Renal Cell Carcinoma Associated With Transcription Factor E3 Expression and Xp11.2 Translocation

Incidence, Characteristics, and Prognosis

Tobias Klatte, MD,1 Berthold Streubel, PhD,2 Friedrich Wrba, MD,2 Mesut Remzi, MD,1,3 Barbara Krammer, MD,4 Michela de Martino, PhD,1 Matthias Waldert, MD,1 Michael Marberger, MD,1 Martin Susani, MD,2 and Andrea Haitel, MD2

Key Words: Transcription factor E3; TFE3; Translocation; Prognosis; Kidney cancer

Abstract

We studied the characteristics and prognosis of renal cell carcinoma (RCC) associated with Xp11.2 translocation and transcription factor E3 (TFE3) expression and determined the need for genetic analysis in routine diagnostics. Of 848 consecutive cases, 75 showed microscopic features suggestive of Xp11.2 translocation RCC or occurred in patients 40 years or younger. Of these cases, 17 (23%) showed strong nuclear TFE3 immunostaining, which was associated with more advanced tumors and inverse prognosis in univariate (P = .032) but not multivariate (P = .404) analysis. With fluorescence in situ hybridization and polymerase chain reaction, only 2 cases showed alterations of the X chromosome and the ASPL-TFE3 gene fusion, respectively. In our laboratory, the predictive value of TFE3 expression for the Xp11.2 translocation was 12%. Strong nuclear TFE3 expression is associated with metastatic spread and a poor prognosis. In our laboratory, TFE3 is not diagnostic for Xp11.2 translocation RCC. Diagnosis of Xp11.2 translocation RCC may be made only genetically.

In the 2004 World Health Organization (WHO) classification of renal tumors, renal cell carcinoma (RCC) associated with Xp11.2 translocation/transcription factor E3 (TFE3) fusion was delineated as a distinct entity.1 These RCCs are defined by several translocations involving the TFE3 gene that is located on chromosome Xp11.2, resulting in a gene fusion between TFE3 and at least 6 possible partners. The most commonly observed translocations are t(X;17)(p11.2;q25), t(X;1)(p11.2;p34), and t(X;1)(p11.2;q21), which lead to gene fusions of TFE3 with ASPL, PSF, and PRCC, respectively.2,3 Since the translocations lead to overexpression of the TFE3 protein, immunohistochemical staining for TFE3 is widely used as a surrogate marker for the Xp11.2 translocation,4 with strong nuclear TFE3 expression on low-power magnification considered characteristic.5 Epithelial markers such as the cytokeratins are expressed in 50% of the cases,1 but few express proteins of the hypoxia-inducible pathway such as HIF-1α and CAIX.6 Clinical studies have shown that the majority of Xp11.2 translocation RCCs occur in children and young adults.7,8 More than half of the patients have metastatic disease, leading to a poor prognosis.8-11 However, there are only a few larger case series reported in the literature, and, thus, clinicopathologic characteristics and outcomes remain poorly understood.4,9,12 In addition, there have been only a few reports evaluating the accuracy of TFE3 immunostaining by direct comparison of immunohistochemical and genetic analyses.5,13 The aims of this study were as follows: (1) determine the incidence of RCC associated with the Xp11.2 translocation and TFE3 expression in our laboratory, (2) characterize its clinicopathologic features and survival, and (3) evaluate the necessity of genetic analysis in diagnosing Xp11.2 translocation RCC.
by direct comparison with TFE3 immunohistochemical analysis. For these aims, we analyzed the data for 848 consecutive patients who underwent surgery for RCC at 1 institution during a 20-year period.

Materials and Methods

Study Population and Classification

Following approval by the institutional review board, a retrospective study was undertaken that included histologic reevaluation, immunohistochemical staining, and genetic evaluation. Between 1991 and 2010, a total of 848 patients underwent surgery for a unilateral, sporadic RCC at our institution. All available slides stained with H&E were reevaluated, retyped, and regraded by 1 dedicated uropathologist (A.H.). Further immunohistochemical and genetic studies were performed in tumors that occurred in young patients (≤40 years) and/or showed microscopic features suggestive of Xp11.2 translocation RCC.2,3,14 In this regard, 58 patients (6.8%) were 40 years or younger; of the 790 tumors in patients older than 40 years, 17 (2.2%) displayed microscopic features suggestive of Xp11.2 translocation RCC, defined by papillary structures lined by clear and/or bloated cells. Thus, a total 75 cases were further evaluated, accounting for 8.8% of the total RCC cohort. Sex, symptoms at diagnosis, TNM stage,15 pathologic tumor size, Fuhrman grade, and subtype were recorded for each case.

Immunohistochemical Analysis

Formalin-fixed, paraffin-embedded tissue was used for immunohistochemical analysis with the primary antibody TFE3 (P-16, sc-5958, Santa Cruz Biotechnology, Santa Cruz, CA) using the manual overnight incubation methodology.13 The TFE3 immunohistochemical assay was validated on a 20-year period.2,3,14 In this regard, 58 patients (6.8%) were 40 years or younger; of the 790 tumors in patients older than 40 years, 17 (2.2%) displayed microscopic features suggestive of Xp11.2 translocation RCC, defined by papillary structures lined by clear and/or bloated cells. Thus, a total 75 cases were further evaluated, accounting for 8.8% of the total RCC cohort. Sex, symptoms at diagnosis, TNM stage,15 pathologic tumor size, Fuhrman grade, and subtype were recorded for each case.

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Fluorescence In Situ Hybridization

A dual-color, break-apart fluorescence in situ hybridization (FISH) assay was performed according to Zhong et al,17 using the BAC clones RP11-107C19 and RP11-528A24. In brief, FISH of interphase nuclei was performed on 4-μm-thick, paraffin-embedded sections. RP11-107C19 and RP11-528A24 were labeled with 5-TAMRA deoxyuridine triphosphate (dUTP) and 5(6)-rhodamine green dUTP, respectively. After sample preparation, hybridization with labeled DNA was performed overnight. Slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI, Vysis, Abbott Park, IL) and analyzed with a fluorescence microscope (Zeiss, Oberkochen, Germany). Image 1D. FISH was regarded as positive when more than 10% of the tumor nuclei had evidence of Xp11 rearrangement.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) was performed to detect the ASPL-TFE3 gene fusion transcript according to established methods.12,18 RNA was extracted from paraffin-embedded tumor material from all patients using the High Pure RNA Paraffin kit (product No. 3270289, Roche Diagnostics, Indianapolis, IN). Following reverse transcription (Superscript III; First strand No. 11904-018, Invitrogen, Vienna, Austria), PCR amplification was performed with a specific primer pair for the translocation ASPL-TFE3 t(X;17), as described previously.12,18 PCR products were separated on an agarose gel and visualized by ethidium bromide staining. The amplified fragments were identified by their size and then subjected to automatic sequencing using a BigDye Terminator Cycling Sequencing kit on a DNA analyzer (Applied Biosystems, Carlsbad, CA).

Statistical Analysis

Categorical data were compared by using the Fisher exact test, and the Student t test was used to compare continuous data. Disease-specific survival (DSS) was calculated from the date of surgery to death of RCC or last follow-up. DSS functions were estimated with the Kaplan-Meier method and compared with log-rank tests. Multivariate Cox proportional hazards models were fit to determine independent predictors of DSS. The statistical package R 2.10.1 (http://cran.r-project.org) was used for all analyses.

Results

Patient and tumor characteristics are summarized in Table 1. Of the 848 RCCs that were reevaluated, 75 were...
further analyzed by immunohistochemical analysis and FISH. In this cohort, none of the patients had a history of malignant tumors. Metastatic spread to regional nodes or distant sites was detected in 12 cases (16%). None of the tumors occurred in a multifocal manner.

**Immunohistochemical Findings**

Of the 75 tumors, 17 (23%) showed strong, diffuse nuclear TFE3 expression in the entire tumor (TFE3+). In stromal or tubular cells, no nuclear staining was found. Occasionally, weak cytoplasmic staining was observed in some tubular structures but not in stromal cells. In addition, the staining intensity was much stronger in the nuclei of the tumor compared with the cytoplasm of the tubular cells (Image 1C).

In patients 40 years or younger, a total of 13 (22%) of 58 tumors were TFE3+. According to age at the time of surgery, the incidence values of TFE3 positivity in the age ranges 0 to 10, 11 to 20, 21 to 30, and 31 to 40 years were 67% (2/3), 75% (3/4), 29% (2/7), and 14% (6/44), respectively ($P < .001$) (Figure 1). (TFE3 negativity was as follows for the age groups: 0-10 years, 1/3 [33%]; 11-20 years, 1/4 [25%]; 21-30 years, 5/7 [71%]; and 31-40 years, 38/44 [86%]).
Clinical and pathologic features were compared between TFE3+ and TFE3– cases and all microscopically negative RCC cases. Patients with TFE3+ RCC more frequently had symptoms, larger tumors, and metastatic disease (Table 1). Of the 6 patients with TFE3+ RCC with distant metastasis, 5 (83%) had concomitant regional lymph node involvement, compared with only 14 (14%) of 98 in the negative cases ($P < .001$). Tumors with an extensive papillary growth pattern were more frequently seen in TFE3+ RCC (35% [6/17] vs 21% [12/58] and 35% [6/17] vs 17.7% [137/773], respectively), although the differences were not statistically significant ($P = .357$ and $P = .219$, respectively). In tumors with positive and negative TFE3, the percentage of tumors staining positively for Lu5 and AE1/3 was similar (Lu5 positive, 71% [12/17] vs 66% [38/58]; $P = .922$; AE1/3, 65% [11/17] vs 53% [31/58]; $P = .586$).

The mean follow-up was 44 months (SD, 41.8 months; range, 1-120 months), during which 85 patients died of the disease, including 6 (35%) of 17 with TFE3+ RCC. The mean ± SE 5-year DSS rates for TFE3+ RCC, TFE3– RCC, and the remaining histologically negative RCC cases were 62% ± 12%, 90% ± 4%, and 82% ± 2%, respectively. The survival difference between TFE3+ and TFE3– cases was statistically significant ($P = .032$) as was the difference between the TFE3+ and remaining histologically negative RCCs ($P = .024$; Figure 2). For multivariate analysis, TFE3– cases and histologically negative cases were pooled because they did not differ in terms of pathologic factors (each $P > .2$) and survival ($P = .313$). In the multivariate model, T stage, N stage, M stage, and grade, but not TFE3 immunostaining, were retained as independent prognostic factors of DSS.

**Genetic Analysis**

All 75 tumors were evaluated by FISH and PCR. Here, 2 tumors (3%) showed an X-chromosome alteration in the FISH...
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Figure 2 Kaplan-Meier survival estimates comparing transcription factor E3 (TFE3)+ with TFE3– renal cell carcinoma (RCC) cases (A) and TFE3+ with histologically negative RCC cases (B). In both analyses, TFE3+ RCCs had significantly worse survival. A, Hazard ratio (HR), 3.34; 95% confidence interval (CI), 1.03-11.11; P = .032 (log rank). B, HR, 2.53; 95% CI, 1.10-5.82; P = .024 (log rank).

Table 2 Multivariable Survival Analysis in Renal Cell Carcinoma Cases

<table>
<thead>
<tr>
<th>Hazard Ratio 95% Confidence Interval</th>
<th>P</th>
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<tbody>
<tr>
<td>Symptoms</td>
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</tr>
<tr>
<td>T stage</td>
<td>1.87</td>
</tr>
<tr>
<td>N stage</td>
<td>1.94</td>
</tr>
<tr>
<td>M stage</td>
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<tr>
<td>Grade</td>
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</tr>
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<tr>
<td>TFE3</td>
<td>1.49</td>
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TFE3, transcription factor E3.

* T stage, N stage, M stage, and grade, but not TFE3 status, were independent predictors of disease-specific survival.

and the ASPL-TFE3 fusion transcript in the sequence. Therefore, these 2 tumors represented Xp11.2 translocation RCC with ASPL-TFE3 gene fusion. In the remaining 73 tumors, neither FISH nor PCR was positive. Both translocation RCCs also showed strong nuclear TFE3 expression. Thus, the sensitivity of TFE3 immunostaining for Xp11.2 translocation RCC was 100% (2/2), with a specificity of 79% (58/73) and a negative predictive value of 100% (58/58). However, the positive predictive value of positive TFE3 staining for Xp11.2 translocation RCC was only 12% (2/17). There were only 2 (0.2%) of 848 RCCs with genetically proven Xp11.2 translocation during a 20-year period.

The first patient was a 5-year-old boy who had a 12-cm renal tumor. A Wilms tumor was suspected, and the patient underwent preoperative chemotherapy without any response, followed by open radical nephrectomy with regional lymph node dissection. TNM stage was pT3a pN1 (2/10) pM1 (liver). Postoperatively, the patient received vaccination with tumor-lysate pulsed dendritic cells in combination with interferon-alfa-2a. The interferon-alfa-2a therapy was maintained for 4.5 years. The patient achieved a complete response and, at last follow-up, was alive without evidence of disease. The second patient was a 42-year-old woman who had an 8-cm renal mass found during the workup for flank pain. Open radical nephrectomy was performed, revealing Fuhrman grade 2 RCC with TNM stage T2N0M0. At 8 years after surgery, she was alive without evidence of disease.

Discussion

We reevaluated the data for 848 patients who underwent surgery for RCC. We found that the incidence of Xp11.2 translocation RCC is very low, that TFE3 is an immunohistochemical marker of metastasis and poor survival, and that, although TFE3 expression is associated with Xp11.2 translocation, only a minority of these tumors shows the Xp11.2 translocation in our laboratory. Therefore, TFE3 should not be used as a surrogate marker for the translocation. In our laboratory, definite diagnosis of Xp11.2 translocation RCC may be made only by genetic analysis.

The overall incidence of Xp11.2 translocation RCC is very low, with an increased risk in children and young adults.\textsuperscript{7,9,11} Within a 20-year period, we observed only 2 cases of this disease (0.2%). These data are in accordance with a multicenter study on 3,423 kidney tumors, in which only 31 translocation RCCs (0.9%) were found.\textsuperscript{9} In another large screening study of 443 adults with RCC, only 7 translocation RCCs (1.6%) were observed.\textsuperscript{4} Bruder et al\textsuperscript{19} specifically analyzed the data for pediatric and adolescent populations. In their study, 8 (20%) of 41 tumors were identified as...
translocation RCC. Reinforcing the concept of low incidence, these 8 tumors were diagnosed at 2 centers during a 25-year period. In pediatric patients, the incidence may be higher after chemotherapy.20

The diagnosis of an Xp11.2 translocation RCC is based on microscopic appearance, TFE3 immunostaining, and genetic analyses. In our hands, TFE3 was a highly sensitive and specific immunohistochemical marker for screening tumors for the Xp11.2 translocation. These data are in line with a previous report on more than 1,500 cases; however, in this large study, no TFE3+ cases without the Xp11.2 translocation were observed.5 In contrast, we and others19,21 identified cases that strongly expressed TFE3 but did not have the Xp11.2 translocation. In total, the incidence of an Xp11.2 translocation among our TFE3+ cases was fairly low, with a positive predictive value of only 12%. Hence, in our laboratory, the diagnostic value of TFE3 was lower than reported previously.5 Methodological differences may partially explain this fact, including differences in the lot of the polyclonal antibody and differences in detection methods. We further suspect that the enhanced detection method used in our study may result in the detection of native TFE3 more frequently. In this regard, Argani et al13 recently noted that the incubation method (automated vs overnight) alters the diagnostic value of TFE3 staining with an increased detection of native TFE3.

Our results are further supported by a recent multicenter study of 252 patients with RCC who were younger than 35 years or older than 80 years.21 The tumors were all reclassified according to the World Health Organization classification, TFE3 was evaluated by immunohistochemical analysis, and comparative genomic hybridization was performed for genetic analysis. On reclassification, only 3 tumors (1.2%) showed histologic features suggestive of translocation RCC. TFE3 stained positively in 12% of tumors from younger patients (<35 years) and 0.5% of older patients (>80 years). It is important to note that no specific genetic alterations could be detected.21 We conclude that, in our laboratory, strong nuclear TFE3 expression may not be caused by translocations only, but also by other biologic pathways that yet have to be identified. TFE3 should not be used as a surrogate marker for the translocation. Definite diagnosis of Xp11.2 translocation RCC may be made only by genetic analysis. Given the conflicting data in the literature, however, further studies are necessary.

Traditionally, Xp11.2 translocation RCC has been associated with advanced tumor stage and a poor prognosis.2,8,11,12 Studies indicate that about half of the patients have metastatic disease8,11,12,22 and that the majority of patients will eventually die of progressive RCC11,12,22 However, our data and the data of others show that some patients have considerable long-term survival, even in the setting of metastatic disease.2,4,23 Furthermore, it was shown in a literature review that ASPL-TFE3 gene fusion RCC is more likely to present at an advanced stage than is PRCC-TFE3 RCC.24 Our 2 genetically confirmed cases of ASPL-TFE3 gene fusion had a favorable outcome in short-term follow-up, while strong TFE3 staining was generally associated with poor survival. This fact may further indicate that in a proportion of tumors with strong TFE3 staining, native TFE3 was detected.

Recently, Malouf et al22 evaluated 53 cases of translocation RCC, of which 23 were metastatic. In the latter group, 21 patients received targeted therapy with vascular endothelial growth factor receptor–targeted agents and/or mTOR inhibitors. Of note, 7 patients (33%) achieved an objective response and median overall survival was 27 months, which is comparable to the general population receiving these agents.25,26 The response to mTOR inhibitors may be partially explained by elevated expression levels of phosphorylated S6.6 Given the relatively low incidence of this entity and the relatively few cases reported in the literature, one may only conclude that the majority of Xp11.2 translocation RCCs occur with metastatic disease; however, it may be too early to conclude clinical outcomes.

There is now considerable evidence that immunohistochemical and genetic markers may assist in determining the prognosis in RCC and, therefore, in tailoring postoperative surveillance and therapy.27-29 In this regard, the value of TFE3 as a routine immunohistochemical marker may be 3-fold: as a prognostic factor, as an indicator of concomitant lymph node involvement, and as a screening marker for Xp11.2 translocation before genetic analysis, as described. We and others12 found a strong association of TFE3 expression with regional lymph node metastasis, which is generally accepted as a poor prognostic marker in the setting of metastatic RCC.30 Thus, TFE3 may assist in assigning risk groups in these cases, which subsequently guides systemic therapy. In addition, TFE3 may indicate the need for regional node dissection in patients with clinically localized disease, which may be of benefit in selected cases.31-33 TFE3 may further predict prognosis, although this marker was not retained as an independent prognostic factor in multivariate analysis, which may be due to the relatively low number of patients with TFE3+ RCC and the subsequent low statistical power. Further studies with larger numbers of cases are necessary to elucidate this role.

There are several issues in the present study that merit discussion. We did not evaluate TFE3 expression in patients older than 40 years with RCC histologically negative for Xp11.2 translocation. Thus, we may have missed some cases, although the overall incidence in this group is low.4 Since our data indicate that TFE3 can be positive in RCCs without the translocation, the incidence of TFE3+ tumors...
and the prognostic relevance of this marker may be underestimated. We did not evaluate fusions that are much less common than ASPL-TFE3, such as PSF-TFE3 and PRCC-TFE3. However, it is unlikely that PCR would have been positive in the absence of positive FISH results. Finally, the retrospective nature and the relatively low number of cases limit the strength of the conclusions, so that all data should be confirmed with larger data sets.

**Conclusions**

The incidence of Xp11.2 translocation RCC is very low. Strong nuclear TFE3 expression is associated with metastatic spread and a poor prognosis; however, it is not diagnostic for Xp11.2 translocation RCC and should, therefore, not be used as a surrogate marker in our laboratory. In our laboratory, definite diagnosis of Xp11.2 translocation RCC may be made only by genetic analysis.

**References**


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