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

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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 translocation 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

<table>
<thead>
<tr>
<th>Case No.</th>
<th>TF3 IHC</th>
<th>Sex</th>
<th>Age (Y)</th>
<th>Tumor size (cm)</th>
<th>Primary location</th>
<th>Histology type</th>
<th>Lymph node metastasis</th>
<th>Distant metastasis</th>
<th>TMN stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive</td>
<td>female</td>
<td>19</td>
<td>2 × 3 × 4.5</td>
<td>left kidney</td>
<td>Xp11.2 translocation RCC</td>
<td>No</td>
<td>negative</td>
<td>T1bN0M0 (I)</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>male</td>
<td>25</td>
<td>8 × 6 × 5</td>
<td>right kidney</td>
<td>Xp11.3 translocation RCC</td>
<td>No</td>
<td>negative</td>
<td>T2N0M0 (II)</td>
</tr>
<tr>
<td>3</td>
<td>Positive</td>
<td>female</td>
<td>20</td>
<td>4.5 × 3.2 × 2.5</td>
<td>right kidney</td>
<td>Xp11.4 translocation RCC</td>
<td>No</td>
<td>negative</td>
<td>T1bN0M0 (I)</td>
</tr>
<tr>
<td>4</td>
<td>Positive</td>
<td>female</td>
<td>58</td>
<td>9 × 9 × 7</td>
<td>right kidney</td>
<td>Xp11.5 translocation RCC</td>
<td>No</td>
<td>negative</td>
<td>T2N0M0 (II)</td>
</tr>
<tr>
<td>5</td>
<td>Positive</td>
<td>female</td>
<td>17</td>
<td>4 × 4.3</td>
<td>right kidney</td>
<td>Xp11.6 translocation RCC</td>
<td>No</td>
<td>negative</td>
<td>T1bN0M0 (I)</td>
</tr>
<tr>
<td>6</td>
<td>Negative</td>
<td>male</td>
<td>77</td>
<td>5.2 × 4.2 × 4</td>
<td>left kidney</td>
<td>ccRCC</td>
<td>No</td>
<td>negative</td>
<td>T1bN0M0 (I)</td>
</tr>
<tr>
<td>7</td>
<td>Negative</td>
<td>female</td>
<td>69</td>
<td>6 × 6 × 5</td>
<td>left kidney</td>
<td>ccRCC</td>
<td>No</td>
<td>negative</td>
<td>T1bN0M0 (I)</td>
</tr>
<tr>
<td>8</td>
<td>Negative</td>
<td>female</td>
<td>76</td>
<td>3.5 × 3.5 × 3</td>
<td>right kidney</td>
<td>ccRCC</td>
<td>No</td>
<td>negative</td>
<td>T1aN0M0 (I)</td>
</tr>
<tr>
<td>9</td>
<td>Negative</td>
<td>female</td>
<td>59</td>
<td>6 × 5.5 × 4</td>
<td>right kidney</td>
<td>ccRCC</td>
<td>No</td>
<td>negative</td>
<td>T4aN0M0 (IV)</td>
</tr>
<tr>
<td>10</td>
<td>Negative</td>
<td>male</td>
<td>60</td>
<td>7 × 7 × 6</td>
<td>left kidney</td>
<td>ccRCC</td>
<td>No</td>
<td>negative</td>
<td>T1bN0M0 (I)</td>
</tr>
<tr>
<td>11</td>
<td>Negative</td>
<td>female</td>
<td>61</td>
<td>4.5 × 4.5 × 4</td>
<td>left kidney</td>
<td>ccRCC</td>
<td>No</td>
<td>negative</td>
<td>T1bN0M0 (I)</td>
</tr>
<tr>
<td>12</td>
<td>Negative</td>
<td>male</td>
<td>61</td>
<td>3.25 × 2.5 × 2.5</td>
<td>right kidney</td>
<td>ccRCC</td>
<td>No</td>
<td>negative</td>
<td>T1aN0M0 (I)</td>
</tr>
<tr>
<td>13</td>
<td>Negative</td>
<td>male</td>
<td>38</td>
<td>3.8 × 3.28</td>
<td>right kidney</td>
<td>pRCC</td>
<td>No</td>
<td>negative</td>
<td>T1aN0M0 (I)</td>
</tr>
<tr>
<td>14</td>
<td>Negative</td>
<td>male</td>
<td>59</td>
<td>2.5 × 2.4 × 1.5</td>
<td>right kidney</td>
<td>pRCC</td>
<td>No</td>
<td>negative</td>
<td>T1aN0M0 (I)</td>
</tr>
<tr>
<td>15</td>
<td>Negative</td>
<td>male</td>
<td>30</td>
<td>6 × 5 × 4.5</td>
<td>right kidney</td>
<td>pRCC</td>
<td>No</td>
<td>negative</td>
<td>T1aN0M0 (I)</td>
</tr>
<tr>
<td>16</td>
<td>Negative</td>
<td>male</td>
<td>55</td>
<td>6 × 5.5</td>
<td>right kidney</td>
<td>pRCC</td>
<td>No</td>
<td>negative</td>
<td>T1bN0M0 (I)</td>
</tr>
<tr>
<td>17</td>
<td>Negative</td>
<td>female</td>
<td>75</td>
<td>4 × 4 × 3</td>
<td>left kidney</td>
<td>pRCC</td>
<td>No</td>
<td>negative</td>
<td>T1aN0M0 (I)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Human tumor type</th>
<th>Case No.</th>
<th>Fold change</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophageal cancer</td>
<td>32</td>
<td>-1.77</td>
<td>87.50%</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>11</td>
<td>-1.96</td>
<td>81.80%</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>12</td>
<td>-2.74</td>
<td>91.70%</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>10</td>
<td>-1.53</td>
<td>90%</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>12</td>
<td>-1.82</td>
<td>66.70%</td>
</tr>
</tbody>
</table>

arranged in alveolar or papillary architecture. Besides, Xp11.2 translocation also occurs in alveolar soft part sarcoma (ASPS) [7], epithelioid hemangioendothelioma [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 diagnosis 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.
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).
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-ΔΔCT method relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The gene-specific primers were as follows:

GAPDH sense 5'-GTCAACGGATTTGGTCTGTAT-3', reverse 5'-AGTCTTCTGGGTGGCAGTGAT-3';
TMED-COG8 sense, 5'-GAGGCACGGAATGCTTTTG-3', reverse 5'-GTCAGGCTATTCCATC-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
TMED6-COG8 and TFE3 translocation renal cell carcinomas

(A) esophagus cancer

(B) esophagus cancer

(C) hepatocellular carcinoma

(D) hepatocellular carcinoma

(E) gastric cancer

(F) gastric cancer
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 ccRCCs...
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 ASPS 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 from 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.

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Disclosure of conflict of interest

None.

References


