($p=0.002$), lymph node metastases ($p<0.001$) and high-grade malignancy ($p<0.001$). Patients with high polysomy or deletion of MET presented an advanced tumour stage (III, IV) in 81.5 % (22/27) and 88.9 % (16/18), respectively. Tumours with copy number gain (CNG) of EGFR ($n=26$, 13.5 %) presented an increased copy number of MET (low and high polysomy and amplification) in 69.2 % (18/26) in contrast to 23.8 % (39/164) MET CNG in EGFR-negative tumours ($p<0.001$). Interestingly, deletion of MET was also associated with EGFR copy number gain (23.1 vs. 4.2 %, $p=0.031$). Tumours with genomic loss of PTEN ($n=48$, 22.6 %) significantly more often ($p<0.001$) showed an aberration of genomic MET (34/48, 70.8 %) compared to regular PTEN gene status (MET aberration in 47/164, 28.7 %). Copy number gain of MET was also strongly associated with loss of PTEN ($p<0.001$). No statistically relevant correlation could be detected between MET gene status and M-stage, respectively, tumour recurrence.

MET gene status correlated with protein status as tumours with CNG of MET more often presented increased immunohistochemical protein expression (31/58, 53.4 %), compared to tumours with disomy or deletion of MET (49/135, 36.3 %, $p=0.038$). Disease-specific overall survival of the patients

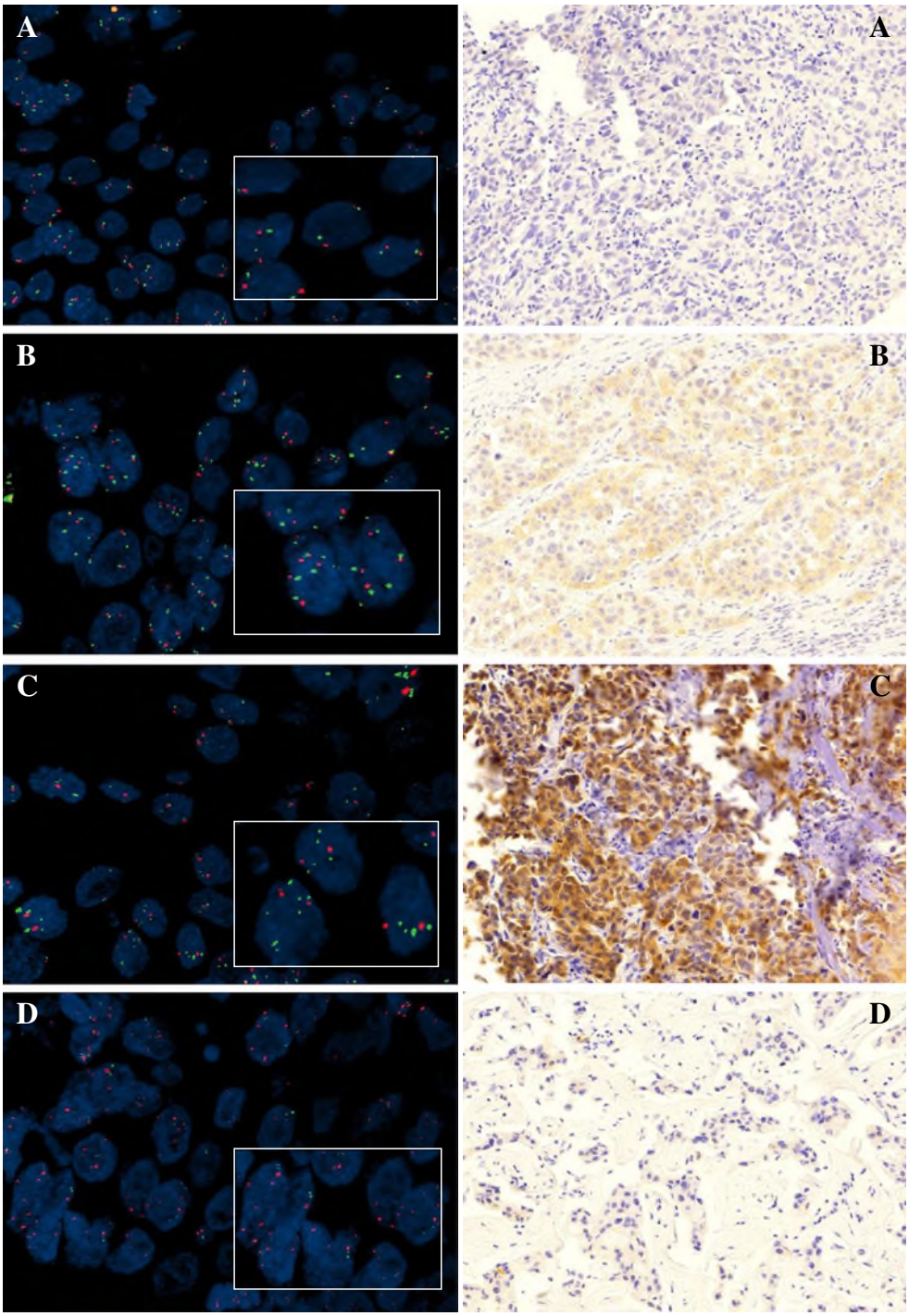

Fig. 1 Samples of FISH analysis of MET in different subtypes of salivary gland carcinomas and the corresponding immunohistochemical staining. **a** Disomy and negative staining (×10) in mucoepidermoid carcinoma. **b** High polysomy and weak cytoplasmatic/membranous staining (IRS 70 points, ×10) in mucoepidermoid carcinoma. **c** Amplification and strong cytoplasmatic staining (IRS 240 points, ×10) in adenocarcinoma NOS. **d** Deletion and negative staining (×20) in salivary duct carcinoma

distinctly differed according to MET gene status ($p<0.001$, Fig. 2). High polysomy but also deletion correlated with survival rates significantly worse than disomy or low polysomy.

MET was immunohistochemically expressed in 41.6 % (92/221) of the carcinomas. Staining of duct cells was found in SDC, ACNOS, ACCC and ADCC; staining of myoepithelial cells was detected in MYEC and ADCC; and staining of acinic cells was observed in ACCCs.

Out of the different subtypes, SQCC most frequently (14/22, 63.6 %) presented MET expression, followed by MEC (19/38, 50.0 %), ACCC (11/25, 44.0 %) and ADCC (17/40, 42.5 %). SDC presented MET staining in 33.3 % (7/21), ACNOS in 34.8 % (8/23) and MYEC in 36.8 % (7/19). A statistically significant correlation was not found ($p>0.05$) between increased immunohistochemical expression of MET and clinicopathological parameters.

Table 1 MET gene status and important clinicopathological parameters

Parameter	Disomy (%)	Low polysomy (%)	MET FISH High polysomy (%)	Amplification (%)	Deletion (%)	Total
Total	144 (61.8)	42 (18.0)	27 (11.6)	2 (0.9)	18 (7.7)	233
Histology						
ACCC	32 (86.5)	5 (13.5)	0	0	0	37
ADCC	31 (88.6)	3 (8.6)	1 (2.8)	0	0	35
MEC	28 (68.3)	8 (19.5)	4 (9.6)	0	1 (2.4)	41
SDC	4 (16.7)	10 (41.7)	4 (16.7)	0	6 (25.0)	24
ACNOS	11 (44.0)	6 (24.0)	5 (20.0)	1 (4.0)	2 (8.0)	25
SQCC	11 (42.3)	3 (11.5)	9 (34.6)	0	3 (11.5)	26
MYEC	10 (50.0)	6 (30.0)	1 (5.0)	1 (5.0)	2 (10.0)	20
PLGAC	6 (85.7)	0	0	0	1 (14.3)	7
OCC	3 (60)	0	0	0	2 (40)	5
BCAC	4 (100.0)	0	0	0	0	4
MMT	1 (33.3)	0	1 (33.3)	0	1 (33.3)	3
EMT	1 (50)	0	1 (50.0)	0	0	2
LCC	2 (100.0)	0	0	0	0	2
UC	0	0	1 (100.0)	0	0	1
CAC	0	1 (100.0)	0	0	0	1
Age						
<70	**97 (71.9)**	**20 (14.8)**	**11 (8.1)**	**2 (1.5)**	**5 (3.7)**	**135**
>70	**31 (46.9)**	**17 (25.8)**	**10 (15.2)**	**0**	**8 (12.1)**	**66**
Gender						
Male	**63 (53.4)**	**22 (18.6)**	**19 (16.1)**	**1 (0.8)**	**13 (11.0)**	**118**
Female	**81 (70.4)**	**20 (17.4)**	**8 (6.7)**	**1 (0.8)**	**5 (4.3)**	**115**
T stage						
T1–T2	**93 (70.9)**	**23 (17.6)**	**9 (6.8)**	**1 (0.7)**	**5 (3.8)**	**131**
T3–T4	**48 (48.9)**	**18 (18.4)**	**18 (18.4)**	**1 (0.1)**	**13 (13.3)**	**98**
N stage						
N0	**112 (73.2)**	**19 (12.4)**	**12 (7.8)**	**2 (1.3)**	**8 (5.2)**	**153**
N1–N3	**27 (37.0)**	**21 (28.8)**	**15 (20.5)**	**0**	**10 (13.7)**	**73**
M stage						
M0	129 (62.6)	34 (16.5)	25 (12.1)	2 (1.0)	16 (7.8)	206
M1	12 (50.0)	8 (33.3)	2 (8.3)	0	2 (8.3)	24
Grading						
G1–G2	**93 (80.9)**	**15 (13.0)**	**6 (5.2)**	**0**	**1 (0.9)**	**115**
G3	**51 (43.2)**	**27 (22.9)**	**21 (17.8)**	**2 (1.7)**	**17 (14.4)**	**118**
Recurrence						
No recurrence	110 (67.1)	22 (13.4)	18 (10.9)	2 (1.2)	12 (7.3)	164
Recurrence	33 (55.0)	16 (26.7)	7 (11.7)	0	4 (6.7)	60
EGFR FISH						
No CNG	**115 (70.1)**	**29 (17.7)**	**9 (5.5)**	**1 (0.6)**	**10 (6.1)**	**164**
CNG	**5 (19.2)**	**6 (23.1)**	**11 (42.3)**	**1 (3.8)**	**3 (11.5)**	**26**
PTEN						
No deletion	**117 (71.3)**	**24 (14.6)**	**13 (7.9)**	**1 (0.6)**	**9 (5.5)**	**164**
Deletion	**14 (29.2)**	**13 (27.1)**	**12 (25.0)**	**1 (2.1)**	**8 (16.7)**	**48**
MET IHC						
0, 1+	72 (62.7)	18 (15.9)	8 (7.1)	1 (0.9)	14 (12.4)	113
2+, 3+	45 (56.3)	18 (22.5)	12 (15.0)	1 (1.3)	4 (5.0)	80

Statistically significant associations are highlighted in bold

ACCC acinic cell carcinoma, *ADCC* adenoid cystic carcinoma, *MEC* mucoepidermoid carcinoma, *SDC* salivary duct carcinoma, *ACNOS* adenocarcinoma NOS, *SQCC* squamous cell carcinoma, *MYEC* myoepithelial carcinoma, *CNG* copy number gain, *FISH* fluorescence in situ hybridisation, *IHC* immunohistochemistry

Discussion

This study investigated the impact of MET aberrations on the behaviour of salivary gland carcinomas. MET (hepatocyte growth factor receptor) was first described in 1984 time [16] and is a proto-oncogene which plays a key role in malignant transformation, particularly in tumours with increased MET gene copy number. Increased MET activity is associated with cell proliferation, cell motility and cell dissociation. Moreover, MET stimulates tissue infiltration and

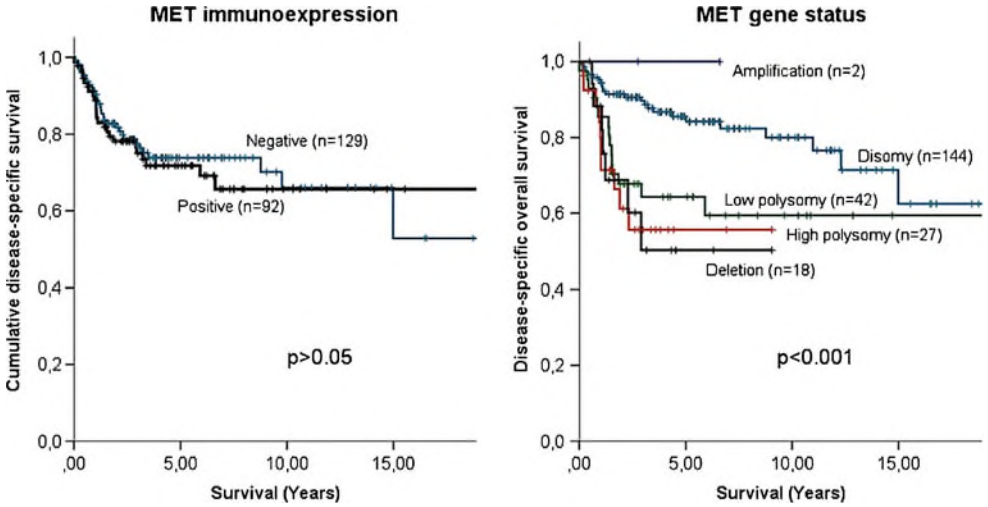

Fig. 2 Kaplan–Meier survival analyses of MET FISH and immunohistochemistry

angiogenesis [17]. These are all associated with metastatic propensity, which is reflected in the correlation of MET aberrations with poor prognosis of various cancer types like bladder, breast, colorectal, gastric, kidney, liver, lung, pancreas, prostate and also head and neck cancer [18].

In our investigated salivary gland carcinomas, MET aberrations occurred in different subtypes, mainly in high-grade carcinomas like salivary duct carcinomas, adenocarcinomas NOS or squamous cell carcinomas. Copy number gain of MET was mainly found as low or high polysomy of chromosome 7, for example, in salivary duct carcinomas or adenocarcinomas NOS, whereas true amplification was only observed in one adenocarcinoma NOS and one myoepithelial carcinoma. Tumours with increased MET gene copy number were more aggressive in that patients presented with advanced tumour stage and showed worse overall survival in comparison to regular MET status.

Apart from gene copy gain, this study is the first report of genomic MET deletions in certain types of salivary gland cancer. Of the MET deletions, 95 % occurred in high-grade variants with worse overall survival pointing to an implication of MET loss in high-grade transformation of the salivary glands. Of the salivary duct carcinomas, 25 % presented a deletion of MET, which highlights the genomic instability of this entity as other aberrations like copy number gain or mutation of HER2 and EGFR or deletion of PTEN have already been described before [14, 15, 19, 20]. We cannot explain the mechanisms of MET deletions in the carcinogenesis of salivary gland carcinomas. In a hepatocyte knockout mouse model (c-met$^{fl/fl}$, AlbCre$^{+/-}$; Met LivKO mice), loss of the hepatocyte growth factor/c-MET signalling pathway accelerated *N*-nitrosodiethylamine-induced hepatocarcinogenesis with the development of significantly more and bigger tumours in comparison with control mice [21]. Loss of MET increased cellular stress with imbalance of redox homeostasis, leading to tumour progression. Moreover, loss of MET was associated with prolonged activation of EGFR signalling in these tumours. In our salivary gland tumours, we observed a correlation between MET deletion and copy number gain of EGFR, but also, gain of genomic MET was associated with increased EGFR gene status, indicating that any form of MET instability in salivary gland cancer goes along with activation of EGFR signalling. In addition, we show that MET aberrations are associated with loss of PTEN function. As mentioned above, PTEN deletions are often found in salivary duct carcinomas but also in other subtypes of high-grade salivary gland carcinomas, associated with worse overall survival [15].

In addition to the genomic MET status, we also studied MET protein expression by immunohistochemistry. A high proportion of MET expressing cases was found in squamous cell carcinoma, mucoepidermoid carcinoma, acinic cell carcinoma and adenoid cystic carcinoma along with increased gene copy number, but without significant associations with clinicopathological parameters or long-term survival. Similar findings have been reported for non-small cell lung cancer as MET protein expression also did not correlate with patient survival, whereas FISH analysed gene status did [22].

We found immunohistochemical expression of MET to correlate with regional lymph node and distant metastasis in high-grade carcinomas [23]. In vitro, stimulation of an adenoid cystic carcinoma cell line by rhHGF induced scattering and promoted tumour invasion [24]. Moreover, enhanced expression of CD151 boosts the HGF-induced impact on tumour characteristics, especially in adenoid cystic carcinomas [25].

In addition to its prognostic value, genomic MET aberration might be important for targeted therapy of salivary gland cancer. MET itself could function as a target for antibody therapy. For example, partial remission was observed in advanced MET-amplified gastro-oesophageal and non-small cell lung cancer when treated with the MET kinase inhibitor crizotinib [26, 27].

MET function might modulate the efficacy of anti-EGFR therapy. In lung cancer, a weak effect of EGFR tyrosine kinase inhibitors like gefitinib and erlotinib has been reported in patients with high MET levels [6, 13, 28, 29]. One reason might be that amplification of MET stimulates the PI3K-AKT-mTOR pathway by activation of HER3, which leads to tumour growth and invasiveness independently from EGF receptor inhibition [6]. In contrast, additional inhibition of MET might increase sensitivity for EGFR TKIs. In vitro studies on lung cancer cells with acquired EGFR resistance triggered by MET amplification showed that incubation with a MET inhibitor restores cell sensitivity to EGFR inhibition [6, 30]. Interestingly, EGFR signalling seems responsible for acquired MET kinase inhibitor resistance as well [31–33]. These findings suggest that combined therapy, consisting of both MET and EGFR inhibitors, might improve patient outcome [29].

We found loss of PTEN, a crucial step in the activation of the PI3K-AKT-mTOR pathway, to be associated with MET aberration and MET copy number gain. In glioblastoma cells, PTEN inhibits MET-induced cell proliferation, migration and invasion. Moreover, restoration of PTEN combined with c-MET blocking additively inhibits tumour proliferation and cell cycle progression [34]. In salivary gland carcinomas, PTEN loss has been found in tumours with increased levels of EGFR and HER2 [15]. Glioblastoma in breast cancer and loss of PTEN in colorectal cancer go along with resistance to anti-EGFR and anti-HER2 therapy [35–37].

During the last years, there have been first clinical phase II studies on targeted therapies against EGFR and HER2 in salivary gland cancer, and response rates, however, are rather disappointing [38–42]. Explanations for this therapy resistance are lacking. One reason might be that most studies include high numbers of adenoid cystic carcinomas in which usually EGFR or HER2 is not up-regulated [14]. In none of these studies, the genomic status of EGFR or HER2 was evaluated prior to the beginning of therapy. Another reason might be the above-described impact of MET aberrations and PTEN loss leading to autonomous stimulation of the PI3K-AKT-mTOR signalling, resulting in tumour growth independently from inhibition of the erbB-1 or erbB-2 receptor. Therefore, personalised therapy might require a combination of approaches targeting MET, PTEN and mTOR. Recently, Piha-Paul et al. published a case report of a 70-year-old patient with cutaneous and mediastinal metastases of a salivary duct carcinoma, who had been unsuccessfully treated by docetaxel, cetuximab and radiotherapy. The patient tumour showed loss of PTEN, and after starting treatment with the mTOR inhibitor temsirolimus (20 mg IV weekly), a partial response (lesion volume decrease of 62 %) was observed at the end of the second cycle [43].

In summary, we report genomic MET aberrations, particularly MET deletions, in certain types of salivary gland cancer associated with unfavourable outcome not only for high MET activity but also for MET deletion. Moreover, aberrations of MET are associated with EGFR and PTEN signalling, which might be relevant for the development of targeted therapy of salivary gland carcinomas.

Conflict of interest The authors declare that they have no conflict of interest.

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