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Original Article

RNA-seq identified a novel functional MET fusion in head and neck cancer 5-8F cell line

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Abstract: The mortality of head and neck squamous cell carcinoma (HNSCC) has remained nearly unchanged for the past two decades. Deepening our understanding of genomic alterations and their influence on biological function of HNSCC cells will help develop effective and personalized treatment. In present study, paired-end whole-transcriptome sequencing (RNA-seq) was performed across 8 HNSCC cell lines. A novel *MET* fusion, *CAPZA2-MET* fusion, was identified by fusion analysis with FusionMap software and validated by nested reverse transcription-polymerase chain reaction (RT-PCR) and Sanger sequencing in HNSCC 5-8F cell line. 5-8F cells harboring this fusion were very sensitive to tivantinib, a specific small molecular inhibitor to MET kinase, demonstrating that this novel MET fusion may serve as a potential therapeutic target for HNSCC patients.

Keywords: Head and neck squamous cell carcinoma, RNA-seq, 5-8F cell line, *CAPZA2-MET* fusion, tivantinib

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the 6th most common cancer, accounting for over 560,000 newly diagnosed cases every year worldwide [1, 2]. Although the genomic alterations in HNSCC have been gradually uncovered with the development of next-generation sequencing [3-7], about 30% of HNSCC patients harbor no alteration of known drugable targets [8, 9]. Despite intense research efforts, the mortality of HNSCC has remained nearly unchanged for the past two decades [10]. Thus, identification of the genetic and molecular factors involved in the tumorigenesis and disease progression are crucial to advance our understanding of HNSCC, and for insight into the development of therapeutic strategies to improve survival in HNSCC patients.

Gene fusions, usually resulting from chromosomal rearrangements, are considered as a genetic hallmark of many neoplasias [11-13]. Tyrosine kinase gene fusions, such as ALK, ROS1, RET, NTRK1, FGFR1-3 fusions, have been identified as strong driver mutations and demonstrate great potential for therapeutic intervention in various types of cancer [14-17]. However, few kinase gene fusions were reported in HNSCC, except the FGFR3-TACC3 fusion [7, 17]. Hence, it is of great interest to discovery novel kinase gene fusions in HNSCC and to investigate the potential of these gene fusions as drugable targets.

Here, RNA-seq analysis was performed across 8 HNSCC cell lines with the average depth of 42×. A novel *MET* fusion was found in 5-8F cell line, which was validated by RT-PCR and Sanger sequencing. The 5-8F cell line, harboring the *MET* fusion, was dramatically sensitive to tivantinib, a small molecular inhibitor to MET, demonstrating that this novel MET fusion may serve as a potential therapeutic target for HNSCC patients.

Materials and methods

Cell culture and compounds

5 cell lines of human nasopharyngeal carcinoma (5-8F, CNE-2, HNE-1, HNE-2, and HONE-1) were provided by the Cell Center of Central South University (Changsha, China). The Hep2 human laryngeal carcinoma cell line, the Fadu

Table 1. Primers for nested RT-PCR

Primer name	Sequence
CAPZA2-E1-OUT-F1	gatctggaggagcagttgtctgat
MET-E5-OUT-R1	ttgactgcaggactggaaatgtct
CAPZA2-E5-IN-F2	tcacttaaggaaggaggcaactga
MET-E4-IN-R2	tcccagtgataaccagtgtgtagc
hACTB	caccatgtaccctggcatt
hACTB	gtacttgcgctcaggaggag

human hypopharyngeal carcinoma cell line, and the KB human epidermal carcinoma of the mouth cell line were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured with RPMI 1640 or DMEM medium supplemented with 10% FBS (Gibco), penicillin (100 IU/ml) and Streptomycin (100 μg/ml) (Life Technologies) in a humidified atmosphere containing 5% CO_2 at 37°C. Cells in the exponential growth phase were used for all the experiments.

Compounds of small molecular inhibitors to MET (tivantinib, crizotinib and cabozantinib) and to JAK (baricitinib, ruxolitinib and momelotinib) were purchased from Selleck (China) and dissolved in DMSO.

Paired-end whole-transcriptome sequencing (RNA-seq) and gene fusions calling

Cells (8×10^4) were seeded in 2 ml of RPMI 1640/DMEM medium containing serum per well in a 6-well plate. 72 h later, all the samples were homogenized with 1 ml Trizol (Invitrogen, Life Technologies) and total RNAs were extracted according to the manufacturer's instruction.

Preparation of cDNA followed the procedure described in Trapnell et al [18]. The cDNA library was size-fractionated on a 2% TAE low melt agarose gel (Lonza catalog # 50080), a narrow slice (~2 mm) of the cDNA lane centered at the 300 bp marker was cut. The slice was extracted using the QiaEx II kit (Qiagen catalog # 20021), and the extract was filtered over a Microcon YM-100 microconcentrator (Millipore catalog # 42409) to remove DNA fragments shorter than 100 bps. One-sixth of the filtered sample volume was used as template for 15 cycles of amplification using the paired-end primers and amplification reagents supplied with the Illumina ChIP-Seq genomic DNA prep kit. Each library was loaded into its own single Illumina flow cell lane, producing an average of 37.5 million pairs of 100-mer reads per lane, or nearly 3.3 Gb of total sequence for each sample. Transcripts were assembled from the mapped fragments sorted by reference position.

To detect fusion genes from RNA-Seq by Illunima, we choose the software of FusionMap [19] with higher sensitivity and specificity, which characterizes fusion junctions at base-pair resolution.

Gene fusions validation: reverse transcription-polymerase chain reaction (RT-PCR) and Sanger sequencing

Total RNAs above isolated were synthesized to cDNA using Prime Script RT reagent kit with gDNA Eraser (Takara, RR074A) for RT-PCR with Random Primer (9 mer). Then, nested RT-PCR was performed with two sets of primer pairs for each gene fusion (**Table 1**). *hActb* was adapted as the input control. The PCR amplification was carried on with Premix Taq (Takara, R004A) according to the following protocol: 95°C for 3 min, 95°C for 15 s, 60°C for 20 s, and 72°C for 1 min, repeated by 32 cycles for the latter three steps. 100-fold dilution of the products of the first run of PCR amplification was used as the template of the second run of PCR. And then, the positive band of gel electrophoresis for the nested PCR products were extracted from the gel with the QIAquick Gel Extraction Kit (Qiagen, 28706) and applied for Sanger sequencing.

Determination of IC50 for small molecular inhibitors to MET by MTS assay

5-8F cells (1×10^3/each well) were grown in 100 μl of RPMI 1640 medium containing serum per well in a 96-well plate. After 24 h, the cells were treated with small molecular inhibitors to MET, tivantinib (0, 0.0030, 0.0095, 0.0301, 0.0952, 0.3009, 0.9507, 3.0043, 9.4937, and 30 μmol/L, respectively), crizotinib (0, 0.0020, 0.0064, 0.0201, 0.0635, 0.2006, 0.6338, 2.0029, 6.3291, and 20 μmol/L, respectively), and cabozantinib (0, 0.0100, 0.0316, 0.100, 0.316, 1.00, 3.16, 10.0, 31.6, 100 μmol/L, respectively) for 144 h. Every treatment was triplicate in the same experiment. Then 20 μl of MTS (CellTiter 96 AQueous One Solution Reagent; Promega) was added to each well for

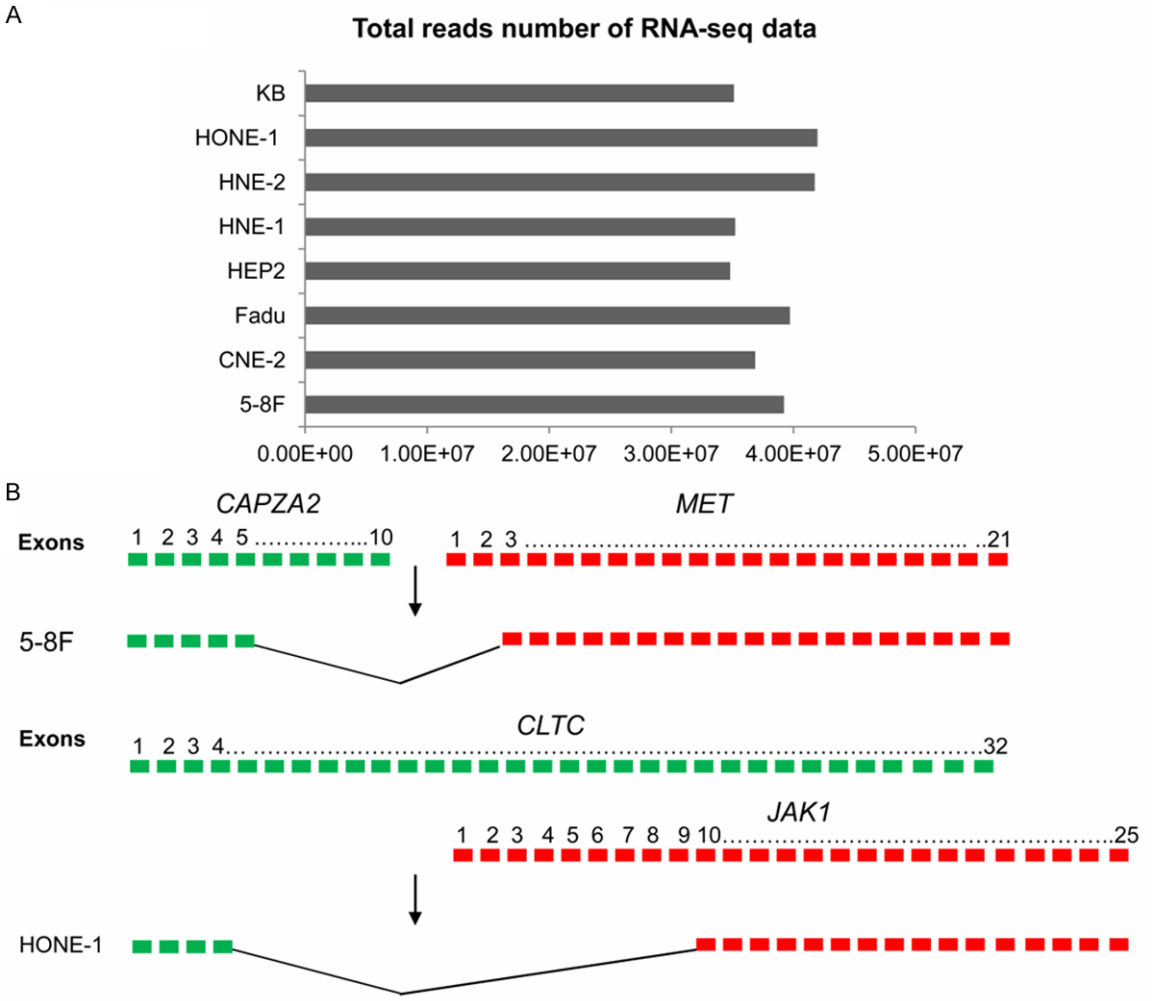

Figure 1. RNA-seq results summary and two potential kinase fusion transcripts in HNSCC cell lines. A. Total reads number of RNA-seq data for 8 HNSCC cell lines. B. Schematic representations of two potential kinase fusion transcripts in 5-8F and HONE-1 cell lines. The numbers indicate exons.

1 to 4 h at 37°C. After incubation, the absorbance was read at a wavelength of 490 nm according to the manufacturer's protocol. The cell viability was calculated relative to the untreated cells, respectively. The IC50 calculation was performed with GraphPad Prism 5.0 software via nonlinear regression.

Results

Gene fusions calling by FusionMap for RNA-seq data predicted that novel MET and JAK1 fusions are present in HNSCC cell lines

From RNA-seq of eight HNSCC cell lines (5-8F, CNE-2, HNE-1, HNE-2, HONE-1, Hep2, Fadu and KB), we obtained 42.38× average read-depth (**Figure 1A**). 44 in-frame candidate fusion mRNA transcripts were identified across the 8 cell lines, including potential fusions of kinases, transcriptional factors, chromatin-modifying proteins and some other known cancer-related proteins (**Table 2**). Unfortunately, the seed reads of majority of these fusion transcripts were so few that it is theoretically difficult to be validated by classic methods, such as RT-PCR. Hence, only a small part of these candidate fusion transcripts would be validated. Furthermore, protein structure analysis by online InterPro software (http://www.ebi.ac.uk/interpro/) showed that the kinase domains of two kinase fusion partners, MET of *CAPZA2-MET* fusion in 5-8F cell line, and JAK1 of *CLTC-JAK1* fusion in HONE-1 cell line (**Figure 1B**), were intact in primary structure and hence be

Table 2. Candidate fusions in 8 HNSCC cell lines

Cell line	Seed Count	Rescued Count	Gene1	Gene2	Fusion Junction Sequence	Fusion Gene
58F	4	2	MET	CAPZA2	TTCACAGCCTGATGAATTTCTCAGAAGTGTagtgcagactccattcgggtaatgttcttt	CAPZA2->MET
CEN_2	3	0	NCK1	IL20RB	CTATTGTGAAAAACCTAAAGGATACCTTAGatgaagtggccattctgcctgcccctcaga	NCK1->IL20RB
CEN_2	1	1	BCL2L2	PABPN1	GCTGACTGGATCCACAGCAGTGGGGGCTGGgagctggaagctatcaaagctcgagtcagg	BCL2L2->PABPN1
FADU	2	2	ZNF264	AURKC	AGGAAGACCTCTCCCAAGACACCTGTCCAGtggctacagcaaaccaaacagcccagcagc	ZNF264->AURKC
FADU	1	1	HNRNPF	BCL3	ATCCCCCTCTGAAGTTCATGTCCGTGCAGCtcttgctagagcgcggtgccgacatcgacg	HNRNPF->BCL3
FADU	2	0	KRT5	BCR	TAATCTCATCCATCAGTGCATCAACCTTGGcctccagctctatctcaaattcctcgttcc	BCR->KRT5
FADU	1	1	NCK1	IL20RB	CTATTGTGAAAAACCTAAAGGATACCTTAGatgaagtggccattctgcctgcccctcaga	NCK1->IL20RB
FADU	2	0	HSP90AA1	DAXX	TGAGGAAACCCAGACCCAAGACCAACCGATagaggaggaggatctggaacagatgcagga	HSP90AA1->DAXX
FADU	2	0	GTF2F1	HDAC2	GCCTGAGGAGGACAAGGAGGAGGAGGAGGAaaagaaagctagaattgaagaagataagaa	GTF2F1->HDAC2
FADU	5	3	BCL7C	SETMAR	GAGCCTGTTCCAGAACTGCTGGAAGCTGAGactatgaaaatgatgttagacaaaaagcaa	BCL7C->SETMAR
FADU	3	2	SKP1	PPP2CA	ATCAGAACTCTGCAACTTAATTGAAGGCATcttctcgcagaggctcttgacctgggactc	PPP2CA->SKP1
FADU	2	0	RAB13	PRKACA	AACAAATGTGACATGGAGGCCAAGAGGAAGgtggaagctcccttcataccaaagtttaaa	RAB13->PRKACA
FADU	2	0	YWHAE	RPA1	ACAACAGATTAACTTTAGCTCAGTCTCAACcgcaatggccccctcgctcagttggccgac	RPA1->YWHAE
FADU	2	0	CCDC72	KDM5C	CTTGAAAGCCTTATCTTCCTCGTCCATCTCttcctcctcctggacctcctcagcctctgg	KDM5C->CCDC72
HEP2	2	0	SRM	TP53	CCGTGGTGGCCTATGCCTACTGCACCATCCactacaactacatgtgtaacagttcctgca	SRM->TP53
HEP2	1	1	MIER1	XRCC6	AACAGGTGGAAATAAGAAACCACTTCATGCagatagtttgatttttttggttgatgcctc	MIER1->XRCC6
HEP2	2	0	SMARCE1	PPP1R15A	CAGGAATTTCCTCTTCTTCTCCTGGTGTCGttcctcttcctctgtgtcctctcctggcca	PPP1R15A->SMARCE1
HNE-1	3	0	CITED1	HDAC8	GAAGTGATGCTGCCAGCTGGAGCTGTTGTGctttgatgtagttgaggatttgttggattc	HDAC8->CITED1
HNE-1	2	0	CDK2AP1	SETD8	TGCTGGTGGAAGGCGAGTGGACACTCCCAGctctagccatggctccactctgtcaccgcc	SETD8->CDK2AP1
HNE-1	2	0	BSG	PARP14	AGTACTCCTGCGTCTTCCTCCCCGAGCCCAtgggcacagccttgtatggaaaggagaagc	BSG->PARP14
HNE-1	2	2	TNFRSF1A	SCNN1A	GGGTATATTCCCACCAACAGCTCCAGGAGCacctcctgagcacccctgccccctcggcct	SCNN1A->TNFRSF1A
HNE-1	5	2	PRKAR1B	ZNHIT1	GGACCGGCGCTGCAGCACGGACGCGGTGCCctgctcctccaacagggcctgaaagttttt	ZNHIT1->PRKAR1B
HNE-1	2	0	PIK3R4	ATP2C1	AAGTTTGTCTTCCTCTTCCTCTGTCATTCCctcaaacaagcgtaagtcagcaggaactct	ATP2C1->PIK3R4
HNE-1	1	1	FGFRL1	PPIA	AGCGCTGAGGGCGGCCAACGAGGGAAGGTCcttgtctgcaaacagctcaaaggagacgcg	PPIA->FGFRL1
HNE-1	2	0	CTNNAL1	C9orf5	ATTATCTTTATGATTAATAAGCGTGGTGATtgaagatttcagatcttcagaaatgtcacg	C9orf5->CTNNAL1
HNE-1	3	0	BCL2L2	PABPN1	GCTGACTGGATCCACAGCAGTGGGGGCTGGgagctggaagctatcaaagctcgagtcagg	BCL2L2->PABPN1
HNE-2	3	0	OAF	POU2F3	AGGAGGATGTCCGGTTCTGGCTGGAGCAAGatatcaagatgagtggggatgtagccgatt	OAF->POU2F3
HNE-2	2	0	BIVM	ERCC5	ATCAGGACTCGGCATGGAAAAAGATGTCTAatattagcatttggttaaaccaagcactta	BIVM->ERCC5
HNE-2	1	1	BRCA2	EEF1A1	CCACCATCAGCCAACTGTATTCCTTTTCCAcccattttggcttttaggggtagttttcac	EEF1A1->BRCA2
HNE-2	2	0	EIF3J	MAP3K14	GCAAGAAAAGCAAAGCAAAGCCAAAAAGAAttggaagggaaagcgtcgcagcaaagcccg	EIF3J->MAP3K14
HNE-2	1	1	CDR2	CTNNA1	GGAGGAGCTGAAGTCATCTGGCCAAGGGAGggctgcagctagaggaatcctgcagaagaa	CDR2->CTNNA1
HNE-2	3	2	TNFRSF1A	SCNN1A	GTATATTCCCACCAACAGCTCCAGGAGCACctcctgagcacccctgccccctcggcctgg	SCNN1A->TNFRSF1A

HNE-2	2	0	TWF2	CTNNA1	CAGGGCCTCGCCTTCCCCCTGCAGCCTGAGcaaaaagcgaagattgcggaacaggtggcc	TWF2->CTNNA1
HNE-2	1	1	C7orf58	WNT16	GGTGGTGTTCAGTGGCTTAATTCCAATCACctgctgccgagcatccgagagggcgcccgg	C7orf58->WNT16
HONE-1	2	0	HSP90AA1	SET	TTGTTTTTTTCTCTTCCTGCTTCTTTTTCTcttcttcatcatccatatcgggaaccaagt	SET->HSP90AA1
HONE-1	2	0	JAK1	CLTC	AGCACGTACATCCCCTCCTCGCTTCCTTCTatcttaaactgtgcaaagctagctgcatgt	CLTC->JAK1
HONE-1	1	1	HIF1A	SNAPC1	GGGCGCCGGCGGCGCGAACGACAAGAAAAAtggcagaatgagaaatttagaaaagaacat	HIF1A->SNAPC1
HONE-1	2	0	HSP90AA1	CALR	ATTTCTGCCTGAAAGGCGAACGTCTCAACCtcctcctctttgcgtttcttgtcttcttcc	CALR->HSP90AA1
HONE-1	2	0	TES	MET	GTCGATGGCATTGTGGCATCCTTGACACACCctattaaagcagtgctcatgattgggtcc	MET->TES
HONE-1	1	1	RPS6KA2	RNASET2	CACATCCAGGTTGAGATCCTCCTCTGTGGTttggtagtaattgatggatggttttatccc	RNASET2->RPS6KA2
HONE-1	3	0	BCL2L2	PABPN1	GCTGACTGGATCCACAGCAGTGGGGGCTGGgagctggaagctatcaaagctcgagtcagg	BCL2L2->PABPN1
KB	3	0	CITED1	HDAC8	GAAGTGATGCTGCCAGCTGGAGCTGTTGTGctttgatgtagttgaggatttgttggattc	HDAC8->CITED1
KB	1	1	TMEM49	RPS6KB1	TGCAGAGATGGACCTATGCCGGGGACAGCAagttcatatggtccaactcccccatggtcc	RPS6KB1->TMEM49
KB	1	1	UBASH3B	ERCC5	TCTCGGGATATCCGATTTGCTAACCATGAGgaggagttggaaactctggagagcaacctc	UBASH3B->ERCC5

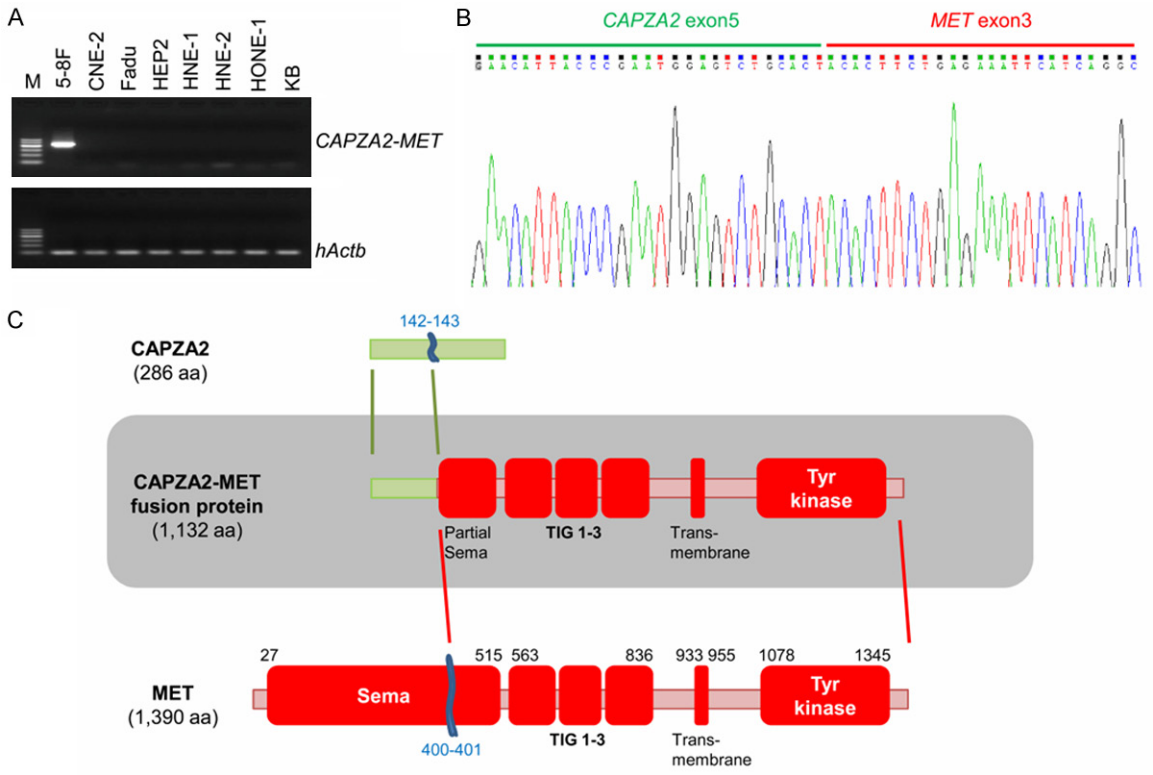

Figure 2. *CAPZA2-MET* fusion in 5-8F cell line confirmed by nested RT-PCR and Sanger sequencing. A. *CAPZA2-MET* fusion was screened in 8 HNSCC cell lines by nested RT-PCR. Only the second run PCR products were illustrated by electrophoresis in 2% agarose gel. The positive band of 5-8F cDNA was 450 bp or so in size, approximately the same as predicted. M, 100 bp ladder DNA marker. B. Sanger sequencing result for PCR product of *CAPZA2-MET* fusion in 5-8F cells. The result showed that the *CAPZA2-MET* fusion was exactly the same as predicted by fusion analysis of RNA-seq data. C. Predicted domains of CAPZA2-MET fusion kinase. The fusion kinase consists of 142 N-terminal residues of CAPZA2 and 990 C-terminal residues of MET kinase.

probably functional. Therefore, these 2 kinase fusions were suitable for the next experiments.

MET fusion was validated by RT-PCR and Sanger sequencing in 5-8F cell line

To validate the deep sequencing results, we performed nested RT-PCR analysis for *MET* fusion across the eight HNSCC cell lines, one of which was predicted to harbor *CAPZA2-MET* fusion transcript. The positive band of gel electrophoresis for the nested PCR products was present only in the predicted cDNA lane, i.e., *MET* fusion band only in 5-8F cDNA lane (**Figure 2A**). Then the positive fusion band was extracted from the gel and applied for Sanger sequencing. The results confirmed an in-frame fusion of the end of exon 5 of *CAPZA2* to the start of exon 3 of *MET* in 5-8F cell line (**Figure 2B**). In addition, the functional domains of CAPZA2-MET fusion kinase were predicted, which harbored partial Sema domain, 3 TIG domains, transmembrane domain and protein tyrosine kinase domain of MET protein (**Figure 2C**).

5-8F cells harboring CAPZA2-MET fusion responded well to t ivantinib, a specific small molecular inhibitor to MET

Then, the sensitivity of the 8 HNSCC cell lines to tivantinib, a specific small molecular inhibitor to MET, was determined by MTS assay, as described in Materials and Methods. The IC50s of these cell lines to tivantinib at 144 h were as follow: 5-8F 0.073 ± 0.012 μmol/L; CNE-2 0.39 ± 0.22 μmol/L; Fadu 0.46 ± 0.08 μmol/L; HEP2 0.52 ± 0.10 μmol/L; HNE-1 0.42 ± 0.23 μmol/L; HNE-2 0.14 ± 0.01 μmol/L; HONE-1 0.15 ± 0.10 μmol/L; KB 0.78 ± 0.23 μmol/L (**Figure 3A** and **3B**). 5-8F cell line was the most sensitive to tivantinib across the 8 HNSCC cell lines. Previous studies showed that malignant pleural mesothelioma H2596 cell line was the most

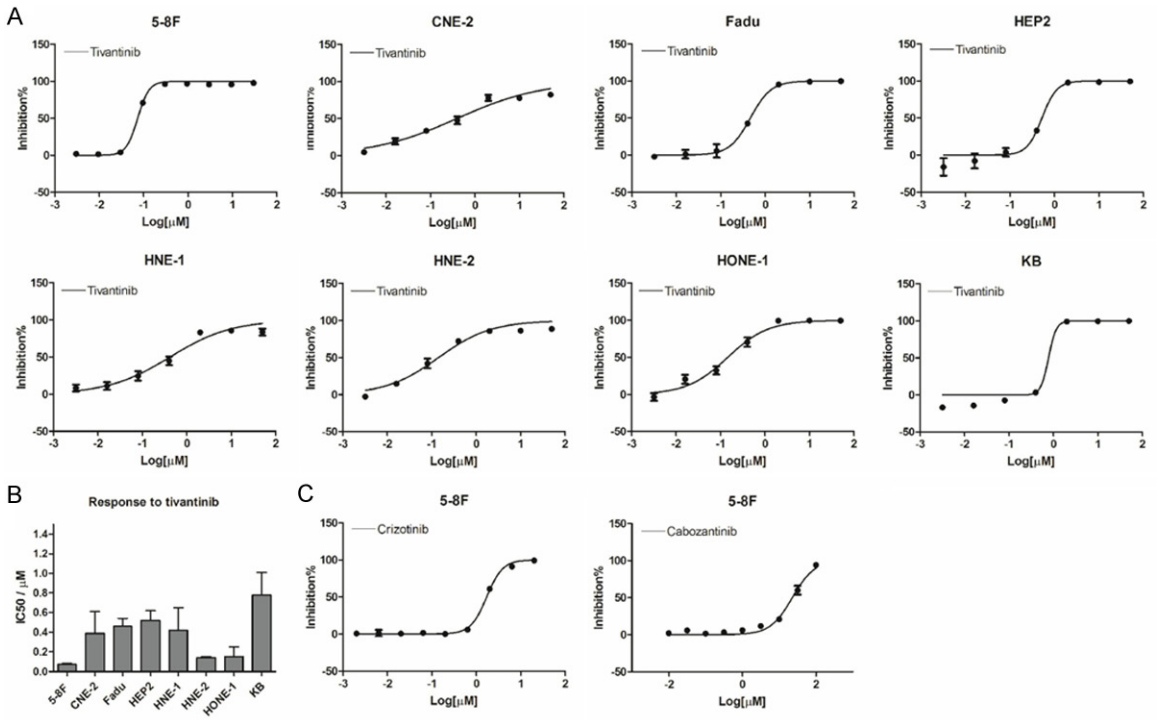

Figure 3. The response of HNSCC cell lines to MET inhibitors. A. IC50 determination of 8 HNSCC cell lines to the specific MET inhibitor, tivantinib. 5-8F cells with *CAPZA2-MET* fusion were the most sensitive to tivantinib. B. Response of 8 HNSCC cell lines to tivantinib was summarized in histogram. C. 5-8F cells were resistant to two non-specific MET inhibitor, crizotinib and cabozantinib.

sensitive cell line to tivantinib with IC50 = 0.11 μmol/L at 72 h [20, 21]. 5-8F cell line exerted a comparable sensitivity with H2596 and hence was almost the most sensitive cell line to tivantinib. However, 5-8F cells did not response to the other two non-specific small molecular inhibitors to MET, crizotinib and cabozantinib (**Figure 3C**). Meanwhile, HONE-1 cells harboring potential *CLTC-JAK1* fusion were resistant to three JAK inhibitors, baricitinib, ruxolitinib and momelotinib (Data not shown). These results proposed this MET fusion as a novel potential therapeutic target for HNSCC patients.

Discussion

In present study, a novel functional kinase fusion, *CAPZA2-MET* fusion, was identified by analysis of RNA-seq data and validated by nested RT-PCR in HNSCC cell line 5-8F. Moreover, 5-8F cells harboring the *CAPZA2-MET* fusion were sensitive to tivantinib (a specific inhibitor to MET) but resistant to crizotinib and cabozantinib (two non-specific small molecular inhibitors to MET kinase).

MET encodes a well-studied proto-oncogene whose activation is triggered by dimerization of the intracellular domain upon binding of hepatocyte growth factor (HGF) [22, 23]. The MET pathway is dysregulated in many human cancers and promotes tumor growth, invasion and dissemination. Abnormalities in MET signalling have been reported to correlate with poor clinical outcomes and drug resistance in patients with cancer. Thus, MET has emerged as a promising anticancer therapeutic target [24, 25]. In HNSCC, *MET* is functionally important with prominent overexpression (58-84%), increased gene copy number (13-39%), and mutations in tyrosine kinase domain (13.5-14%) [26-28]. However, there were no reports on *MET* fusion in HNSCC so far. Actually, a transforming *TPR-MET* fusion was generated *in vitro* via carcinogen-induced chromosomal rearrangement fusing the dimerization domain of TPR to the kinase domain of *MET* in 1986 and subsequently found to be recurrent mutation in gastric cancer [29, 30]. A dozen of *MET* fusions were identified in various tumors in the last two years with the development of next-

generation sequencing [31-33], including *PTPRZ1-MET* fusion in secondary giloblastomas [31], *TRIM4-MET* and *ZKSCAN1-MET* in melanoma [33], *KIF5B-MET* fusion in lung adenocarcinoma [32]. These *MET* fusions fuse exon 2, exon 13, exon 14 or exon 15 of *MET* gene to the 5'-partner, all reserving intact tyrosine kinase domain. Among which, *TPR-MET*, *PTPRZ1-MET*, *TRIM4-MET* and *ZKSCAN1-MET* fusions were proven to be functional in kinase activity.

We described here a novel functional *MET* fusion, *CAPZA2-MET* fusion, in HNSCC cell line 5-8F. *CAPZA2* encodes a member of the F-actin capping protein alpha subunit family. It is the alpha subunit of the barbed-end actin binding protein Cap Z. By capping the barbed end of actin filaments, Cap Z regulates the growth of the actin filaments at the barbed end. According to the data from Genecards (http://www.genecards.org) and BioGps database (http://www.biogps.org), the mRNA expression level of *CAPZA2* in majority of normal tissues and cancer cell lines is greater by 3-20 fold than that of *MET*, the fusion of *CAPZA2* to *MET* may increase the expression of *MET* in 5-8F cells, which need to be validated by qPCR assay in future.

Interestingly, 5-8F cells respond only to specific inhibitor (tivantinib) but not to the other two non-specific inhibitors of MET kinase (crizotinib and cabozantinib). Tivantinib is a non-ATP competitive and highly selective inhibitor of MET that binds to the non-phosphorylated inactive form of MET. Tivantinib is currently being investigated in four phase III clinical trials in non-small cell lung cancer (NSCLC) and liver cancer, and a phase II clinical trial in HNSCC (http://www.clinicaltrials.gov). Preclinical studies show that tivantinib inhibits MET activation in multiple cancer cell lines [34]. Most of malignant pleural mesothelioma cell lines responded well to tivantinib with IC50 values ranging from 0.1 to 0.4 μmol/L at 72 h [20], while K562, CEM and KB3-1 cell lines exerted almost the same sensitivity to tivantinib with IC50 values of 0.4 μmol/L at 72 h [21]. IC50 value of 5-8F cells to tivantinib at 144 h was 0.075 μmol/L, demonstrating 5-8F cell line was almost the most sensitive cell line to tivantinib so far. As to crizotinib and cabozantinib, they are approved by Food and Drug Administration (FDA) for treatment of NSCLC with anaplastic lymphoma kinase (ALK) fusion and progressive metastatic medullary thyroid cancer, respectively. Crizotinib targets to ALK and MET, while cabozantinib is a multi-targets inhibitor. The targets of cabozantinib include VEGFR2, c-Met, Ret, Kit, Flt-1/3/4, Tie2 and AXL. Crizotinib and cabozantinib are both non-specific MET inhibitors, it is anticipated that 5-8F cells respond partially to these two compounds. However, 5-8F cells were resistant to these two compounds according to our data. It may be in that some other molecules hinder the binding of these two compounds to MET kinase in 5-8F cells, which warrants further investigation by immunoblotting.

Considering that *CAPZA2* and *MET* are neighboring genes in the same coding orientation located in the same chromosomal region 7q31, it warrant further experiments to determine the fusion event resulting from DNA rearrangement or read-through gene fusion, a novel fusion type identified in prostate cancer and breast cancer [35-38]. Usually, the DNA breakpoint information for gene fusion should be submitted, especially for a novel identified gene fusion. Unfortunately, the *CAPZA2-MET* fusion occurred between the intron 5-6 of *CAPZA2* and the intron 2-3 of *MET*, involving 33 kb DNA sequence. The distance is too long to detect the breakpoint by classic PCR assay with genomic DNA of 5-8F cells. Therefore, targeted whole genome sequencing or southern blot with specifically-designed probes is needed to investigate whether DNA rearrangement between *CAPZA2* and *MET* occurs in 5-8F cells. If DNA rearrangement cannot be detected, knockdown of CTCF to examine whether the expression level of *CAPZA2-MET* is influenced by CTCF binding to insulator sequence(s) between *CAPZA2* and *MET* may help confirm that the fusion is caused by read-through event [37].

Furthermore, the *CAPZA2-MET* fusion should be screened in HNSCC tumor samples to identify the occurrence of this fusion in HNSCC patients and to improve the targeted therapy of MET inhibitors for HNSCC patients.

In summary, a novel functional *MET* fusion was identified and validated in HNSCC 5-8F cell line. 5-8F cells harboring this fusion were sensitive to tivantinib, a specific inhibitor to MET kinase, proposing MET fusion may serve as a potential therapeutic target for HNSCC patients.

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Disclosure of conflict of interest

None.

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