Human Cancer Biology

Genomic Heterogeneity of Translocation Renal Cell Carcinoma

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Abstract

**Purpose:** Translocation renal cell carcinoma (tRCC) is a rare subtype of kidney cancer involving the TFE3 genes. We aimed to investigate the genomic and epigenetic features of this entity.

**Experimental Design:** Cytogenomic analysis was conducted with 250K single-nucleotide polymorphism microarrays on 16 tumor specimens and four cell lines. LINE-1 methylation, a surrogate marker of DNA methylation, was conducted on 27 cases using pyrosequencing.

**Results:** tRCC showed cytogenomic heterogeneity, with 31.2% and 18.7% of cases presenting similarities with clear-cell and papillary RCC profiles, respectively. The most common alteration was a 17q gain in seven tumors (44%), followed by a 9p loss in six cases (37%). Less frequent were losses of 3p and 17p in five cases (31%) each. Patients with 17q gain were older (P = 0.0006), displayed more genetic alterations (P < 0.003), and had a worse outcome (P = 0.002) than patients without it. Analysis comparing gene-expression profiling of a subset of tumors bearing 17q gain and those without suggest large-scale dosage effects and TP53 haploinsufficiency without any somatic TP53 mutation identified. Cell line–based cytogenetic studies revealed that 17q gain can be related to isochromosome 17 and/or to multiple translocations occurring around 17q breakpoints. Finally, LINE-1 methylation was lower in tRCC tumors from adults compared with tumors from young patients (71.1% vs. 76.7%; P = 0.02).

**Conclusions:** Our results reveal genomic heterogeneity of tRCC with similarities to other renal tumor subtypes and raise important questions about the role of TFE3 translocations and other chromosomal imbalances in tRCC biology. Clin Cancer Res; 19(17); 4673–84. ©2013 AACR.

**Introduction**

Translocation renal cell carcinoma (tRCC) is a subtype of renal cell carcinoma (RCC) that was recognized in the 2004 World Health Organization classification of renal tumors as a genetically distinct entity (1). Originally described in pediatric patients, the spectrum of the disease has recently been expanded to include adults (2). Recent studies showed that tRCC represents one third of pediatric RCCs, up to 15% of RCCs in patients younger than 45 years old, and up to 5% of all RCCs regardless of age (3–5). The hallmark of tRCC is the fusion of the TFE3 gene, located in Xp11, with various partners, including PRCC in t(X;1)(p11.2;q21), SF3B1 in t(X;1)(p11.2;p34), ASPSCR1-TFE3 in t(X;17)(p11.2;q25), NONO in inv(X)(p11.2q12), and CLTC in t(X;17)(p11.2;q23) (6). tRCC can also be related to translocations involving the TFE gene (7).

We and others have reported that the disease behaves differently in adult and pediatric patients (2, 8–10). Similarly, better outcomes in young patients were reported for alveolar soft part sarcoma, a tumor characterized by ASPSCR1-TFE3 translocation (11). Recently, we showed that patients with tRCC who had metastatic disease at presentation were older (median age 36 years) and predominantly male, whereas patients who had loco-regional...
**Translational Relevance**

Translocation renal cell carcinoma (tRCC) is a rare kidney cancer subtype that mainly arises in children and young adults and often has both papillary and clear-cell pathologic features. Little is known about whether additional genetic alterations are associated with tRCC. By single-nucleotide polymorphism–array profiling and LINE-1 methylation, we found genomic heterogeneity of tRCC that included alterations common with clear-cell RCC (e.g., 3p loss) and papillary RCC (e.g., trisomy 7 and/or 17). When compared with young patients (<18 years), adults with tRCC displayed distinct genomic and epigenetic aberrations, exemplified by lower LINE-1 methylation and frequent 17q partial gain, which were consistent with a large-scale dosage effect affecting RCC carcinogenesis. Our results show that besides TFE3/TFEB translocations, tRCC shares alterations commonly present in other RCC histologic subtypes and these are associated with patient outcomes. Furthermore, our study suggests that targeting tRCCs according to their genetic profile may have therapeutic relevance.

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**Patients and Methods**

**Patient selection and classification of cases included in single-nucleotide polymorphism-array analysis**

Tissue specimens from 21 patients with a histopathologic diagnosis of tRCC supported by TFE3 positivity on immunohistochemistry were collected after approval of the Institutional Review Board of each of the participating centers. TFE3 and TFEB immunostaining were conducted at each individual institution as previously described (7, 17). The flow chart of patient selection, describing the different tests conducted is depicted in Supplementary Fig. S1.

Among the total of 21 cases, 15 were confirmed to be tRCC by FISH, conventional karyotyping, or reverse transcription PCR (RT-PCR) analysis for all known specific fusion partners. One patient whose tumor had classical tRCC morphology and TFE3 immunohistochemical positivity was also included in the final cohort (n = 16). We collected the following clinicopathologic information for each patient: age, sex, ethnicity, tumor–node–metastasis (TNM) classification, tumor size, lymph node involvement, Fuhrman grade, and survival time (Supplementary Table S1). The clinicopathologic data from 5 of the patients (numbers T31 through T35) were previously reported (9). Four patients with positive TFE3 immunohistochemistry but negative by FISH analysis for TFE3 translocations were included in the single-nucleotide polymorphism (SNP) array studies as a control group.

**Patient selection and LINE-1 methylation analysis**

DNA was available for studying LINE-1 methylation in 12 patients for whom SNP-array analysis was conducted (all except the following 4 cases: LOY009, MRCC106, MRCC107, and MRCC117). In addition, DNA was extracted from 15 formalin-fixed paraffin-embedded (FFPE) tissues of patients for whom we previously reported pathologic features and outcome (9, 18). Seven of those patients had confirmed translocation by karyotyping and/or RT-PCR, and the remaining had their diagnosis confirmed by TFE3 immunostaining, as previously described (18). Furthermore, DNA extracted from 16 samples belonging to adjacent normal kidney of patients operated on for renal cell carcinoma was included to study LINE-1 methylation variability. DNA isolation was done using the Gentra Systems Puregene DNA Purification Kit according to the manufacturer’s instructions. LINE-1 repetitive element, a surrogate of global DNA methylation, was assessed by pyrosequencing as described previously (19). Assay replicates for this assay were highly correlated as previously shown (19).

**Generation of novel cell lines from adult patients**

Two novel cell lines, HCR-59 and MDA-92, were derived from 2 adults treated at MD Anderson Cancer Center (MDACC; Houston, TX), as previously reported for other RCC subtypes (20). HCR-59 was derived from a 20-year-old Caucasian female, and MDA-92 was derived from a 51-year-old Hispanic male. For both of those cell lines, karyotyping was conducted according to previously published methods (21). Spectral karyotyping (SKY) was also conducted on HCR-59 by using the human chromosome HiSKY probe (Applied Spectral Imaging, Inc.).

The tRCC cell lines UOIK109 and UOIK146, derived, respectively, from a 39-year-old male and a 42-year-old female (22), were kindly provided by Dr. W. Marston Linehan (U.S. NIH, Bethesda, MD). Both cell lines were originally reported as pRCC cell lines before tRCC became a recognized pathologic entity. The UOIK109 line has been shown to carry the NONO-TFE3 translocation (23), and the UOIK146 cell line carries the translocation t(X;1)(p11.2; q21.2) (22).
Cytogenomic analysis

DNA obtained from frozen tissues or from manually microdissected FFPE containing at least 70% tumor cells was quantified on a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc.). The DNA was analyzed on Affy250K Nsp SNP genotyping arrays (Affymetrix, Inc.) following the previously reported modified protocol (24). LOH and copy number estimates were obtained using the publicly available Copy Number Analyzer for Affymetrix GeneChip Arrays package (CNAG v3.0; ref. 25). The number of genetic alterations was defined as the number of gains and losses of segmental chromosomal regions.

RNA sequencing

For 4 translocation Xp11 cases (T31, T32, T34, T1), RNA of good quality with RNA integrity number values between 8.0 and 10.0 was available. Briefly, preparation of mRNA-seq libraries for each case was conducted according to Illumina procedures at the MDACC Genomic Core Facility, and sequencing was done on a HiSeq 2000 system (Illumina, Inc.). After quality control, reads were mapped and then aligned to the UCSC hg19 version of the reference genome. Gene expression counts were normalized using the RPKM (reads/kb/million) method (26).

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) is a computational method that assesses whether a defined set of genes shows statistically significant differences between two conditions (24). The average degree of change between the normalized RPKM values of 2 samples with partial 17q gain and 2 samples without was calculated, and genes were ranked by order of expression in the 2 samples with partial 17q gain. The list was uploaded as a pre-ranked list to GSEA v2.04 (Broad Institute, Cambridge, MA; ref. 27), and GSEA was conducted using the default normalization mode. Genes were classified according to the positional gene set (C1) in the Molecular Signatures Database (http://www.broadinstitute.org/gsea/msigdb/index.jsp).

Mutation of TP53 and VHL genes

For the TP53 gene, mutations occurring in exons 5 to 8 were assessed using five sets of oligonucleotide primers as previously described (28). VHL mutations were assessed using four primers pairs as previously described (29). Overall, 12 cases with available DNA were analyzed as shown in Supplementary Fig. S1.

Statistical analysis

Associations with clinicopathologic factors were analyzed with \( \chi^2 \) and t test. OS was calculated from the date of nephrectomy to the date of death or last visit (censored). Survival probabilities were estimated with the Kaplan-Meier method, and log-rank testing was applied to compare survival curves. Spearman correlation coefficient was used to assess the correlation between the LINE-1 methylation and number of genetic aberrations. All statistical tests were two-sided and conducted at the significance level of 0.05 using Prism version 5 (GraphPad Software, Inc.).

Results

Patient and tumor characteristics

Patient and tumor characteristics are summarized in Supplementary Table S1. With a median follow-up time of 29 months (range, 3–92 months), 5 patients (31%) were dead of disease, 8 patients had no evidence of disease, and 3 patients were alive with metastatic disease.

tRCC shows cytogenomic heterogeneity

Translocation carcinomas showed significant heterogeneity in cytogenomic profiles (Fig. 1 and Table 1). The most common alteration observed was a gain of 17q, which was present in seven (44%) of the tumors. Less frequent alterations were gains of chromosomes 12 and 7, observed in 6 (37%) and 5 (31%) patients, respectively. The most commonly observed cytogenetic losses were of 9p in 6 (37%) patients and of 3p, 1p, 17p, and 18q, each of which occurring in 5 (31%) patients. Three of 10 women in our cohort (30%) lost one X chromosome.

Overall, the 16 cases of tRCC with a confirmed translocation could be classified into four groups on the basis of their similarities to cytogenomic profiles of other renal epithelial tumors (15).

- **Group I**: 5 cases had profiles similar to those of ccRCC, owing to 3p loss and other imbalances (including the TFEB case). Three of those 5 cases were available for histologic review with 2 showing mixed clear-cell/papillary features, and 1 showing only clear-cell morphology.
- **Group II**: 3 cases had profiles similar to those of pRCC with gains of chromosomes 7, 12, and 17. All 3 cases showed mixed ccRCC and pRCC histology.
- **Group III**: 5 cases had novel cytogenomic profiles, that is, not associated with those of other subsets of renal tumors.
- **Group IV**: 3 cases had a balanced chromosomal complement.

As Fig. 1 shows, tRCC tumors had heterogeneous chromosome copy number profiles. 3p loss was associated with 9p loss (\( P < 0.04 \)) but not associated with the loss of 1p, 17p, or 18q (\( P = 0.24 \) for each comparison). There was no significant difference in the number of genetic alterations between patients with 3p loss and those without (11.8 vs. 6.7; \( P = 0.23 \)).

Of the cases (44%) that had a gain of 17q, two tumors had a confirmed t(X;17)(p11;q25) translocation and 1 case had a TFEB translocation. Interestingly, in 4 of 7 cases with 17q gain, 17p loss has been shown, consistent with the generation of an isochromosome 17q, or i(17q). In contrast, none of 4 cases overexpressing TFE3 but negative by FISH had gain of 17q or loss of 17p, and 2 of them had 3p loss (Supplementary Table S2).
Tumors of adult patients (age ≥ 18 years) showed more genetic abnormalities than tumors from younger patients (age < 18 years; \( P = 0.006 \)), with no differences found in sex (\( P = 0.31 \)), TNM stage (\( P = 0.60 \)), and Fuhrman grade (\( P = 0.62 \)).

**Unsupervised clustering reveals two distinct groups of tRCC**

To gain insights into the biology of tRCC, we conducted unsupervised clustering analysis using the most frequent segmental alterations (losses or gains). That analysis revealed two main clusters (Fig. 2A). The median number of genetic alterations in cluster A was 0 (range, 0–5), compared with 14 in cluster B (range, 5–22). The two clusters showed no significant differences in terms of pT stage, lymph node involvement, metastasis, TNM classification, gender, and Fuhrman grade (\( P = 0.62 \)).

Of note, 7 of 10 patients who were older than 18 years had a 17q gain, but none of the 2 older patients in cluster A did.

**Association between chromosomal imbalances and pathologic features and outcome**

As noted earlier, a gain of 17q was the most frequent alteration (44%) we found in tRCC with a minimally gained region of 11.9 Mb (17q24.3-q25.3). Four of these cases had 17p deletions, with a minimally deleted region of 22.1 Mb (entire p arm), including the TP53 locus. No associations were found between 17q gain and any other frequently occurring genetic gain or loss. The mean number of alterations in patients with 17q gain was 14.4 ± 2.4 versus 3.7 ± 2.4 in patients without 17q gain (\( P < 0.003 \); Fig. 2B). In addition, patients with 17q gain were older than those without: their median age was 40.2 (range, 20.4–54.6) versus 16.7 years (range, 9.1–33.1; \( P = 0.0006 \)). The gain of 17q was also found more frequently in men than in women (71.4% vs. 11.1%; \( P = 0.03 \)). No associations were found between 17q gain and other clinicopathologic variables, i.e., tumor size, ethnicity, pT stage, lymph node involvement,
Table 1. Genetic/genomic characteristics of translocation RCC cases

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chromosomal translocation</th>
<th>Diagnostic method</th>
<th>Gene</th>
<th>Fusion partner</th>
<th>Gains</th>
<th>Losses</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>NA</td>
<td>FISH</td>
<td>TFE3</td>
<td>Unknown</td>
<td>+5(p33.3-q34), +5(q34-q35.3), +17(q21.31-q25.3)</td>
<td>–1p31.1-p21.1), –1p13.3-p11.2), –3(p26.3-p11.2), –9q24.3-p21.1), –17(p13.3-p11.2), –18q11.2-q11.12), –18q12.1-q23</td>
</tr>
<tr>
<td>T2</td>
<td>NA</td>
<td>FISH</td>
<td>TFE3</td>
<td>Unknown</td>
<td>+2p25.3-q37.3, +7p22.3-q36.3, +12p13.33-q24.33, +17(p13.3-q25.3), +20p13-q13.33</td>
<td>No</td>
</tr>
<tr>
<td>T25</td>
<td>NA</td>
<td>RT-PCR</td>
<td>TFE3</td>
<td>NONO</td>
<td>No</td>
<td>No</td>
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<td>T31</td>
<td>t(X;1)(p11.2;q21)</td>
<td>RT-PCR</td>
<td>TFE3</td>
<td>PRCC</td>
<td>No</td>
<td>–X</td>
</tr>
<tr>
<td>T32</td>
<td>t(X;1)(p11.2;q21)</td>
<td>RT-PCR</td>
<td>TFE3</td>
<td>PRCC</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>T33</td>
<td>t(X;1)(p11.2;q21)</td>
<td>RT-PCR</td>
<td>TFE3</td>
<td>PRCC</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>T35</td>
<td>t(X;1)(p11.2;2q1)</td>
<td>RT-PCR</td>
<td>TFE3</td>
<td>PRCC</td>
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<td>No</td>
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<td>RT-PCR</td>
<td>TFE3</td>
<td>PRCC</td>
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<td>No</td>
</tr>
<tr>
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<td>FISH</td>
<td>TFE3</td>
<td>Unknown</td>
<td>+12p13.33-q24.33, +2p25.3-q37.3</td>
<td>–1p36.33-p34.3), –6p25.3-q27, –9(p24.3-q34.3), –13(q11-q34), –14(q11.2-q32.33), –18p11.1-q23, –19p13.3-q13.43, –X(p11.4-q28), –4p16.3-q35.2, –7(q22.3-q26.3),</td>
</tr>
<tr>
<td>T106</td>
<td>t(X;1)(p7.2q7)</td>
<td>Karyotype</td>
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<td>Unknown</td>
<td>+2p21.2-q37.1, +12, +13q14.3-q34, +14</td>
<td>–1p36.33-p34.3), –6p25.3-q27, –9(p24.3-q34.3), –13(q11-q34), –14(q11.2-q32.33), –18p11.1-q23, –19p13.3-q13.43, –X(p11.4-q28), –4p16.3-q35.2, –7(q22.3-q26.3), –11p15.5-q25, –22q11.1-q13.33</td>
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<tr>
<td>T107</td>
<td>t(X;1)(p11.2;p34)</td>
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<td>Presumably PSF</td>
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<td>No</td>
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<tr>
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<td>NA</td>
<td>FISH</td>
<td>TFE3</td>
<td>Unknown</td>
<td>7</td>
<td>–3, –15, –17(p13.3-p11.2), –2op</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available; IHC, immunohistochemistry; NED, no evidence of disease.
TNM classification, or Fuhrman grade (data not shown). Finally, patients with 17q gain had a more adverse prognosis, with a 19.9-fold higher risk of RCC-specific death (95% confidence intervals, 3.1–129; \( P = 0.002 \); Fig. 2C).

Patients with 3p loss had a higher pT stage than did those without 3p loss (\( P = 0.03 \)). However, in contrast to the 17q gain, no association was found between 3p loss and clinicopathologic variables such as age, gender, and TNM classification. Patients with 3p loss also had a shorter OS time than did those without 3p loss, with median 12.7 months versus not reached (\( P = 0.001 \)). To assess whether 3p loss was associated with VHL mutations, as is the case for ccRCC, somatic mutations of VHL were analyzed in 12 cases, but no mutations were identified.

Patients with 9p loss had more genetic alterations than did patients without 9p loss: 15.0 ± 2.3 versus 4.3 ± 1.9 (\( P = 0.003 \)). No statistically significant associations were found between 9p loss and age, gender, TNM stage classification, pT stage, lymph node involvement, or metastasis; patients with 9p loss had a shorter OS time than did those without 9p loss, with median OS of 26 versus 66 months. However, this difference did not reach statistical significance (\( P = 0.13 \)).

Cell lines recapitulate cytogenomic profiles from tumors

We used the established cell lines UOK109 and UOK146 and the novel cell lines HCR-59 and MDA-92. The UOK109 line, which was derived from a tumor originally reported as pRCC, had a pRCC-like cytogenomic profile, with chromosome 7 gain (Supplementary Fig. S2A). In contrast, the UOK146 cell line, also derived from a pRCC tumor, had a ccRCC-like cytogenomic profile, with loss of 1p, 3p, chromosome 4, 9p, and chromosome 18 and a gain of chromosomes 7 and 17q (Supplementary Fig. S2B).

To assess whether the 17q gain is related to unbalanced translocation or is the result of an isochromosome 17, we conducted G-banding karyotyping of the UOK146 cells. Besides a 3p loss, an i(17q) was present. This is consistent with a model in which a 17q gain in cancer often occurs as the result of i(17q) formation and is accompanied by allelic loss at 17p (Supplementary Fig. S2C).

The results of FISH analysis for TFE3 (Supplementary Fig. S2D) as well as conventional karyotyping of the MDA-92 cell line (derived from patient 13) confirmed the TFE3 translocation (data not shown) and showed a near-tetraploid karyotype, with 1p and 3p losses, as is the case for the primary tumor. We also observed two interchromosomal

Figure 2. A, unsupervised clustering analysis of most frequently gained and lost regions in patients with tRCC showing two main subtypes. B, more alterations occurred in patients with 17q gain than in patients without the gain. **, \( P = 0.0026 \). C, Kaplan–Meier survival estimates for patients with and without 17q gain.
translocations involving 17q that led to 17q gains (Fig. 3A). Similarly, SKY of the HCR-59 cell line (derived from patient T1) showed, besides 3p and 17p losses, 6 translocated chromosomes with 3 of them involving 17q (Fig. 3B). All the genetic gains and losses reported by SNP cytogenomic array analysis of the primary tumor samples [\(\text{gain}\), \(\text{loss}\)] are located around the breakpoints of the six translocations and are consistent with imbalanced translocations.

**17q partial gain may be related to a large-scale dosage effect and p53 haploinsufficiency**

Because partial 17q gain was the most common abnormality after TFE3 translocations and because patients with 17q had a poorer outcome than did patients without 17q, we investigated the expression pattern on chromosome 17. To identify chromosomal regions differentially expressed in cases with partial 17q gain and those without, we conducted global gene expression analysis using RNA-seq in 4 Xp11 cases for which RNA was available. Two cases with partial 17q gain, including patients T34 and T1, were compared with 2 cases with balanced virtual karyotypes, including patients T31 and T32.

GSEA showed significant enrichment of genes located in the 17q arm as follows: 17q25 (false discovery rate (FDR) = 0.0001; \(\text{P} < 0.0001\)), 17q23 (FDR = 0.07; \(\text{P} < 0.001\)), 17q21 (FDR = 0.08; \(\text{P} < 0.001\)), 17q24 (FDR = 0.10; \(\text{P} < 0.001\)), and 17q22 (FDR = 0.10; \(\text{P} < 0.001\); Fig. 4A), and no enrichment for 17p genes. Interestingly, when genes were classified according to the C6 oncogenic signature in the Molecular Signatures Database, we found enrichment for genes downregulated by TP53 in NCI-60 panel of cell lines with mutated TP53 as compared with those classified as normal (Fig. 4B).

As gain of 17q was frequently associated with loss of 17p, which contains the p53 tumor suppressor gene, and as p53 loss is commonly associated with a poor prognosis in various malignancies, we assessed for the presence of TP53 mutations. Sanger sequencing was thus conducted on 12 tRCC including 7 cases bearing gain of 17q, but no mutation of TP53 was identified in any of those cases (not shown). Interestingly, Ingenuity Pathway Analysis identified TP53 inactivation as the top transcription regulator.
factor predicted to be inactivated in the 2 cases (RCC-T34 and RCC-T11) with concomitant partial 17q gain as compared with the 2 cases (RCC-T31 and RCC-T32) with balanced karyotype (Supplementary Table S3). Overall, there were 51 of 89 genes whose expression direction was consistent with inhibition of TP53 (z-score = −3.84; \( P = 5.37 \times 10^{-11} \); Fig. 4C).

Also consistent with TP53 inactivation, GSEA showed enrichment for multiple pathways related to mitosis as follows: the M-phase of the mitotic cycle (FDR = 0.10; \( P < 0.001 \)), mitosis (FDR = 0.10; \( P < 0.001 \)), and the M-phase (FDR = 0.10; \( P < 0.001 \)), in keeping with the aggressive phenotype of cases with partial 17q gain (Supplementary Fig. S3A).

KEGG (Kyoto Encyclopedia of Genes and Genomes) and BioCarta (BioCarta LLC) pathways (C2 gene set) using GSEA and Ingenuity Pathway Analysis (Ingenuity Systems, Inc.) showed consistent results with T-cell activation in the subgroup of patients with partial 17q gain. In particular, BioCarta pathways showed enrichment of the CTLA-4 pathway (FDR < 0.001; \( P < 0.001 \)), and the KEGG pathway showed enrichment of the T-cell receptor signaling pathway (FDR = 0.01; \( P < 0.001 \)). Those results were also obtained using Ingenuity Pathway Analysis, which showed activation of inducible costimulator–inducible costimulator ligand signaling in T-helper cells (\( P = 2.4E^{-4} \)) and activation of the CTLA-4 signaling pathway (\( P = 1.6E^{-3} \)). Of note, 17 of 95 genes (17.9%) related to the CTLA-4 pathway were upregulated in patients with 17q gain (Supplementary Fig. S3B). Immunohistochemistry for CD3 and CD5 T-cell markers showed increase of T lymphocytes in the 2 cases with 17q gain (10%–20%) as compared with less than 1% to 2% in the 2 cases without 17q gain (not shown).

**LINE-1 methylation is lower in adults than in young patients (≤18 years old)**

We conducted LINE-1 methylation analyses in a total of 27 tRCC cases (12 of which had SNP array results). LINE-1
methylation levels correlated inversely with the number of genetic abnormalities in cases with both SNP-array and LINE-1 methylation data (Spearman $\rho = -0.6; P = 0.04$; Fig. 5A). Consistent with the greater number of genetic abnormalities in adult patients, in the 27-patient cohort, adults showed lower LINE-1 methylation, compared with younger patients (71.1% vs. 76.7%; $P = 0.02$; Fig. 5B). Methylation levels of normal kidney tissue showed little variability with an average methylation of 73.07% and SD of $\pm 0.50$, and no association with age or gender.

**Discussion**

To our knowledge, this is the first study to describe cytogenomic aberrations and DNA methylation status in a cohort of pediatric, adolescent, and adult patients with translocation RCC. Our study shows cytogenomic and epigenetic heterogeneity of tRCC and unexpectedly reveals that these tumors share genomic alterations that are common to ccRCCs and pRCCs. Our results show that tRCC tumors from young patients (<18 years) display fewer genetic alterations and higher levels of global DNA methylation, compared with tumors from adult patients (≥18 years). Moreover, our data show that a partial 17q gain is frequent in tumors from adult patients, particularly in men, and this genetic lesion is associated with poor outcome.

Although the identification of significant correlations between cytogenetic alterations and histopathologic subtypes of RCC is well established for common renal cancer subtypes, such as ccRCC (e.g., 3p loss) and pRCC (e.g., trisomy 7 and/or 17; refs. 15, 30), little is known about chromosome copy number alterations in tRCC. Here, we show that a large proportion of tRCCs present with chromosome copy number changes similar to those observed in ccRCC or pRCC, but almost 30% of them display cytogenomic profiles that cannot be related to these or characterized RCC subtypes.

Morphologic diagnosis of tRCC is challenging, especially in adult patients. Sukov and colleagues showed that tRCC tumors have significant histologic variability (31), and Klatt and colleagues showed that only 2 of 75 cases with microscopic features suggestive of Xp11.2 translocation RCC or occurring in patients 40 years or younger have identifiable translocations with FISH and/or RT-PCR (32). Historically, the translocation involving TFE3 (or TFEB) is considered as the defining feature for this disease. Mechanistic studies have shown the transforming effect of the $TFE3$ and $TFEB$ translocations in cell lines (33, 34). Mathur and Samuels conducted knockdown of PSF-TFE3 in UOK-145 cell line and showed that it led to impaired growth, proliferation, invasion potential, and long-term survival (33). The same authors also showed that the expression of PSF-TFE3 in normal proximal tubular cells leads to initiation and maintenance of an oncogenic phenotype (33). Moreover, in the UO109 cell line (NONO-TFE3 translocation), TFE3-directed short hairpin RNA (shRNA) prevented all colony growth (35). The identification of "ccRCC-like" and "pRCC-like" cytogenomic profiles in tRCC cases raises the question of whether tumors harboring such profiles are in reality ccRCC and pRCCs that have acquired a TFE3 translocation during tumor progression or whether tRCC tumors (initiated by a TFE3 translocation) can then develop different types of chromosomal instability leading to different cytogenomic profiles. Are 3p loss and gain of 7/17 important for the initiation of tRCC tumors or are these changes acquired during tumor progression? Are tumors in these tRCC subgroups, carcinomas that initiated as ccRCC and pRCC, and then acquired $TFE3/TFEB$ translocations as they progressed? Or are these tumors initiated by a $TFE3/TFEB$ translocation, which acquired patterns of chromosomal imbalances similar to those observed in ccRCC and pRCC? The 3p loss is a required event in ccRCC, as it leads to loss of the wild-type VHL allele and abrogation of VHL-hypoxia-regulating activity. VHL mutations do not seem to play a role in tRCC, as VHL mutations were not identified in our cohort. However, other tumor suppressor genes with roles in RCC biology have been recently identified in 3p (PBRM1, SETD2, and BAP1), and thus 3p loss in tRCC tumors might be associated with inactivation of one or more of these genes (36–38). Alternatively, tumor initiation by the $TFE3/TFEB$ translocation might trigger chromosomal instability pathways that resemble those in ccRCC and pRCC subtypes. Future studies are needed to clarify whether the presence of these translocations is enough to determine a tRCC diagnosis and if tumor behavior (and response to therapy) depends on the overall genomic profile. Interestingly, patients with tRCC
with 3p loss (ccRCC-like profile) had worse outcomes compared with those without 3p loss. In contrast, 9p loss in our study was not associated with a poor prognosis, as it is in early-stage ccRCC (32).

Another interesting finding of our study is the greater number of genetic abnormalities and the lower level of global methylation found in tRCC tumors from adult patients, compared with tumors from young patients. These data are concordant with the significant inverse correlation between the degree of genomic alterations and the LINE-1 methylation levels reported in other tumor types (39). Although the difference in methylation levels between adult and young patients is small, it achieved statistical significance due to the robustness of the assay used. This is shown by the fact that methylation levels of normal kidneys showed little variability with an average methylation of 73.07% (±0.50%). Using this assay, no significant associations were previously found between LINE-1 methylation and age, gender, or diet in colonic tissues (19); thus, we believe that the observed differences are related to intrinsic characteristics of tRCC in adult and young patients, rather than to age. Additional studies with a larger group of patients are necessary to confirm these findings.

The cytogenomic differences between tumors from adult and young patients were independent of classical clinicopathologic prognostic factors such as TNM stage and Fuhrman grade. Although those results might be related to a small number of cases, cytogenomic array analysis in 3 of our 4 young patients with American Joint Committee on Cancer (AJCC) stage III–IV disease found no genetic abnormalities. Another age-related difference is that gains in 17q are associated with age remains unclear; and whether adults with tRCC without a 17q gain have a different prognosis than adults with the 17q gain remains unknown. A larger cohort is needed to answer this question. Nonetheless, our results strongly suggest that 17q gain is more frequent in adult tRCCs and our preliminary GSEA analysis suggest a dosage effect of genes on 17q and p53 haploinsufficiency. It is important to note that CML that bear an i(17q) have a poor prognosis (44), and medulloblastomas with an i(17q) or a 17q gain also have a poor outcome (43). In our study, GSEA analysis of a subset of Xp11 cases with and without partial 17q gain found enrichment of pathways involved in mitosis and the cell cycle in patients with 17q gain, suggesting a proliferative advantage in tumors harboring this chromosomal abnormality. It is notable that those cases also had gene expression patterns consistent with activation of T-helper cells and the CTLA-4 signaling pathway. Further studies are required to confirm these findings.

Abnormalities involving 17q have been previously described in tRCC but to date 17q gain had not been recognized as a recurrent abnormality in these tumors, nor its possible association with outcomes. Among our 14 previously reported cases (9), we found 1 case, that of a 2-year-old girl, who had a t(5;17)(q25;17q25), in addition to a t(X;17)(p11.2;17q25) leading to the ASPSCR1-TFE3 fusion, consistent with multiple translocations occurring on the 17q arm. This patient had lymph node involvement and lung metastasis at the time of her diagnosis, which is rare in pediatric cases of tRCC (9). A recent case report of the t(X;17)(p11.q25) translocation, in a 24-year-old Japanese woman (45), also showed the presence of a second translocation involving 17q: t(13;17)(p11.q11). Moreover, in the original report by Argani and colleagues that described tRCC (13), they identified a complex nonreciprocal t(X;17)(p11.2;q25), involving not only the translocation of Xp11 to 17q25 but also of 17q25 to another chromosome. Our results and these reports in the literature suggest an important role of 17q in the biology of a subset of tRCC tumors.

Although we did not study whether there is intratumoral heterogeneity with respect to the presence of 17q and other chromosomal abnormalities identified in this study, we have previously shown that intratumoral heterogeneity at the level of chromosomal abnormalities is seen in only up to 20% of RCC cases (46), in contrast with the level of mutational heterogeneity reported by Gerlinger and colleagues (47). However, intratumoral heterogeneity studies might be helpful to understand whether 17q is an initiating event or acquired during tumor progression, as cytogenomic heterogeneity in our prior studies is restricted to abnormalities acquired during tumor progression (46).

In conclusion, our data show that tRCC is genetically heterogeneous and share cytogenomic profiles with other renal cell tumors, raising important questions about the role for TFE3/TFE3 translocations in tumor initiation and/ or progression. Furthermore, we have shown that cytogenomic and methylation differences exist between tRCC tumors arising in adult and pediatric/adolescent patients.
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These data may be used to develop prognostic biomarkers in tRCC patients.

Disclosure of Potential Conflicts of Interest
F.A. Monzon has honoraria from Speakers Bureau of Affymetrix. No potential conflicts of interest were disclosed by the other authors.

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