KIT/PDGFRA/KDR amplification defines a novel molecular subtype of adenoid cystic carcinoma patients who may benefit from treatment with tyrosine kinase inhibitors

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Background: Adenoid cystic carcinoma (ACC) is a rare cancer with an aggressive phenotype and the high incidence of recurrence and distant metastasis severely affects the overall survival of ACC patients. Understanding the molecular mechanisms that drives ACC could improve the treatment and outcomes of patients with this disease.

Methods: Actionable genetic alterations in 52 surgically resected ACC tissue samples were identified using targeted next generation sequencing (NGS). Expression of c-KIT/PDGFRα/VEGFR2 was assessed by immunohistochemistry (IHC). Sunitinib, a multi-targeted small molecule inhibitor of receptor tyrosine kinases (RTKs), was used off-label in one ACC patient harboring the *KIT/PDGFRA/KDR* amplification.

Results: Potentially actionable genetic alterations were detected in 61.5% (32/52) of patients. In addition to the common actionable targets identified in NOTCH signaling and FGF/PI3K pathway, multiple novel gene fusions were detected in 7.7% (4/52) of ACC patients. Specifically, the *KIT/PDGFRA/KDR* amplification was identified in 2 of 52 (3.8%) cases and triple positive c-KIT/PDGFRα/VEGFR2 by IHC was associated with a significantly higher likelihood of distant metastasis. Furthermore, an advanced ACC patient with the *KIT/PDGFRA/KDR* amplification and who was positive for three encoded proteins showed a partial response to sunitinib.

Conclusions: A total of 61.5% of ACC patients were found to harbor at least one actionable genetic alteration via a targeted NGS in this study. The *KIT/PDGFRA/KDR* amplification as well as triple positive c-KIT/PDGFRα/VEGFR2 defined a distinctive molecular phenotype that was characterized by distant metastasis. Clinical trials investigating the application of RTKs in ACC patients with the *KIT/PDGFRA/KDR* amplification or triple positive c-KIT/PDGFRα/VEGFR2 are warranted.

Keywords: Adenoid cystic carcinoma (ACC); *KIT/PDGFRA/KDR*; sunitinib; actionable genetic alteration; next generation sequencing (NGS)

Introduction

Adenoid cystic carcinoma (ACC) is an uncommon malignant tumor that most frequently arises in salivary glands and other locations (1). Despite of its slow growth kinetics, ACC has a poor prognosis due to its high rate of perineural invasion, recurrence and distant metastasis (2). Extended local excision with radiation therapy is the main primary treatment choice followed by additional resection as required for the local recurrence (2). Owing to chemotherapy refractory for ACC, there is no standard of systemic treatment for advanced disease, representing a major unmet need (3). In terms of 19 reported clinical trials of targeted therapy, only 5.5% of the ACC cases achieved objective response (OR) (4). Given the recent advances in DNA sequencing and targeted agents, uncovering the molecular basis of ACC may facilitate the identification of effective treatments.

It has been reported that *MYB-NFIB* fusion, *MYBL1-NFIB* fusion, high MYB expression and high c-KIT expression represent the fundamental signature of ACC (5-8). However, the exact biological roles of these events are not yet entirely clear. Until recently, multiple genetic mutations selectively involved in chromatin remodeling, DNA damage and protein kinase A signaling were identified via whole genome and whole exome sequencing, which have highlighted the critical roles of these aberrations in ACC oncogenesis (9-12). Targeted alterations of the FGF/IGF/PI3K pathway and Notch signaling (9,10,13,14), were frequently identified in ACC, suggesting potential new avenues for this disease. In particular, the frequency of targeted genetic alterations in ACC appears to be much lower than that seen in the common solid tumors (13,15,16), irrespective of disease site of origin. In the present study, targeted next generation sequencing (NGS) was performed for 52 ACC cases to further identify additional targets for potential therapeutic application. Furthermore, the clinical significance of high c-KIT/PDGFRα/VEGFR2 expression in ACC was analyzed.

We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/tcr-20-637).

Methods

Patients and samples

From January 2014 to December 2016, 52 ACC cancer tissues were collected. The diagnosis of ACC was confirmed by hematoxylin and eosin (HE) staining. Patient characteristics are shown in *Table 1*. This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. The number of Ethic Approval was 20160713. The investigations were carried out following the rules of the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all patients included in the study, and all the experiments were carried out in accordance with the National Health and Family Planning Commission of the PRC's guidelines.

NGS-based assay

In this study, paraffin-embedded tissue samples were obtained for NGS assay. Tumor DNA and library construction were performed as previously described (17). Target enrichment was performed with a custom sequence capture-probe (Nimblegen, USA) that targeted 7,708 exons of 508 cancer-related genes and 78 introns from 19 genes recurrently rearranged in solid tumor, representing ~1.7 Mb of the human genome in total (17). Sequencing was performed with 2×101 bp paired-end reads and 8-bp index reads on an Illumina Hiseq 2500 platform (Illumina, San Diego, USA) using the manufacturer's protocols.

Primary sequence data were first processed by filtering adaptor sequences and removing low-quality reads using the SOAPnuke software (http://soap.genomics.org.cn/) developed by BGI, and then reads were aligned to build hg19 of the NCBI reference genome assembly using BWA aligner v0.6.2-r126. PCR duplicate reads were removed by PICARD v1.98. Local realignment and base quality score recalibration were performed using GATK v2.3-9, and poorly mapped reads were removed based on the recalibration results. SNVs were detected by Mutect and SOMATK-SNV (developed by BGI, manuscript in preparation), and Indels (small insertions and deletions) were detected by GATK and SOMATK-INDEL (developed by BGI, manuscript in preparation). A minimal amplicon coverage of 300 was defined, and a variant allele frequency of 1% was used as the minimal threshold to provide reliable diagnostic analysis. CNV calling was performed by CONTRA v2.0.4 (18). We defined a gene as showing copy number gain when its coverage fold ratio was ≥2.0, and loss was defined when it was ≤0.5.

Immunohistochemistry (IHC)

Paraffin-embedded tissues were serially sectioned at 5 mm

Table 1 Clinical characteristics of the 52 adenoid cystic carcinoma patients

Patient characteristic	No. of patients (N=52)	Proportion (%)
Age (yr) [range]	51.8 [23–78]	–
Sex		
Male	21	40.4
Female	31	59.6
Smoking history		
Never	36	69.2
Former/current	16	30.8
Primary tumor site		
Major salivary gland	21	40.4
Minor salivary gland	19	36.5
Trachea	6	11.5
Other sites	6	11.5
Histological subtype		
Tubular/cribriform	42	80.8
Solid	10	19.2
Disease stage at diagnosis		
I/II/III	29	55.8
IV	19	36.5
Unknown	4	7.7
Perineural invasion		
Yes	18	34.6
No	34	65.4
Tissue sample source		
Primary	42	80.8
Recurrence	7	13.5
Metastatic	3	5.8
Disease recurrence/metastasis		
Yes	20	38.5
No	18	34.6
Unknown	14	26.9
Recurrence/metastasis site (N=20)		
Local	7	13.5
Distant metastasis	13	25.0
Treatment modality to the primary tumor		
Surgery	31	59.6
Surgery plus radioactive seed implants	11	21.2
Surgery plus adjuvant radiation therapy	5	9.6
Others	5	9.6

and IHC was performed. Antigen retrieval was performed by boiling the slides in 10 mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature for 20 min. Each section was incubated with primary antibodies overnight at 4 ℃. The following primary antibodies and dilutions were used: c-KIT (#ZA-0523, ZSGB-Bio, Beijing, China, diluted 1:200), PDGFRα (#E2690, Amsbio, Oxon, UK, diluted 1:400) and VEGFR2 (#E3710, Amsbio, Oxon, UK, diluted 1:200). Staining was completed with a DAB detection kit (ZLI-9019, ZSGB-Bio, Beijing, China) and counterstained with hematoxylin (ZLI-9615, ZSGB-Bio, Beijing, China). Two investigators evaluated the IHC staining based on the modified histoscore protocols published previously (19,20). The expression of c-KIT, PDGFRα and VEGFR2 was evaluated according to the percentage of the stained cells and staining intensity. The percentages of stained cancer cells were divided into 3 categories, (<5%, 5–50%, >50%). The staining intensity was divided into 3 categories, (negative, pale brown, brown). A tumor tissue sample was considered to be positive if only the percentage of brown stained cancer cells was greater than 50%. We defined samples with less than 5% and 5–50% stained cancer cells as negative and weakly positive expression respectively. Staining intensity was ignored for interpreting negative or weakly positive results. Finally, any tumor sample with more than 50% pale brown stained cancer cells was classified as weakly positive.

Statistical analysis

The experimental data are presented as the means ± SEM. Pearson's Chi-squared test, Fisher's exact test and Student's *t*-test were used to compare the gene expression status and patient characteristics between the two groups. The Kaplan-Meier method was used to estimate relapse-free survival (RFS). For the analysis of RFS, data for patients who did not have surgical treatment and who were lost to follow-up were excluded. P values were two tailed for all tests. P<0.05 was used to define statistical significance.

Results

Clinical characteristics and NGS assay

Fifty-two ACC cases were selected for NGS assays, and the patient characteristics are listed in *Table 1*. The mean age was 51.8 years (range, 23–78 years). The ACC cases included carcinomas of the major salivary gland (40.4%),

minor salivary gland (36.5%), trachea (11.5%) and other sites (11.5%), comprised the entire ACC patients studied. The tumor tissues for NGS assay included 42 primary, 7 relapsed and 3 metastatic samples. Genetic alterations were found in 260 genes among all 52 patients (mean 10.1±4.2, range, 2–20) with a median average sequencing depth of 626±287 for tumor DNA from each sample. Nineteen genes were detected in more than 5 samples (*Figure 1*), including those with targeted alterations and without available targeted agents. The most frequently mutated gene was *Notch1* (n=19, 36.5%).

Molecular profiling of ACC identified by NGS

In total, 32 of 52 (61.5%) patients had at least one potentially actionable alteration (*Table S1*), while 38.5% (20/52) of patients had no actionable somatic alterations. The mean number of potentially actionable alterations per patient was 1.1 (1.1±1.2, range, 0–4). Molecular profiling of the 52 patients identified by the NGS assay is shown in *Figure 2*. The specific alterations that were observed in the most common actionable targets included the following: *Notch1* (N=7, 13.5%), *HER2* (N=5, 9.6%), *PIK3CA* (N=5, 9.6%), *PTEN* (N=4, 7.7%) and *FGFR2* (N=3, 5.8%) (*Figure 2*). In addition, gene arrangements, which included *ETV6-ABL1*, *ETV6-NTRK3*, *KDR-PDGFRA* and *RET-KIAA1217*, were identified in 4 of 52 (7.7%) ACC patients. *RAS* mutations (1*KRAS* and 2*HRAS*) were identified in 7.0% (3/43) of patients with ACC in the head and neck region, which might not benefit from anti-EGFR agents. Furthermore, 21.0% (9/43) of ACC in the head and neck harboring somatic mutations or copy number variations (CNV) in *PIK3CA*, *PTEN* and *HER2* might be associated with a low response to anti-EGFR therapy.

KIT/PDGFRA/KDR amplification and protein expression in ACC

Notably, amplification of the three receptor tyrosine kinase (RTK)-encoding oncogenes *KIT*, *PDGFRA* and *KDR*, closely spaced at 4q12, were identified in 2 of 52 (3.8%) ACC patients (*Figure 2*). By IHC assessment, high protein expression of all three RTKs was designated as triple-positive ACC. Fifteen of the 52 (28.8%) evaluated ACC tumors were c-KIT/PDGFRα/VEGFR2 triple-positive, including the aforementioned 2 cases with increased gene copy number of *KIT/PDGFRA/KDR* (*Figure 3A*).
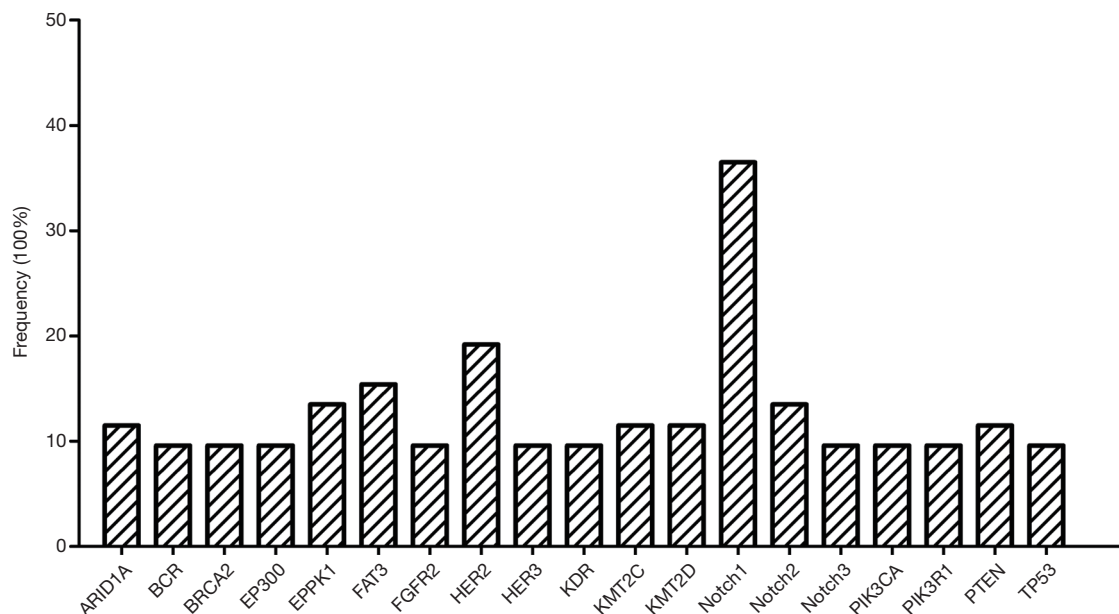
Figure 1 Genetic alterations identified in more than 5 samples by next generation sequencing.

Representative IHC staining of c-KIT, PDGFRα and VEGFR2 is shown in *Figure 3B*. Correlation analysis between clinical characteristics and c-KIT/PDGFRα/ VEGFR2 protein expression status is presented in *Table 2*. Compared with non-triple positive ACC patients, those with triple positive c-KIT/PDGFRα/VEGFR2 were more likely to develop distant metastasis in the lung, liver, bone and other atypical sites (63.6% *vs.* 22.2%, P=0.024) (*Table 2*, *Figure 3C*). No differences in other clinical characteristics were seen between triple positive and non-triple positive patients. RFS data were obtained for 36 ACC patients. However, the median RFS did not significantly differ between the triple positive and the non-triple positive groups (P=0.793) (*Figure 3D*). Remarkably, triple positive ACC patients were prone to developing distant metastasis than non-triple positive patients (23.1 months *vs.* NA, P=0.043) (*Figure 3E*).

Sunitinib exhibited clinical activity in a patient with KIT/ PDGFRA/KDR amplified ACC

A 30-year-old female was diagnosed with ACC of the right soft palate by biopsy. She received extended resection to the right soft palate and pharynx tumor, dissection of right cervical lymph nodes and radioactive seed implantation. Due to the high expression of EGFR confirmed by IHC, she was then treated with 6 cycles of the anti-EGFR antibody

nimotuzumab. CT and MRI scans, respectively, revealed bilateral pulmonary metastasis and left cervical lymph nodes metastasis, 2.5 years after the first surgery. She then received dissection of the left cervical lymph nodes followed by local radioactive seed implantation. Two cycles of intro-thoracic cis-platin chemotherapy were performed to treat massive pleural effusion. After further disease progression, the NGS assay of the primary tumor sample identified amplification of *KIT/PDGFRA/KDR* (*Figure 4A*) and triple positive for the encoded RTKs was demonstrated by IHC (*Figure 4B*). The patient was treated with sunitinib (37.5 mg, 4/2 W), a TKI targeting c-KIT/PDGFR/VEGFR2 and achieved a partial response (PR) after 2 cycles of treatment. Unfortunately, the patient experienced several Grade 2–3 adverse events including hypertension, fatigue, diarrhea, depression, stomatitis and skin discoloration during sunitinib targeted therapy. Although these adverse events led to sunitinib discontinuation and disease progression, the patient eventually had a duration of response of 6.5 months (*Figure 4B*).

Discussion

ACC is characterized by progressive local growth, a propensity for perineural invasion and high incidence of distant metastasis (21). While there have been many discoveries on its molecular biology, there is no standard
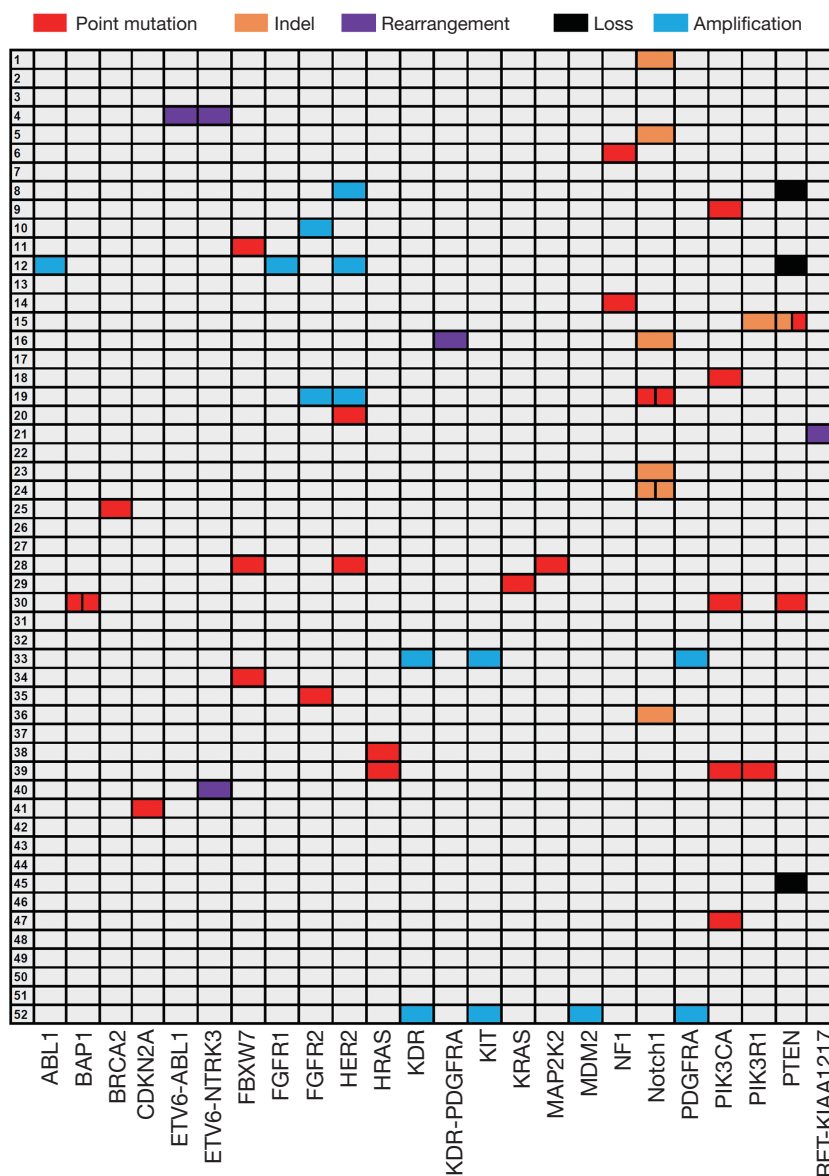
Figure 2 Comprehensive annotation of targeted genetic alterations in the 52 adenoid cystic carcinoma cases. Targeted genetic alterations detected in the 52 adenoid cystic carcinoma cases by next generation sequencing were shown. The divided boxes in cases 15, 19, 24 and 30 mean compound mutations.

systemic treatment for the management of metastatic or locally recurrent ACC (3). Sequencing of ACC has shed light on the genetic landscape of this disease, revealed genetic mutations associated with chromatin remodeling and provided evidence for actionable genetic alterations in the FGF/IGF/PI3K pathway and NOTCH signaling (9,10,13). In this study, we also found frequent actionable targets in the FGF/PI3K pathway and Notch1 signaling. Interestingly, *Notch1* was the most frequently mutated gene

in our study with a prevalence of potentially activating mutations in *Notch1* of 13.5%. A recent study found *Notch1* mutations in 14.7% of 102 ACC patients and the majority was assessed to be activating by IHC (14). *Notch1* mutations in ACC were associated with solid histology, liver and bone metastasis, poor prognosis, and potential responsiveness to Notch1 inhibitors in ACC (14). All these findings hint that *Notch1* activating mutations might be a valuable therapeutic target for ACC. Consistent with the previous studies, gene
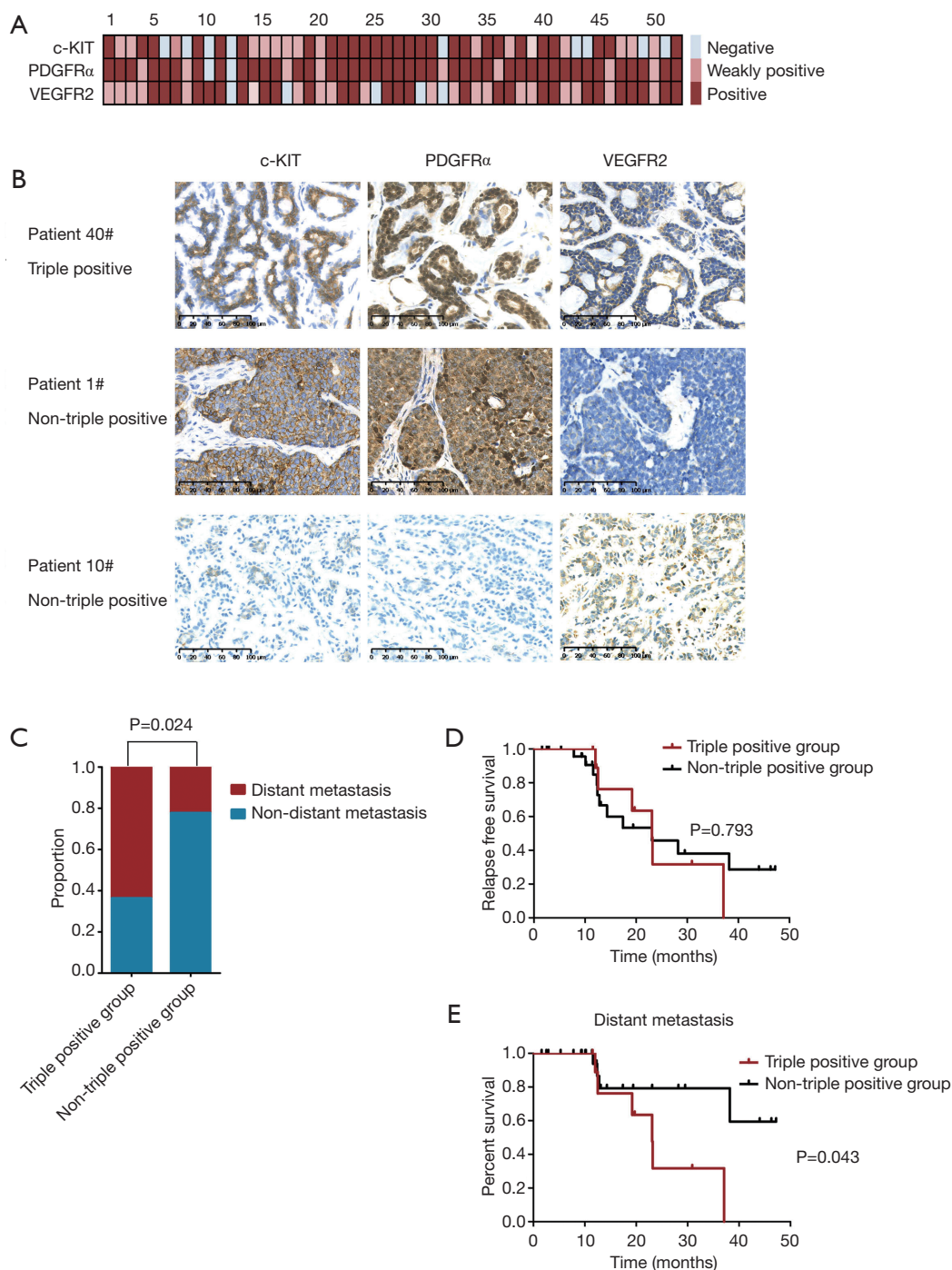
Figure 3 Expression of c-KIT/PDGFRα/VEGFR2 in adenoid cystic carcinoma. (A) Summary of c-KIT/PDGFRα/VEGFR2 by immunohistochemistry in 52 adenoid cystic carcinoma cases. (B) Representative immunohistochemistry staining for different expression levels of c-KIT/PDGFRα/VEGFR2 in adenoid cystic carcinoma. Original magnification ×200. (C) Comparative analysis of the occurrence of distant metastasis between the patients with and without triple positive c-KIT/PDGFRα/VEGFR2. (D) Kaplan-Meier estimate of relapse free survival for patients with triple positive c-KIT/PDGFRα/VEGFR2 versus non-triple positive c-KIT/PDGFRα/VEGFR2. (E) Kaplan-Meier estimate of occurrence of distant metastasis for patients with triple positive c-KIT/PDGFRα/VEGFR2 versus non-triple positive c-KIT/PDGFRα/VEGFR2.

Table 2 Correlation between clinical characteristics and c-KIT/PDGFRα/VEGFR2 expression

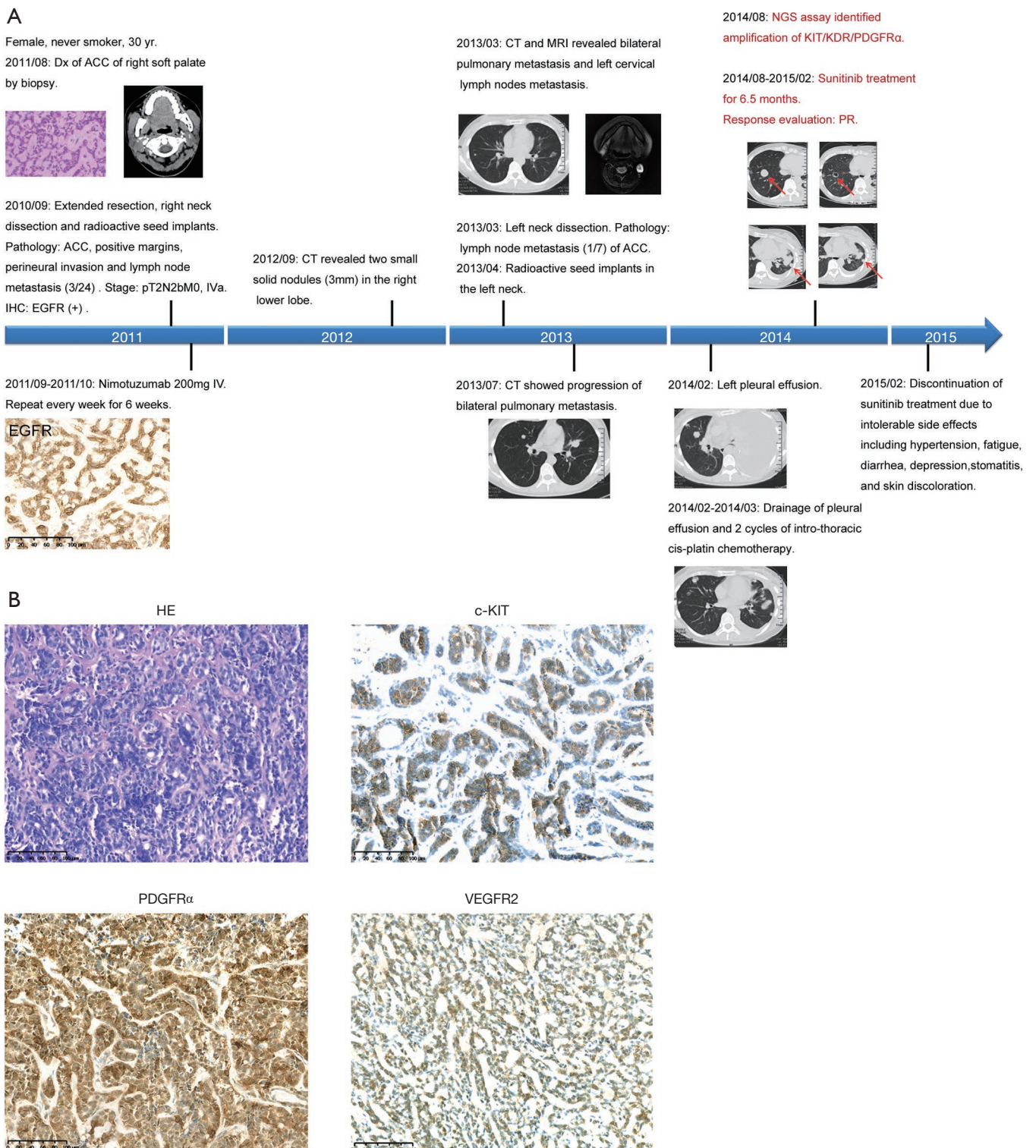| Patient characteristic	Triple positive (N=15)		Non-triple positive (N=37)		P
	No.	%	No.	%	
Primary tumor site					0.209
Major salivary gland	5	33.3	16	43.2	
Minor salivary gland	4	26.7	15	40.5	
Trachea	3	20.0	3	8.1	
Other sites	3	20.0	3	8.1	
Histological subtype					0.448
Tubular/cribriform	11	73.3	31	83.8	
Solid	4	26.7	6	16.2	
Disease stage at diagnosis					1.000[a]
I/II/III	8	53.3	21	56.8	
IV	6	40.0	13	35.1	
Unknown	1	6.7	3	8.1	
Perineural invasion					1
Yes	5	33.3	13	35.1	
No	10	66.7	24	64.9	
Tissue sample source					0.336
Primary	11	73.3	31	83.8	
Recurrence	2	13.3	5	13.5	
Metastatic	2	13.3	1	2.7	
Disease recurrence/metastasis					0.485[b]
Yes	7	46.7	13	35.1	
No	4	26.7	14	37.8	
Unknown	4	26.7	10	27.0	
Recurrence/metastasis site (20/36)					
Local	2[c]	18.2	7	25.9	0.690
Distant metastasis	7	63.6	6	22.2	0.024

[a], compared PDGFRα/VEGFR2/c-KIT expression between stage IV and stages I to III. [b], patients without available follow-up data were excluded from statistical analysis. [c], 2 patients with triple positive PDGFRα/VEGFR2/c-KIT recurred locally first and then developed distant metastasis finally.

mutations linked to chromatin remodeling such as *ARID1A*, *BRCA2*, *EP300*, *KMT2C* and *KMT2D* were also frequently detected in our study, confirming the importance of chromatin remodeling dysfunction in ACC tumorigenesis (4,9-11).

MYB-NFIB and *MYBL1-NFIB* arrangements have been thought to be signature molecular events of ACC (5). *MYB-NFIB* arrangement was found to account for about half of ACC patients (10,13). In 35% of the *MYB-NFIB*-negative ACC cases, another study identified novel inter- and intra-chromosomal rearrangements of the *MYBL1-NFIB* (8). In terms of prognosis, an association was found between *MYB* alterations, rather than *MYBL1* alterations, and poor patient outcome (8). Most importantly, one recent study found

A

Female, never smoker, 30 yr.
2011/08: Dx of ACC of right soft palate
by biopsy.

2013/03: CT and MRI revealed bilateral
pulmonary metastasis and left cervical
lymph nodes metastasis.

2014/08: NGS assay identified
amplification of KIT/KDR/PDGFRα.

2014/08-2015/02: Sunitinib treatment
for 6.5 months.
Response evaluation: PR.

2010/09: Extended resection, right neck
dissection and radioactive seed implants.
Pathology: ACC, positive margins,
perineural invasion and lymph node
metastasis (3/24) . Stage: pT2N2bM0, IVa.
IHC: EGFR (+) .

2012/09: CT revealed two small
solid nodules (3mm) in the right
lower lobe.

2013/03: Left neck dissection. Pathology:
lymph node metastasis (1/7) of ACC.
2013/04: Radioactive seed implants in
the left neck.

| 2011 | 2012 | 2013 | 2014 | 2015 |

2011/09-2011/10: Nimotuzumab 200mg IV.
Repeat every week for 6 weeks.

EGFR

2013/07: CT showed progression of
bilateral pulmonary metastasis.

2014/02: Left pleural effusion.

2015/02: Discontinuation of
sunitinib treatment due to
intolerable side effects
including hypertension, fatigue,
diarrhea, depression,stomatitis,
and skin discoloration.

2014/02-2014/03: Drainage of pleural
effusion and 2 cycles of intro-thoracic
cis-platin chemotherapy.

B

HE

c-KIT

PDGFRα

VEGFR2

Figure 4 A case of adenoid cystic carcinoma with the *KIT/PDGFRA/KDR* amplification showed a significant response to sunitinib treatment. (A) Timeline of treatments for a female patient with adenoid cystic carcinoma. The patient harbored the *KIT/PDGFRA/KDR* amplification and achieved a partial response of 6.5 months upon treatment with sunitinib. (B) Triple positive c-KIT/PDGFRα/VEGFR2 expression in this *KIT/ PDGFRA/KDR* amplified patient was confirmed by immunohistochemistry. Original magnification ×200. Red arrows: pulmonary metastasis.

that *MYB-NFIB* acted as an oncogenic driver and a key therapeutic target in ACC by regulating AKT-dependent IGF1R signaling (22). Despite these advances, the therapeutic targeting of MYB and MYBL1 arrangements remains a major challenge. Because of their absence from the NGS gene panel used in the present study, the prevalence of *MYB-NFIB* and *MYBL1-NFIB* arrangements were not determined. However, our NGS results discovered several novel gene arrangements in 7.7% of all the ACC patients, including *ETV6-ABL1*, *ETV6-NTRK3*, *KDR-PDGFRA* and *RET-KIAA1217*. These gene arrangements produced oncogenically activated fusion kinases in multiple solid tumors and provide new important insights into the targeted treatment of ACC (23-26).

The lack of efficacy of chemotherapeutic regimens for systemic ACC has prompted a significant emerging interest in identifying effective targeted agents for this disease (1). A variety of drugs currently used for other more common cancers such as imatinib, dasatinib, sunitinib, nintedanib and axitinib have been assessed in ACC patients in phase I or II clinical trials (4,27-29), yet the OR rate was only about 10%. Given their limited activity and substantial toxicity, further studying these agents in unselected ACC patients is not recommended (30). Recently, a phase II trial was conducted to evaluate the efficacy of axitinib in patients with incurable ACC and simultaneously an exploratory analysis of correlating biomarkers that predict drug benefit was performed (31). Although the primary end point was not met, the study suggested that 4q12 amplification is correlated with clinical responsiveness to axitinib therapy. By NGS, 3 of 11 patients were revealed to harbor 4q12 amplification and two achieved stable disease along with one with the longest progression free survival (PFS) on record (31). Specifically, our study identified the *KIT/PDGFRA/KDR* amplification, located at 4q12, in 2 of 52 ACC patients. This result was consistent with three previous reports on ACC, in which the *KIT/PDGFRA/KDR* amplification was identified in 1 of 28, 2 of 60 and 1 of 17 cases, respectively (10,13,32). The findings of the present are particularly notable, which suggested that the *KIT/PDGFRA/KDR* amplification may be a distinctive molecular phenotype rather than an accidental event in ACC. The increased gene copy number was not highly correlated well with elevated protein expression of the RTKs (19). Using IHC analysis, triple-positive c-KIT/PDGFRα/VEGFR2 was detected in 28.8% of the total ACC patients, including the 2 cases with the *KIT/PDGFRA/KDR* amplification. Although triple positive c-KIT/PDGFRα/VEGFR2 was

not related to RFS, patients with triple positive c-KIT/PDGFRα/VEGFR2 were more likely to develop distant metastasis. All these results imply that the amplification and high expression of *KIT/PDGFRA/KDR* are associated with specific phenotypes of ACC.

Notably, an advanced ACC patient with the *KIT/PDGFRA/KDR* amplification had a PR response to sunitinib in our study, yet no OR were observed in a previous phase II study of sunitinib in unselected ACC (33). In addition to ACC, simultaneous amplification of the *KIT/PDGFRA/KDR* genes also occurs in a small fraction of glioblastoma and malignant peripheral nerve sheath tumors (34,35). In an *in vitro* experiment, malignant peripheral nerve sheath tumor cell line S462 harboring amplification of the *KIT/PDGFRA/KDR* was extremely sensitive to sunitinib (36). We also analyzed the prevalence of the *KIT/PDGFRA/KDR* amplification in malignant tumors using data from cBioPortal for Cancer Genomics (37,38). The *KIT/PDGFRA/KDR* amplification was reported in 34 of 169 cancer studies (*Table S2*). In addition to ACC, the *KIT/PDGFRA/KDR* amplification was also frequently identified in glioma, glioblastoma, neuroendocrine prostate cancer and sarcoma. Consequently, further investigation to clarify the importance of simultaneous amplification or high expression of *KIT/PDGFRA/KDR* in ACC and other tumors is encouraged. The *KIT/PDGFRA/KDR* amplification or high expression might serve as a predictive marker for the efficacy of related TKIs targeting the RTKs described above.

Conclusions

In conclusion, NGS identified potentially actionable genetic alterations in 61.5% of ACC patients. The *KIT/PDGFRA/KDR* amplification is an underappreciated clinical biomarker of ACC and defines a novel subtype. Clinical trials of RTKs inhibitors are urgently warranted for the molecularly defined subpopulation of ACC with the *KIT/PDGFRA/KDR* amplification and/or high protein expression.

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Footnote

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Data Sharing Statement: Available at http://dx.doi.org/10.21037/tcr-20-637

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-20-637). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. The number of Ethic Approval was 20160713. The investigations were carried out following the rules of the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all patients included in the study, and all the experiments were carried out in accordance with the National Health and Family Planning Commission of the PRC's guidelines.

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Table S1 Genetic alterations identified in 52 cases of ACC

Case Number	Tissue sample source	Primary tumor site	Disease stage	Perineural invasion	Distant metastasis	Number of genomic alterations	Actionable alterations
1	Primary	Minor salivary gland	IV	No	Cervical vertebra	6	Notch1 p.[L2451Hfs*32]
2	Primary	Major salivary gland	IV	Yes	No	7	–
3	Primary	Minor salivary gland	III	No	No	2	
4	Primary	Trachea	I	No	No	6	ETV6-ABL1, ETV6-NTRK3
5	Primary	Minor salivary gland	I	No	Unknown	11	Notch1 p.[G2430Afs*5]
6	Primary	Major salivary gland	II	No	No	10	NF1 p.[R1241*]
7	Primary	Minor salivary gland	II	Yes	No	9	
8	Metastasis	Major salivary gland	IV	No	Lung metastasis	11	HER2 amplification, PTEN loss
9	Primary	Other sites	II	Yes	No	10	PIK3CA p.[E545A]
10	Primary	Major salivary gland	II	No	No	10	FGFR2 amplification
11	Primary	Major salivary gland	III	No	No	7	FBXW7 p.[R479Q]
12	Primary	Minor salivary gland	IV	No	Unknown	9	ABL1 amplification, FGFR1 amplification, HER2 amplification, PTEN loss
13	Primary	Trachea	I	No	Lung metastasis	9	–
14	Primary	Major salivary gland	III	No	No	7	NF1 p.[S436*]
15	Primary	Major salivary gland	II	No	No	16	PI3KR1 p.[N564_P568dup], PTEN p.[Q171*], PTEN p.[K6Rfs*4]
16	Primary	Minor salivary gland	III	No	Unknown	14	KDR-PDGFRA, Notch1
17	Recurrence	Major salivary gland	I	No	No	5	
18	Recurrence	Minor salivary gland	II	No	No	12	PIK3CA p.[H1047L]
19	Primary	Major salivary gland	I	No	Lung, brain, liver and spleen metastasis	10	FGFR2 amplification, HER2 amplification, Notch1 p.[S2492*], Notch1 p.[L1574P]
20	Primary	Major salivary gland	II	No	No (cervical lymph nodes metastasis)	9	HER2 p.[R896C]
21	Primary	Minor salivary gland	II	No	No (cervical lymph nodes metastasis)	13	RET-KIAA1217
22	Primary	Major salivary gland	I	No	No	5	–
23	Primary	Major salivary gland	IV	Yes	Unknown	16	Notch1 p.[S2432Afs*3]
24	Primary	Major salivary gland	II	Yes	Skull and lung metastasis	11	Notch1 p.[Q1455Rfs*133], Notch1 p.[T2466Sfs*11]
25	Primary	Minor salivary gland	IV	No	No	12	BRCA2 p.[I2105Yfs*14]
26	Primary	Minor salivary gland	III	Yes	No	7	–
27	Recurrence	Major salivary gland	IV	Yes	Lung metastasis	10	–
28	Primary	Other sites	Unknown	No	Unknown	9	HER2 p.[S310F], FBXW7 p.[R278*], MAP2K2 p.[F57C]
29	Primary	Other sites	II	Yes	Unknown	6	KRAS p.[G12D]
30	Primary	Minor salivary gland	IV	No	No	16	BAP1 p.[Q40*], BAP1 p.[Q253*], PIK3CA p.[T1025A], PTEN
31	Primary	Trachea	IV	No	No	4	–
32	Primary	Minor salivary gland	I	Yes	No	15	
33	Primary	Minor salivary gland	IV	Yes	Lung, pleura, liver, spleen and kidney metastasis	10	KDR amplification, KIT amplification, PDGFRA amplification
34	Primary	Other sites	Unknown	Yes	Lung and pelvic	12	FBXW7 p.[R479Q]
35	Primary	Minor salivary gland	IV	No	Unknown	11	FGFR2 p.[P253R]
36	Primary	Minor salivary gland	IV	Yes	No	18	Notch1 p.[S2439Rfs*39]
37	Recurrence	Major salivary gland	IV	Yes	No	18	–
38	Primary	Major salivary gland	III	Yes	Unknown	9	HRAS p.[Q61K]
39	Primary	Major salivary gland	IV	Yes	Lung metastasis	17	HRAS p.[Q61R], PIK3CA p.[E542K], PIK3R1 p.[D569_T576delinsA]
40	Primary	Minor salivary gland	IV	Yes	Unknown	6	ETV6-NTRK3
41	Recurrence	Trachea	I	No	Lung metastasis	11	CDKN2A p.[G23D]
42	Recurrence	Major salivary gland	Unknown	No	Unknown	10	–
43	Primary	Major salivary gland	II	Yes	No (cervical lymph nodes metastasis)	9	–
44	Primary	Major salivary gland	II	No	No	9	–
45	Metastasis	Other sites	IV	No	Lung, liver, mediastinum, pleura, pericardium and abdominal wall metastasis	9	PTEN loss
46	Primary	Trachea	I	No	Unknown	6	–
47	Primary	Other sites	II	No	Skull and head skin metastasis	11	PIK3CA p.[H1047L]
48	Recurrence	Minor salivary gland	III	No	Unknown	20	
49	Primary	Minor salivary gland	IV	No	Unknown	19	–
50	Primary	Major salivary gland	IV	Yes	No	6	–
51	Primary	Minor salivary gland	IV	No	Unknown	2	–
52	Metastasis	Trachea	IV	No	Liver metastasis	7	KIT amplification, KDR amplification, PDGFRA amplification, MDM2 amplification

ACC, adenoid cystic carcinoma.

Table S2 Prevalence of the *KIT/PDGFRA/KDR* amplification in malignant tumors

Study	Tumor type	KIT/PDGFRA/KDR amplified cases	Total cases	Frequency (%)
MSK-IMPACT Clinical Sequencing Cohort (MSKCC, *Nat Med* 2017)	PanCancer studies	61	10,366	0.59
Cancer Cell Line Encyclopedia (Novartis/Broad, *Nature* 2012)	Cell lines	17	995	1.71
Bladder Urothelial Carcinoma (TCGA, Provisional)	Bladder urothelial carcinoma	2	235	0.85
Mutational profiles of metastatic breast cancer (France, 2016)	Invasive breast carcinoma	1	213	0.47
Breast Cancer (METABRIC, *Nature* 2012 & *Nat Commun* 2016)	Invasive breast carcinoma	17	2,051	0.83
Breast Invasive Carcinoma (TCGA, Provisional)	Invasive breast carcinoma	7	963	0.73
Breast cancer patient xenografts (British Columbia, *Nature* 2014)	Invasive breast carcinoma	1	15	6.67
The Metastatic Breast Cancer Project (Provisional, October 2017)	Invasive breast carcinoma	1	78	1.28
Merged Cohort of LGG and GBM (TCGA, *Cell* 2016)	Diffuse glioma	32	794	4.03
Brain Lower Grade Glioma (TCGA, Provisional)	Diffuse glioma	8	283	2.83
Glioblastoma Multiforme (TCGA, Provisional)	Glioblastoma	18	273	6.59
Pheochromocytoma and Paraganglioma (TCGA, Provisional)	Miscellaneous neuroepithelial tumor	1	162	0.62
Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma (TCGA, Provisional)	Cervical squamous cell carcinoma	1	191	0.52
Esophageal Carcinoma (TCGA, Provisional)	Esophageal adenocarcinoma	3	184	1.63
TCGA data for Esophagus-Stomach Cancers (TCGA, *Nature* 2017)	Esophagogastric adenocarcinoma	4	265	1.51
Stomach Adenocarcinoma (TCGA, Provisional)	Stomach adenocarcinoma	4	393	1.02
Adenoid Cystic Carcinoma (MSKCC, *Nat Genet* 2013)	Salivary adenoid cystic carcinoma	2	60	3.33
Adenoid Cystic Carcinoma (FMI, *Am J Surg Pathl* 2014)	Salivary adenoid cystic carcinoma	1	28	3.57
Recurrent and Metastatic Head & Neck Cancer (MSKCC, *JAMA Oncol* 2016)	Head and neck	5	132	3.79
Head and Neck Squamous Cell Carcinoma (TCGA, Provisional)	Head and neck squamous cell carcinoma	2	504	0.40
Pan-Lung Cancer (TCGA, *Nat Genet* 2016)	PanCancer studies	28	1,114	2.51
MSK-IMPACT Clinical Sequencing Cohort for Non-Small Cell Cancer (MSK, *Cancer Discovery* 2017)	Lung adenocarcinoma	1	860	0.12
Lung Squamous Cell Carcinoma (TCGA, Provisional)	Lung squamous cell carcinoma	9	178	5.06
Ovarian Serous Cystadenocarcinoma (TCGA, Provisional)	Ovarian epithelial tumor	7	594	1.18
Pancreatic Cancer (UTSW, *Nat Commun* 2015)	Pancreatic adenocarcinoma	1	109	0.92
Prostate Adenocarcinoma (Fred Hutchinson CRC, *Nat Med* 2016)	Prostate adenocarcinoma	1	54	1.85
Metastatic Prostate Cancer, SU2C/PCF Dream Team (Robinson *et al.*, *Cell* 2015)	Prostate adenocarcinoma	2	150	1.33
Neuroendocrine Prostate Cancer (Trento/Cornell/Broad 2016)	Neuroendocrine prostate cancer	11	77	14.29
Prostate Adenocarcinoma (MSKCC, *Cancer Cell* 2010)	Prostate adenocarcinoma	1	103	0.97
Sarcoma (MSKCC/Broad, *Nat Genet* 2010)	Sarcoma	4	207	1.93
Sarcoma (TCGA, Provisional)	Sarcoma	6	248	2.42
Skin Cutaneous Melanoma (TCGA, Provisional)	Cutaneous melanoma	7	287	2.44
Testicular Germ Cell Cancer (TCGA, Provisional)	Non-seminomatous germ cell tumor	4	149	2.68
Genomic Profile of Patients with Advanced Germ Cell Tumors (MSK, *JCO* 2016)	Germ cell tumors	2	180	1.11

The prevalence of the *KIT/PDGFRA/KDR* amplification in malignant tumors was analyzed using data from cBioPortal for Cancer Genomics (http://www.cbioportal.org/index.do; December 26, 2017) (37,38).