Original Article
TMED6-COG8 is a novel molecular marker of TFE3 translocation renal cell carcinoma

Yongcan Xu, Qiu Rao, Qiuyuan Xia, Shanshan Shi, Qunli Shi, Henghui Ma, Zhenfeng Lu, Hui Chen, Xiaojun Zhou

Department of Pathology, Nanjing Jinling Hospital, Nanjing University School of Medicine, Nanjing, China

Abstract: TFE3 translocation renal cell carcinoma is a highly aggressive malignancy which often occurs primarily in children and young adults. The pathognomonic molecular lesion in this subtype is a translocation event involving the TFE3 transcription factor at chromosome Xp11.2. Hence, the pathological diagnosis of an Xp11.2 translocation RCC is based upon morphology, TFE3 immunohistochemistry, or genetic analyses. However, due to the false-positive immunoreactivity for TFE3 IHC and expensive for TFE3 break-apart FISH assay, additional molecular markers are necessary to help provide early diagnose and individualization treatment. Owing to recent advances in microarray and RNA-Seq, Pflueger et al. have discovered that TMED6-COG8 is dramatically increased in TFE3 translocation RCCs, compared with clear cell RCCs and papillary RCCs, implying that TMED6-COG8 might be a new molecular tumor marker of TFE3 translocation RCCs. To extend this observation, we firstly validated the TMED6-COG8 expression level by qRT-PCR in RCCs including Xp11.2 translocation RCCs (n = 5), clear cell RCCs (n = 7) and papillary RCCs (n = 5). Then, we also examined the expression level of TMED6-COG8 chimera in Xp11.2 translocation alveolar soft part sarcoma. We found that TMED6-COG8 chimera expression level was higher in Xp11.2 translocation RCCs than in ASPS ($P < 0.05$). What's more, the expression levels of TMED6-COG8 chimera in esophagus cancers (n = 32), gastric cancers (n = 11), colorectal cancers (n = 12), hepatocellular carcinomas (n = 10) and non-small-cell lung cancers (n = 12) were assessed. Unexpectedly, TMED6-COG8 chimera was decreased in these five human types. Therefore, our observations from this study indicated that TMED6-COG8 chimera might act as a novel diagnostic marker in Xp11.2 translocation RCCs.

Keywords: Renal cell carcinomas, TMED6-COG8 chimera, TFE3 translocation renal cell carcinomas, diagnostic marker

Introduction

Kidney cancer remains a major health challenge both worldwide and in China. According to the National Cancer Institute at the National Institutes of Health (http://www.cancer.gov/cancertopics/types/kidney), it estimated that kidney cancer strikes close to 64,000 Americans every year and kills over 13,800, which suggests that kidney cancer is the ninth common cause of cancer-associated death in men [1]. Renal cell carcinoma (RCC), accounting for approximately 80% of cases, is the main type of kidney cancer [2]. Despite of recent improvements in surgical and anti-cancer drugs, the prognosis of RCC is still very poor, especially Xp11.2 translocation/TFE3 gene RCC (Xp11.2/TFE 3 RCC or Xp11.2 transloca-

tion RCC), which is newly accepted as a distinctive entity in the 2004 World Health Organization renal tumor classification [3]. These tumors are characterized by chromosomal translocations involving the TFE3 transcription factor located at chromosome Xp11.2 [4]. Xp11.2 translocation RCC is an uncommon subtype of RCC and accounts for 20%-75% and 1%-5% among childhood and adults RCCs, respectively [5]. Although these new entities are more rare than clear cell RCC (ccRCC) or papillary RCC (pRCC), meta-analyses of cases in the literature have demonstrated that adult cases of Xp11.2 translocation RCC have a more aggressive clinical course and poor prognosis [6]. However, Xp11.2 translocation RCCs frequently share common histopathologic characteristics with ccRCC and pRCC, including voluminous clear cytoplasm

Table 1. Characteristics of 17 renal carcinoma cases

Case No.	TFE3 IHC	Sex	Age (Y)	Tumor size (cm)	Primary location	Histology type	Lymph node metastasis	Distant metastasis	TMN stage
1	Positive	female	19	2 × 3 × 4.5	left kidney	Xp11.2 translocation RCC	No	negative	T1bN0M0 (I)
2	Positive	male	25	8 × 6 × 5	right kidney	Xp11.3 translocation RCC	No	negative	T2N0M0 (II)
3	Positive	female	20	4.5 × 3 × 2.5	right kidney	Xp11.4 translocation RCC	No	negative	T1bN0M0 (I)
4	Positive	female	58	9 × 9 × 7	right kidney	Xp11.5 translocation RCC	No	negative	T2N0M0 (II)
5	Positive	female	17	5 × 4 × 3	right kidney	Xp11.6 translocation RCC	No	negative	T1bN0M0 (I)
6	Negative	male	77	5.2 × 4.2 × 4	left kidney	ccRCC	No	negative	T1bN0M0 (I)
7	Negative	male	69	6 × 6 × 5	left kidney	ccRCC	No	negative	T1bN0M0 (I)
8	Negative	male	76	3.5 × 3.5 × 3	right kidney	ccRCC	No	negative	T1aN0M0 (I)
9	Negative	female	59	6 × 5.5 × 4	right kidney	ccRCC	No	negative	T4aN0M0 (IV)
10	Negative	male	60	7 × 7 × 6	left kidney	ccRCC	No	negative	T1bN0M0 (I)
11	Negative	female	61	4.5 × 4.5 × 4	left kidney	ccRCC	No	negative	T1bN0M0 (I)
12	Negative	male	61	3 × 2.5 × 2.5	right kidney	ccRCC	No	negative	T1aN0M0 (I)
13	Negative	male	38	3.8 × 3 × 2.8	right kidney	pRCC	No	negative	T1aN0M0 (I)
14	Negative	male	59	2.5 × 2.4 × 1.5	right kidney	pRCC	No	negative	T1aN0M0 (I)
15	Negative	male	30	6 × 5 × 4.5	right kidney	pRCC	No	negative	T1bN0M0 (I)
16	Negative	male	55	6 × 5.5 × 5	right kidney	pRCC	No	negative	T1bN0M0 (I)
17	Negative	female	75	4 × 4 × 3	left kidney	pRCC	No	negative	T1aN0M0 (I)

Table 2. Expression levels of TMED6-COG8 chimera in other five human tumor types

| Human tumor type	Case No.	Fold change	Percentage
Esophagus cancer	32	-1.77	87.50%
Gastric cancer	11	-1.96	81.80%
Colorectal cancer	12	-2.74	91.70%
Hepatocellular carcinoma	10	-1.53	90%
Non-small cell lung cancer	12	-1.82	66.70%

arranged in alveolar or papillary architecture. Besides, Xp11.2 translocation also occurs in alveolar soft part sarcoma (ASPS) [7], epithelioid hemangioendotheliomas [8] and perivascular epithelioid cell tumor [9]. Hence, morphology and nuclear immunoreactivity for TFE3 protein might not be enough to diagnose Xp11.2 translocation RCC and it is urgent to find additional molecular analyses (FISH, PCR, or other techniques) helping provide early diagnose and individualization treatment [10-13].

Over the past few years, owing to recent developments in high-throughput sequence capture methods and next-generation whole transcriptome sequencing (RNA-Seq), gene fusion products could be identified in cancer transcriptomes [14, 15]. Accumulating reports of dysregulated fusion transcripts or chimera products expression in numerous cancer types imply that the new transcripts could serve as not only novel clinical biomarkers for diagnosis but also therapeutic targets in cancer. The most famous paradigm is Philadelphia chromosome product, BCR-ABL1 protein. Rowley found in 1973 that BCR-ABL1 transcript is aberrant expression consistently with chromosomal abnormality in chronic myelogenous leukemia (CML) [16]. Sequential studies showed the corelationship between BCR-ABL1 transcript levels and CML prognosis [17]. Another remarkable example is EML4-ALK gene fusion protein, which was first described as oncogenic driver mutations by Soda and colleagues in 2007 in Japanese NSCLC patients [18]. Additionally, several studies have suggested that ALK rearrangement might be crucial for predicting the responsiveness to ALK TKIs and the resistance to EGFR TKIs [19, 20]. More recently, Pflueger et al, analyzed gene expression profile, fusion transcripts and mutations in Xp11.2 translocation RCCs using RNA-Seq [4]. They identified that the expression of an RNA read-through chimera between TMED6 and COG8 was significantly higher in Xp11.2 translocation or TFE3-expressing/non-translocated RCCs compared to ccRCCs and pRCCs. However, limited data are available on the expression profile of TMED6-COG8 chimera in Xp11.2 translocation alveolar soft part sarcoma or other various human tumors, such as non-small-cell lung cancer (NSCLC), esophagus cancer, hepatocellular carcinoma, gastric carcinoma and colorectal cancer.
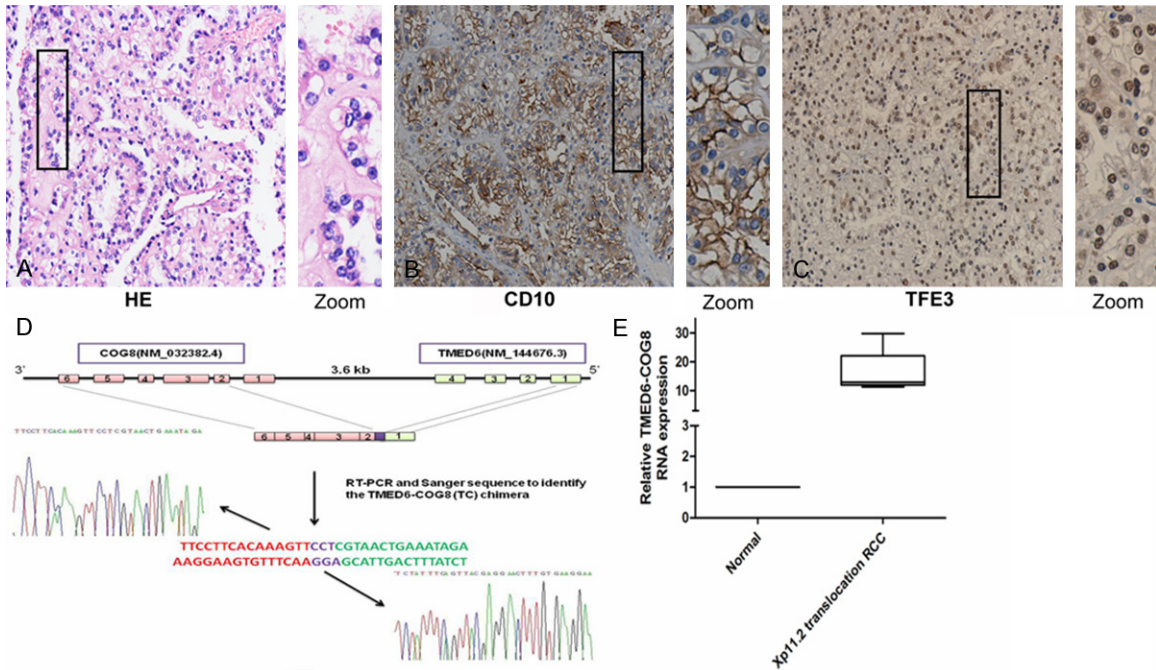
Figure 1. Identification of TMED6-COG8 chimera in Xp11.2 translocation RCCs. A. Histology of the Xp11.2 translocation RCC case (left panel) with a zoom (right panel). B. CD10 IHC of the Xp11.2 translocation RCC case (left panel) with a zoom (right panel). C. TFE3 IHC of the Xp11.2 translocation RCC case (left panel) with a zoom (right panel). D. Schematic representations of the chimeric TMED6-COG8 formation containing all but the first exon of COG8 following the first exon of TMED6 verified by reverse transcript PCR (RT–PCR) and Sanger sequencing. E. The relative expression level of chimeric TMED6-COG8 in Xp11.2 translocation RCC.

Therefore, in this study we validated the TMED6-COG8 chimera expression level by qRT-PCR in RCCs including Xp11.2 translocation RCCs (n = 5), ccRCCs (n = 7) and pRCCs (n = 5). Furthermore, to confirm the diagnostic role of TMED6-COG8 chimera in Xp11.2 translocation RCCs, we also examined the expression level of TMED6-COG8 chimera in esophagus cancers (n = 32), gastric cancers (n = 11), colorectal cancer (n = 12), hepatocellular carcinomas (n = 10) and NSCLCs (n = 12). Our findings firstly showed TMED6-COG8 chimera was increased in RCCs, particularly in Xp11.2 translocation RCCs (16.2-fold, P < 0.05) while decreased in other human tumor types, which suggested TMED6-COG8 chimera might serve as a good diagnostic marker in Xp11.2 translocation RCCs.

Materials and methods

### Human cancer tissues collection

All cancer tissues and corresponding adjacent normal tissue samples were obtained from patients who underwent primary surgical resection dating from September 2009 and October 2014. The tissue samples were retrieved from

the Department of Pathology at Nanjing Jinling Hospital, Nanjing University School of Medicine. None of the patients received preoperative treatment, including chemotherapy or radiotherapy. All cases were pathological diagnosed by two experienced pathologists. The clinicopathologic characteristics of the patients with renal carcinoma (TFE3 tRCC, n = 5; ccRCC, n = 7 and pRCC, n = 5) (**Table 1**), ASPS (n = 4), NSCLC (lung adenocarcinoma, n = 6, lung squamous carcinoma, n = 6), gastric cancer (n = 12) and hepatocellular carcinoma (n = 12) were available (**Table 2**). The study protocol was approved by the Institutional Review Board of Nanjing University, and all of the participants signed an informed consent form.

RNA isolation and reverse transcription

Total RNA was extracted from FFPE tissue using RNeasy FFPE Kit (QIAGEN, Germany, Dusseldorf), according to the manufacturer's instructions. For TMED6-COG8 chimera detection, a 1-µg total RNA was reverse transcribed in a final volume of 20 µl using random primers under standard conditions using the PrimeScript RT Master Mix (Takara, Dalian, China, Cat. #RR036A).
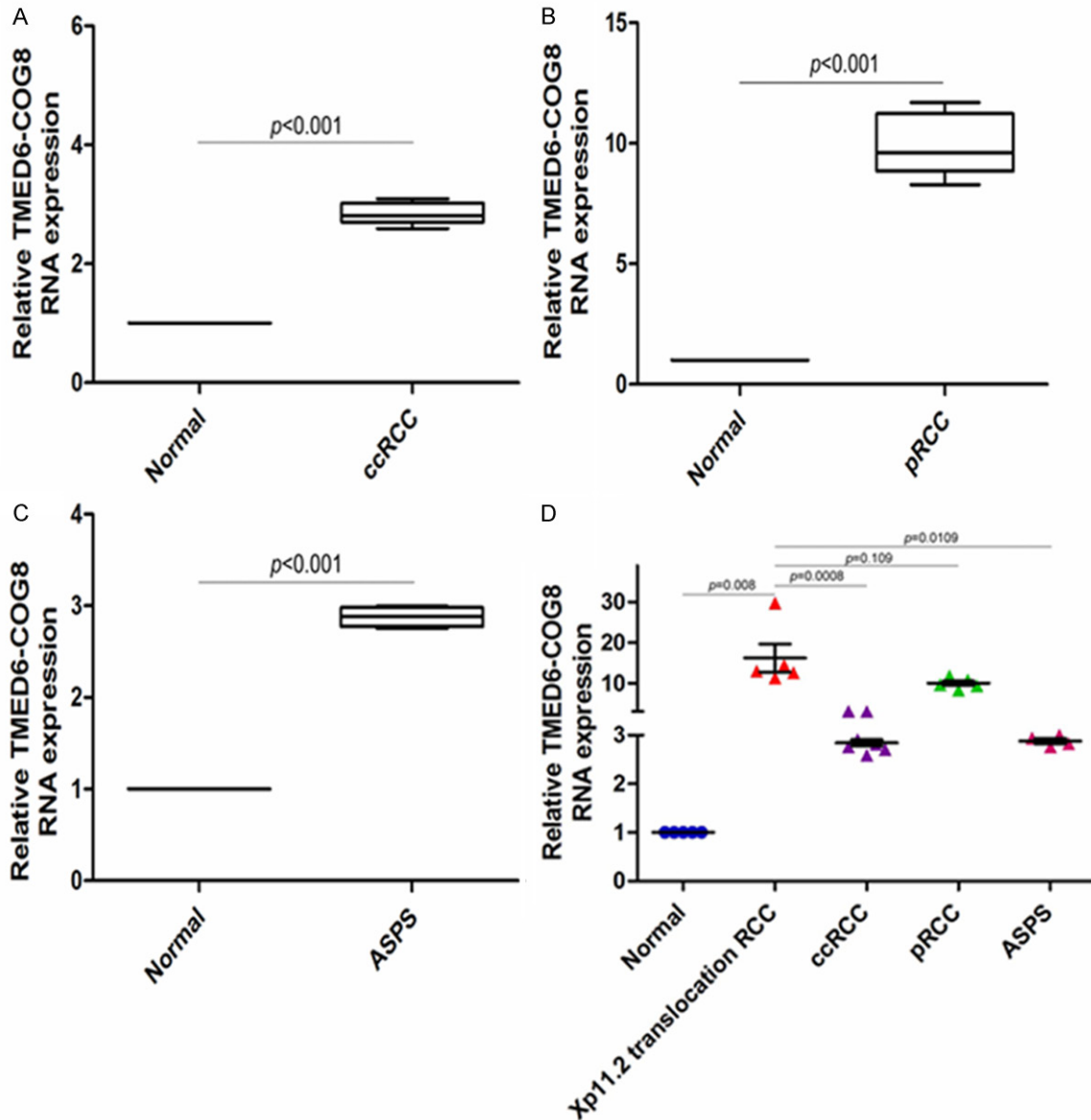
Figure 2. The relative expression level of TMED6-COG8 chimera in ccRCCs. (A) pRCCs (B), Xp11.2 translocation ASPS (C) and an overview of TMED6-COG8 chimera expression levels in three subtypes of RCCs and ASPS (D).

Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) analyses

TMED6-COG8 was quantified by qRT-PCR using SYBR Premix Ex Taq II (Perfect Real Time) (TaKaRa, Dalian, China) according to the manufacturer's instructions. The PCR reaction was conducted at 95°C for 30 s followed by 40 cycles of 95°C for 5 s and 60°C for 34 s in the ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). All of the qRT-PCRs were performed in duplicate. The relative quantification of TMED6-COG8 chimera expression was calculated using the $2-\Delta\Delta CT$

method relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The gene-specific primers were as follows:

GAPDH sense 5'-GTCAACGGATTTGGTCTGTAT-T-3', reverse 5'-AGTCTTCTGGGTGGCAGTGAT-3'; TMED-COG8 sense, 5'-GAGGCACGGAATGCTT-TTG-3', reverse 5'-GTCAGGCTATTCATCCGGC-3';
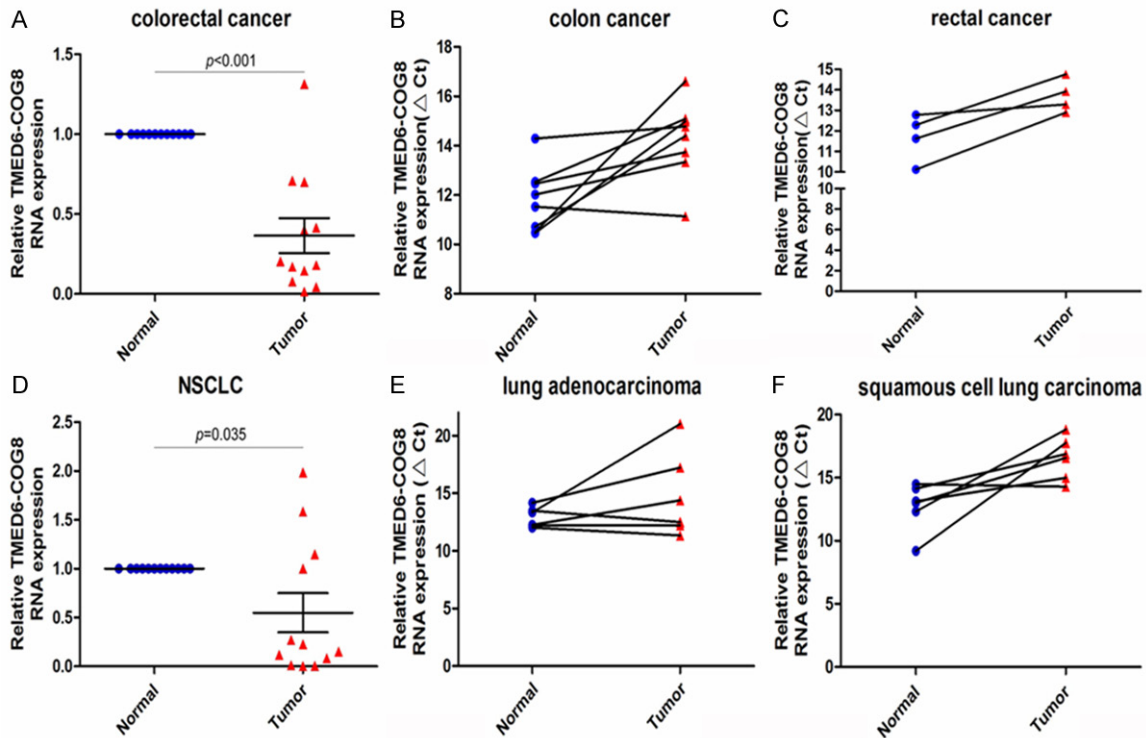
Statistical analysis

Student's t-test (two-tailed) was performed to analyze the in vitro and in vivo data using SPSS version 18 software (Chicago, IL, USA). The results are expressed as the mean ± S.D. A *P*
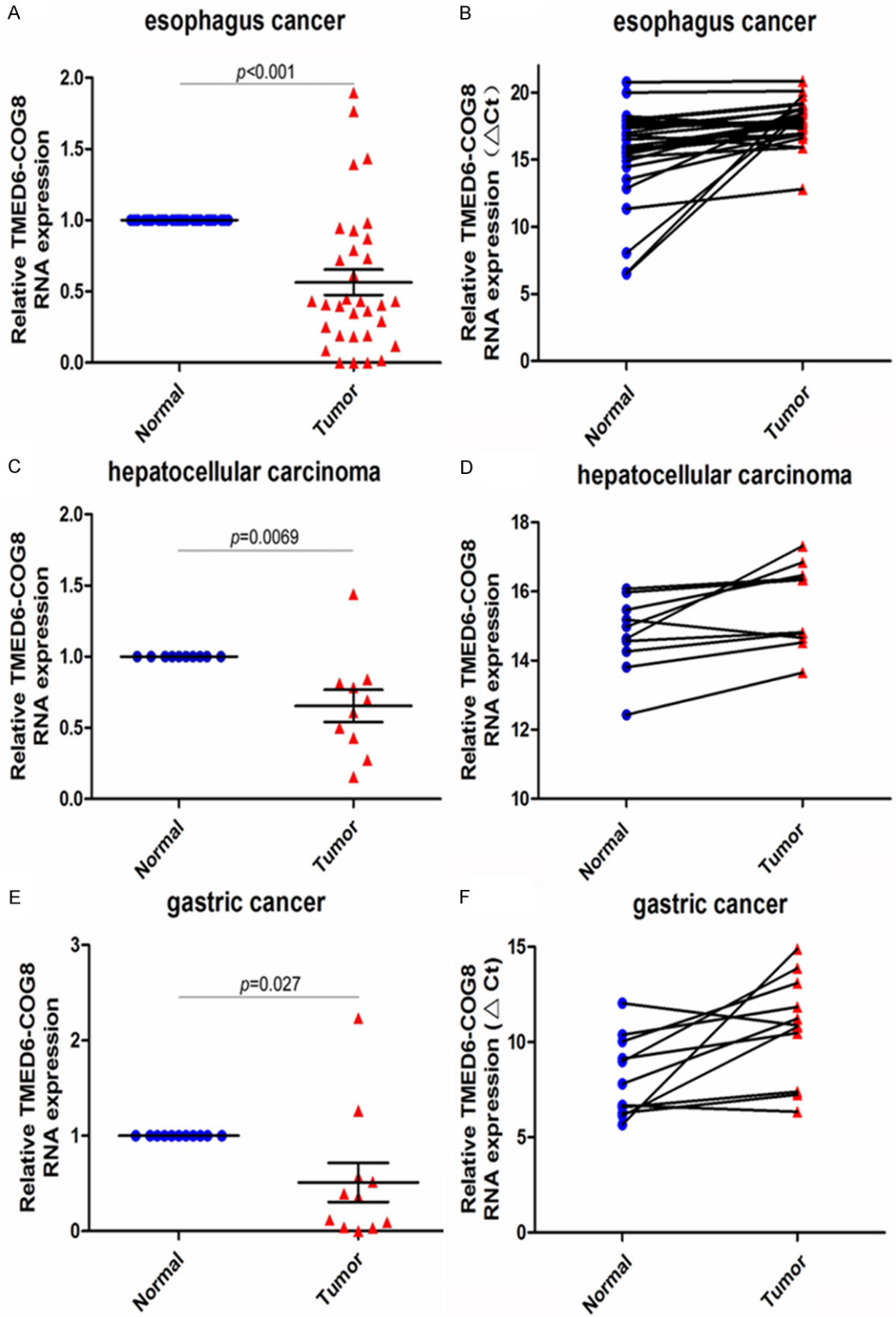
Figure 3. Relative expression level of TMED6-COG8 chimera in other five human cancers. esophageal cancer (A, B), hepatocelluar carcinoma (C, D) and gastric cancer (E, F).

Figure 4. Relative expression level of TMED6-COG8 chimera in other five human cancers. Colorectal cancer (A-C) and NSCLC (D-F).

value less than 0.05 was considered to be statistically significant.

Results

Verification expression of TMED6-COG8 chimera in Xp11.2 translocation RCC

Given previous study have identified TMED6-COG8 chimera as a new marker for Xp11.2 translocation RCC through FusionSeq algorithm applied to RNA-Seq data [4], we verification TMED6-COG8 chimera expression levels in five Xp11.2 translocation RCC tissues from patients who underwent primary surgical resection in the Department of Urinary Surgery, Jinling Hospital, Nanjing University School of Medicine, China. All of five Xp11.2 translocation RCC tissues exhibited histopathologic characteristics of mixed clear cell/papillary architecture (**Figure 1A**) and showed strong plasma membrane expression of CD10 (**Figure 1B**) and nuclear expression of TFE3 by IHC (**Figure 1C**). Since there are three different isoforms of the TMED6-COG8 chimera, RT-PCR and Sanger

sequencing was performed. As presented in **Figure 1D**, TMED6-COG8 chimera contains all but the first exon of COG8 following the first exon of TMED6. Then, we performed qRT-PCR to examine the expression level of TMED6-COG8 chimera in Xp11.2 translocation RCC tissues and adjacent normal renal tissues. The result showed that TMED6-COG8 chimera was significantly up-regulated in clinical Xp11.2 translocation RCC specimens (T) compared to adjacent normal renal tissues (N), (16.2-fold, *P* < 0.05, **Figure 1E**), which were consistent with the result of previous study.

Assessment the expression level of TMED6-COG8 chimera in ccRCC, pRCC and Xp11.2 translocation ASPS

Because Xp11.2 translocation RCCs frequently share common morphologic features with ccRCCs and pRCCs, we also assessed TMED6-COG8 chimera in ccRCCs (n = 7) and pRCCs (n = 5). We found that compared with corresponding adjacent normal renal tissues TMED6-COG8 chimera was also increased in ccRCC tis-

sues (2.84-fold, **Figure 2A**) and pRCC tissues (9.95-fold, **Figure 2B**). Furthermore, to determine the expression level of TMED6-COG8 chimera in other Xp11.2 translocation human tumors, we selected ASPS as the expression model. In surprise, though TMED6-COG8 chimera was up-regulated in ASPS (2.88-fold, **Figure 2C**), the fold change was much lower than that in Xp11.2 translocation RCCs.

On the basis of these results (**Figure 2D**), we speculated that increased TMED6-COG8 chimera might be associated with renal progression and serve as a good diagnostic biomarker for Xp11.2 translocation RCCs.

Expression level of TMED6-COG8 chimera in other types of human tumors

In order to extend previous observations of the strong expression of TMED6-COG8 chimera in Xp11.2 translocation RCCs, in the present study, our attention also focused on the relationship between TMED6-COG8 chimera expression levels and other types of human tumors (**Table 2**), including esophagus cancer (n = 32), gastric cancer (n = 11), colorectal cancer (n = 12), hepatocellular carcinoma (n = 10) and NSCLC (n = 12). Statistical analysis revealed that TMED6-COG8 chimera expression level was weaker in these five human tumor types than that in renal tumor, especially, Xp11.2 translocation RCCs. We found TMED6-COG8 chimera expression level was decreased in the most esophagus cancer (87.5%, -1.77-fold, $P < 0.001$, **Figure 3A**), compared to normal tissues and increased just in 4 of 32 (12.5%) esophagus cancer cases (**Figure 3B**). Analogously, there is nine tissues sample in ten hepatocellular carcinoma tissues (90%) showing a decrease in TMED6-COG8 chimera expression, compared to normal tissues (-1.53-fold, $P = 0.007$, **Figure 3C** and **3D**). In addition, the expression levels of TMED6-COG8 chimera were also down-regulated in other three human tumor types (gastric cancer, colorectal cancer and NSCLC). The fold changes are -1.96, -2.74 and -1.82, respectively (**Figures 3E**, **3F** and **4**).

Taken together, given the results from our observation, we raised the possibility that TMED6-COG8 chimera was down-regulated in human tumors, compared with normal tissues, suggesting TMED6-COG8 chimera might play an important role in RCCs, especially Xp11.2 translocation RCC tissues.

Discussion

Xp11.2 translocation RCC is a really rare subtype of RCCs and usually occurs in children and adolescents [21-23]. To date, there are only a few cases about adult Xp11.2 translocation RCC reported [24, 25]. In regard of asymptomatic, painless renal mass and poor prognosis of Xp11.2 translocation RCC, it is important to distinguish this subtype tumor from other renal cell carcinomas, particularly in cases involving elderly patients. Nowadays, the pathological diagnosis of an Xp11.2 translocation RCC is based upon morphology, TFE3 immunohistochemistry, or genetic analyses [26]. What's more, previous studies has reported that there are 5 defined fusion gene partners, including ASPL on 17q25 [27], PRCC on 1q21 [28], PSF on 1q34 [29], NonO on Xq12 [29] and CLTC on 17q23 [30]. Despite of the improvement in diagnostic method of Xp11.2 translocation RCC, the diagnosis sometimes remain confused because of false-positive immunoreactivity for TFE3 IHC and expensive for TFE3 break-apart FISH assay. Hence, find a novel molecular marker to diagnosis Xp11.2 translocation RCC conveniently and cheaply is more necessary.

With the development of whole genome and RNA Sequencing, more and more RNA read-through chimeras were discovered in different human tumor types. Pflueger et al. identified an RNA read-through chimera between TMED6 and COG8 in Xp11.2 translocation RCCs [4]. They found that the expression level of the chimera was relatively higher in Xp11.2 translocation RCCs than that in ccRCCs and pRCCs. Therefore, in order to extend previous observations that TMED6-COG8 chimera might function as a new marker for Xp11.2 translocation RCCs, we firstly verified TMED6-COG8 chimera expression level in five Xp11.2 translocation RCCs, seven ccRCCs and five pRCCs. We discovered that high levels of the TMED6-COG8 RNA chimera in RCCs, compared with normal tissues, especially in Xp11.2 translocation RCCs. Given that Xp11.2 translocation also occur in other human tumors, we examined the expression level of chimera transcript in ASPS. Unexpectedly, TMED6-COG8 RNA chimera is really higher in Xp11.2 translocation RCCs than in ASPS, which suggested TMED6-COG8 RNA chimera might a new diagnostic molecular marker of Xp11.2 translocation RCCs. Furthermore, we also found that this transcript

was decreased in esophagus cancer, gastric cancer, colorectal cancer, hepatocellular carcinoma and NSCLC, compared to the corresponding normal tissues. It might confirm the diagnostic role of TMED6-COG8 RNA chimera in Xp11.2 translocation RCCs. However, there is no significantly difference of TMED6-COG8 chimera expression level between in Xp11.2 translocation RCCs and in pRCCs. It is possibly because the number of cases is too small. Thus, detection the expression level of TMED6-COG8 chimera in a larger number of cases is more necessary.

Transmembrane emp24 protein transport domain 6 (TMED6) is a protein involved in protein transportation form the endoplasmic reticulum (ER). It has been reported that TMED6 is expressed in pancreatic islets selectively and plays a key role in hormone production or secretion [31]. Additionally, Camparo et al. identified TMED6 in the Xp11 translocation carcinoma gene signature list [32]. While, conserved oligomeric Golgi 8 (COG8) is a subunit of COG protein complex, which is responsible for intracellular trafficking and glycosylation [33]. Hence, the future studies should be performed to assess the biological role of TMED6-COG8 chimera in Xp11.2 translocation RCCs.

In summary, we verified TMED6-COG8 chimera expression levels in RCCs, especially in Xp11.2 translocation RCCs. What's more, we also examined the expression levels of TMED6-COG8 chimera transcript in other human cancers, including Xp11.2 translocation ASPS, esophagus cancer, gastric cancer, colorectal cancer, hepatocellular carcinoma and NSCLC. Based on our observation, TMED6-COG8 chimera might function as a potential diagnostic and therapeutic molecular marker in Xp11.2 translocation RCCs.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiaojun Zhou, Department of Pathology, Nanjing Jinling Hospital, Nanjing University School of Medicine, 305 East Zhongshan Road, Nanjing 210002, Jiangsu Province, China. Tel: +86-25-80860191; Fax: +86-25-80860191; E-mail: zhouxj3456@163.com

References

[1] Siegel R, Ma J, Zou Z and Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.

[2] Chow WH, Dong LM and Devesa SS. Epidemiology and risk factors for kidney cancer. Nat Rev Urol 2010; 7: 245-257.

[3] Lopez-Beltran A, Scarpelli M, Montironi R and Kirkali Z. 2004 WHO classification of the renal tumors of the adults. Eur Urol 2006; 49: 798-805.

[4] Pflueger D, Sboner A, Storz M, Roth J, Comperat E, Bruder E, Rubin MA, Schraml P and Moch H. Identification of molecular tumor markers in renal cell carcinomas with TFE3 protein expression by RNA sequencing. Neoplasia 2013; 15: 1231-1240.

[5] Ross H and Argani P. Xp11 translocation renal cell carcinoma. Pathology 2010; 42: 369-373.

[6] Malouf GG, Camparo P, Oudard S, Schleiermacher G, Theodore C, Rustine A, Dutcher J, Billemont B, Rixe O, Bompas E, Guillot A, Boccon-Gibod L, Couturier J, Molinie V and Escudier B. Targeted agents in metastatic Xp11 translocation/TFE3 gene fusion renal cell carcinoma (RCC): a report from the Juvenile RCC Network. Ann Oncol 2010; 21: 1834-1838.

[7] Folpe AL and Deyrup AT. Alveolar soft-part sarcoma: a review and update. J Clin Pathol 2006; 59: 1127-1132.

[8] Antonescu CR, Le Loarer F, Mosquera JM, Sboner A, Zhang L, Chen CL, Chen HW, Pathan N, Krausz T, Dickson BC, Weinreb I, Rubin MA, Hameed M and Fletcher CD. Novel YAP1-TFE3 fusion defines a distinct subset of epithelioid hemangioendothelioma. Genes Chromosomes Cancer 2013; 52: 775-784.

[9] Argani P, Aulmann S, Illei PB, Netto GJ, Ro J, Cho HY, Dogan S, Ladanyi M, Martignoni G, Goldblum JR and Weiss SW. A distinctive subset of PEComas harbors TFE3 gene fusions. Am J Surg Pathol 2010; 34: 1395-1406.

[10] Malouf GG, Monzon FA, Couturier J, Molinie V, Escudier B, Camparo P, Su X, Yao H, Tamboli P, Lopez-Terrada D, Picken M, Garcia M, Multani AS, Pathak S, Wood CG and Tannir NM. Genomic heterogeneity of translocation renal cell carcinoma. Clin Cancer Res 2013; 19: 4673-4684.

[11] Macher-Goeppinger S, Roth W, Wagener N, Hohenfellner M, Penzel R, Haferkamp A, Schirmacher P and Aulmann S. Molecular heteroge-

neity of TFE3 activation in renal cell carcinomas. Mod Pathol 2012; 25: 308-315.

[12] Kim SH, Choi Y, Jeong HY, Lee K, Chae JY and Moon KC. Usefulness of a break-apart FISH assay in the diagnosis of Xp11.2 translocation renal cell carcinoma. Virchows Arch 2011; 459: 299-306.

[13] Mosquera JM, Dal Cin P, Mertz KD, Perner S, Davis IJ, Fisher DE, Rubin MA and Hirsch MS. Validation of a TFE3 break-apart FISH assay for Xp11.2 translocation renal cell carcinomas. Diagn Mol Pathol 2011; 20: 129-137.

[14] Maher CA, Palanisamy N, Brenner JC, Cao X, Kalyana-Sundaram S, Luo S, Khrebtukova I, Barrette TR, Grasso C, Yu J, Lonigro RJ, Schroth G, Kumar-Sinha C and Chinnaiyan AM. Chimeric transcript discovery by paired-end transcriptome sequencing. Proc Natl Acad Sci U S A 2009; 106: 12353-12358.

[15] Maher CA, Kumar-Sinha C, Cao X, Kalyana-Sundaram S, Han B, Jing X, Sam L, Barrette T, Palanisamy N and Chinnaiyan AM. Transcriptome sequencing to detect gene fusions in cancer. Nature 2009; 458: 97-101.

[16] Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973; 243: 290-293.

[17] Mahon FX and Etienne G. Deep molecular response in chronic myeloid leukemia: the new goal of therapy? Clin Cancer Res 2014; 20: 310-322.

[18] Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K, Hatanaka H, Bando M, Ohno S, Ishikawa Y, Aburatani H, Niki T, Sohara Y, Sugiyama Y and Mano H. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007; 448: 561-566.

[19] Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, Solomon B, Stubbs H, Admane S, McDermott U, Settleman J, Kobayashi S, Mark EJ, Rodig SJ, Chirieac LR, Kwak EL, Lynch TJ and Iafrate AJ. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 2009; 27: 4247-4253.

[20] Guo Y, Ma J, Lyu X, Liu H, Wei B, Zhao J, Fu S, Ding L and Zhang J. Non-small cell lung cancer with EML4-ALK translocation in Chinese male never-smokers is characterized with early-onset. BMC Cancer 2014; 14: 834.

[21] Argani P and Ladanyi M. The evolving story of renal translocation carcinomas. Am J Clin Pathol 2006; 126: 332-334.

[22] Argani P, Lae M, Hutchinson B, Reuter VE, Collins MH, Perentesis J, Tomaszewski JE, Brooks JS, Acs G, Bridge JA, Vargas SO, Davis IJ, Fisher DE and Ladanyi M. Renal carcinomas with the t(6;11)(p21;q12): clinicopathologic features and demonstration of the specific alpha-TFEB gene fusion by immunohistochemistry, RT-PCR, and DNA PCR. Am J Surg Pathol 2005; 29: 230-240.

[23] Argani P and Ladanyi M. Translocation carcinomas of the kidney. Clin Lab Med 2005; 25: 363-378.

[24] Wu A, Kunju LP, Cheng L and Shah RB. Renal cell carcinoma in children and young adults: analysis of clinicopathological, immunohistochemical and molecular characteristics with an emphasis on the spectrum of Xp11.2 translocation-associated and unusual clear cell subtypes. Histopathology 2008; 53: 533-544.

[25] Argani P, Olgac S, Tickoo SK, Goldfischer M, Moch H, Chan DY, Eble JN, Bonsib SM, Jimeno M, Lloreta J, Billis A, Hicks J, De Marzo AM, Reuter VE and Ladanyi M. Xp11 translocation renal cell carcinoma in adults: expanded clinical, pathologic, and genetic spectrum. Am J Surg Pathol 2007; 31: 1149-1160.

[26] Klatte T, Streubel B, Wrba F, Remzi M, Krammer B, de Martino M, Waldert M, Marberger M, Susani M and Haitel A. Renal cell carcinoma associated with transcription factor E3 expression and Xp11.2 translocation: incidence, characteristics, and prognosis. Am J Clin Pathol 2012; 137: 761-768.

[27] Argani P, Antonescu CR, Illei PB, Lui MY, Timmons CF, Newbury R, Reuter VE, Garvin AJ, Perez-Atayde AR, Fletcher JA, Beckwith JB, Bridge JA and Ladanyi M. Primary renal neoplasms with the ASPL-TFE3 gene fusion of alveolar soft part sarcoma: a distinctive tumor entity previously included among renal cell carcinomas of children and adolescents. Am J Pathol 2001; 159: 179-192.

[28] Argani P, Antonescu CR, Couturier J, Fournet JC, Sciot R, Debiec-Rychter M, Hutchinson B, Reuter VE, Boccon-Gibod L, Timmons C, Hafez N and Ladanyi M. PRCC-TFE3 renal carcinomas: morphologic, immunohistochemical, ultrastructural, and molecular analysis of an entity associated with the t(X;1)(p11.2;q21). Am J Surg Pathol 2002; 26: 1553-1566.

[29] Clark J, Lu YJ, Sidhar SK, Parker C, Gill S, Smedley D, Hamoudi R, Linehan WM, Shipley J and Cooper CS. Fusion of splicing factor genes PSF and NonO (p54nrb) to the TFE3 gene in papillary renal cell carcinoma. Oncogene 1997; 15: 2233-2239.

[30] Argani P, Lui MY, Couturier J, Bouvier R, Fournet JC and Ladanyi M. A novel CLTC-TFE3 gene fusion in pediatric renal adenocarcinoma with t(X;17)(p11.2;q23). Oncogene 2003; 22: 5374-5378.

[31] Wang X, Yang R, Jadhao SB, Yu D, Hu H, Glynn-Cunningham N, Sztalryd C, Silver KD and Gong DW. Transmembrane emp24 protein transport domain 6 is selectively expressed in pancreatic islets and implicated in insulin secretion and diabetes. Pancreas 2012; 41: 10-14.

[32] Camparo P, Vasiliu V, Molinie V, Couturier J, Dykema KJ, Petillo D, Furge KA, Comperat EM, Lae M, Bouvier R, Boccon-Gibod L, Denoux Y, Ferlicot S, Forest E, Fromont G, Hintzy MC, Laghouati M, Sibony M, Tucker ML, Weber N, Teh BT and Vieillefond A. Renal translocation carcinomas: clinicopathologic, immunohistochemical, and gene expression profiling analysis of 31 cases with a review of the literature. Am J Surg Pathol 2008; 32: 656-670.

[33] Smith RD and Lupashin VV. Role of the conserved oligomeric Golgi (COG) complex in protein glycosylation. Carbohydr Res 2008; 343: 2024-2031.