Genomic Heterogeneity of Translocation Renal Cell Carcinoma

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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 pathological 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.
Abstract (word count: 250)

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

**Experimental design:** Cytogenomic analysis was performed with 250K single-nucleotide polymorphism microarrays on 16 tumor specimens and 4 cell lines. $LINE-1$ methylation, a surrogate marker of DNA methylation, was performed 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 7 tumors (44%), followed by a 9p loss in 6 cases (37%). Less frequent were losses of 3p and 17p in 5 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 to 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 $TFEB/TFE3$ translocations and other chromosomal imbalances in tRCC biology.
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 < 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), SFPQ in t(X;1)(p11.2;p34), ASPSCR1 in t(X;17)(p11.2;q25), NONO in inv(X)(p11.2q12), and CLTC in t(X;17)(p11;q23) (6). tRCC can also be related to translocations involving the TFEB 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 demonstrated 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 disease were younger (median age 16 years) and predominantly female (9). These data are in line with previous reports, suggesting a more aggressive disease in adults, especially in men (8, 12). However, the basis for the biologic difference in tRCC between adult and pediatric patients has not been elucidated.

Since the first description of tRCC by Argani et al., there have been few reports about a detailed cytogenetic characterization of these tumors (13). Other renal tumors are characterized by specific chromosomal imbalances, such as 3p loss in clear-cell RCC (ccRCC) and trisomies 7 and 17 in papillary RCC (pRCC) (15, 16).

The aims of our study were to investigate whether there are any additional genetic/epigenetic alterations beside TFE3/TFEB translocations, and assess whether there are
associations between specific chromosomal imbalances and classical clinicopathologic factors and overall survival (OS).

Patients and Methods

Patient selection and classification of cases included in single-nucleotide polymorphism (SNP)-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 performed at each individual institution as previously described (7, 17). The flow chart of patient selection, describing the different tests performed is depicted in Supplementary Figure 1.

Among the total of 21 cases, 15 were confirmed to be tRCC by fluorescence in situ hybridization (FISH), conventional karyotyping, or reverse transcription polymerase chain reaction RT-PCR analysis for all known specific fusion partners. One patient whose tumor had classical tRCC morphology and TFEB 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 1). The clinico-pathologic 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 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 performed (all except the following 4 cases: LOY009, MRCC106, MRCC107, and MRCC117). Additionally, DNA was extracted from 15 formalin-fixed paraffin-embedded (FFPE)
tissues of patients for whom we previously reported pathological 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 were 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), 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 performed according to previously published methods (21). Spectral karyotyping (SKY) was also performed on HCR-59 by using the human chromosome HiSKY probe (Applied Spectral Imaging, Inc., Carlsbad, CA).

The tRCC cell lines UOK109 and UOK146, derived, respectively, from a 39-year-old male and a 42-year-old female (22), were kindly provided by Dr. W. Marston Linehan (United States National Institutes of Health, Bethesda, MD). Both cell lines were originally reported as papillary RCC (pRCC) cell lines before tRCC became a recognized pathologic entity. The UOK109 line has been shown to carry the NONO-TFE3 translocation (23), and the UOK146 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., Waltham MA). The DNA was analyzed on Affy250KNsp SNP genotyping arrays (Affymetrix, Inc., Santa Clara, CA) following the previously reported modified protocol (24). Loss of heterozygosity and copy number estimates were obtained by using the publicly available Copy Number Analyzer for Affymetrix GeneChip Arrays package (CNAG v3.0) (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 performed according to Illumina procedures at the MDACC Genomic Core Facility, and sequencing was done on a HiSeq 2000 system (Illumina, Inc., San Diego, CA). 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 (GSEA)**

Gene set enrichment analysis (GSEA) is a computational method that assesses whether a defined set of genes shows statistically significant differences between 2 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) (27), and GSEA was performed 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 showed in Supplementary Figure 1.

**Statistical analysis**

Associations with clinicopathologic factors were analyzed with chi-square 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 2-sided and performed at the significance level of 0.05 using Prism version 5 (GraphPad Software, Inc., La Jolla, CA).

**Results**

**Patient and tumor characteristics**

Patient and tumor characteristics are summarized in supplementary Table 1. With a median follow-up time of 29 months (range, 3–92 months), five patients (31%) were dead of disease, eight 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 (Figure 1/Table 1). The most common alteration observed was a gain of 17q, which was present in 7 (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 ten women in our cohort (30%) lost 1 X chromosome.

Overall, the 16 cases of tRCC with a confirmed translocation could be classified into 4 groups on the basis of their similarities to cytogenomic profiles of other renal epithelial tumors (15).

- **Group I**: five 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 histological review with 2 showing mixed clear-cell/papillary features, and one showing only clear-cell morphology.
- **Group II**: three 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**: five cases had novel cytogenomic profiles, i.e., not associated with those of other subsets of renal tumors.
- **Group IV**: three 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 7 cases (44%) that had a gain of 17q, 2 tumors had a confirmed t(X;17)(p11;q25) translocation and 1 case had a TFEB translocation. Interestingly, in four of the seven cases with 17q gain, 17p loss has been demonstrated 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 2).

Tumors of adult patients (age ≥ 18 years) showed more genetic abnormalities than tumors from younger patients (age < 18 years) (P = 0.006) (Fig. 2A), with no differences found in sex (P = 0.31), TNM stage (P = 0.60), and Fuhrman grade (P = 0.62).

**Unsupervised clustering reveals 2 distinct groups of tRCC**

To gain insights into the biology of tRCC, we performed unsupervised clustering analysis using the most frequent segmental alterations (losses or gains). That analysis revealed 2 main clusters (Fig. 2A). The median number of genetic alterations in cluster A was 0 (range, 0–5), compared to 14 in cluster B (range, 5–22). The 2 clusters showed no significant differences in terms of pT stage, lymph node involvement, metastasis, TNM classification, gender, and Fuhrman grade. The only statistical difference between clusters was age at diagnosis: the median age of the seven patients in cluster A was 16.7 years (range, 9.1–33.1 years), compared to 34.2 years (range, 16–54 years) for the 9 patients in cluster B (P = 0.01). Overall, 5 of the 7 (71%) patients in cluster A were younger than age 18, compared with 1 of the 9 (11%) patients in cluster B. Of note, 7 of the 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 above, 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 vs. 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 years (range, 20.4–54.6) vs. 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, 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 vs. 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 vs. 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 months vs. 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 papillary RCC, had a pRCC–like cytogenomic profile, with chromosome 7 gain (Supplementary Fig. 2A). 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. 2B).

To assess whether the 17q gain is related to unbalanced translocation or is the result of an isochromosome 17, we performed 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. 2C).

The results of FISH analysis for TFE3 (Supplementary Fig. 2D) as well as conventional karyotyping of the MDA-92 cell line (derived from patient T3) 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 2 interchromosomal translocations involving 17q that led to 17q gains (Fig. 3A). Similarly, spectral karyotyping 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 [−1(p31.1-p21.1), −1(p13.3-p11.2), −3(p26.3-p11.2), +5(q33.3-q34), +5(q34-q35.3), −9(p24.3-p21.1), −17(p13.3-p11.2), +17(q21.31-q25.3), −18(q11.2-q23), and −X(p11.1-q28)] are located around the breakpoints of the 6 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 performed 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; P < 0.0001], 17q23 (FDR = 0.07; P < 0.001), 17q21 (FDR = 0.08; P < 0.001), 17q24 (FDR = 0.10; P < 0.001), and 17q22 (FDR = 0.10; 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 down-regulated by TP53 in NCI-60 panel of cell lines with mutated TP53 as compared to 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 performed 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 two cases (RCC-T34 and RCC-T1) with concomitant partial 17q gain as compared to the two cases (RCC-T31 and RCC-T32) with balanced karyotype (Supplementary Table 3). Overall, there were 51 out of 89 genes whose expression direction were consistent with inhibition of TP53 (z-score= -3.84; P = 5.37 x 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 Figure 3A).

KEGG (Kyoto Encyclopedia of Genes and Genomes) and BioCarta (BioCarta LLC, San Diego, CA) pathways (C2 gene set) using GSEA and Ingenuity analysis (Ingenuity Systems, Inc., Redwood City, CA) 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 ICOS-ICOSL 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 Figure 3B). 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 1-2\% in the 2 cases without 17q gain (not shown).

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

We performed 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’s [\( \rho \)] = –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 standard variation of +/- 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 to 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 histopathological 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) (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 et al. showed that tRCC tumors have significant histologic variability (31), and Klatte and collaborators showed that only 2 out 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 demonstrated the transforming effect of the TFE3 and TFEB translocations in cell lines (33, 34). Mathur et al. performed knock-down of PSF-TFE3 in UOK-145 cell line and showed that it leads 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 UOK109 cell line (NONO-TFE3 translocation), TFE3 directed 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? 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 don’t appear 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, tRCC patients with 3p loss (ccRCC-like profile) had worse outcomes compared to 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 employed. This is demonstrated 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 clinicopathological 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 AJCC stage III–IV disease found no genetic abnormalities. Another age-related difference is that gains in 17q were identified in tumors from 70% (n = 7/10) of all our adult patients, whereas this abnormality was not found in younger patients. Patients with 17q gain were more frequently men, displayed more genetic alterations, and had worse outcomes. Five of those patients had a partial gain of 17q, whereas 2 had trisomy 17. Of interest 17q gains are rare in ccRCC and pRCC, in contrast to trisomy 7 and 17, which are very frequent in pRCC (15). Based on our study of patient derived cell lines, 17q gains seem to arise from i(17q) and/or unbalanced translocations and complex rearrangements. Together with G-banding and SNP results available for 3 cell lines (HCR-59, MDA-92, and UOK146), our data showed that 2 cases had multiple translocations involving 17q, leading to 17q gain concomitant with i(17q) formation in 1 case, and 1 case had only an i(17q) without other associated translocations. Two cases had breakpoints in 17p11.2 and were thus considered isodicentric chromosomes. Of note, 17p11.2 has been reported to contain a cluster region characterized by large palindromic and low copy-repeat sequences in chronic myeloid leukemia (CML), medulloblastoma with idic(17)(p11), and high hyperdiploid childhood acute lymphoblastic leukemia (40-43). This complex architecture suggests that i(17q) does not occur randomly, but rather because of susceptibility in the genomic structure. However, the reason why 17q is 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 one case, that of a 2-year-old girl, who had a t(5;17)(q23;q25), 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 et al. 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 demonstrated 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 collaborators (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/TFEB 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. These data may be used to develop prognostic biomarkers in tRCC patients.

Acknowledgments

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References


42. Herou E, Biloglav A, Johansson B, Paulsson K. Partial 17q gain resulting from isochromosomes, unbalanced translocations and complex rearrangements is associated with gene overexpression, older age and shorter overall survival in high hyperdiploid childhood acute lymphoblastic leukemia. Leukemia.
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<td>RT-PCR</td>
<td>TFE3</td>
<td>ASPSCR1</td>
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</table>

Abbreviations: NA, not available; IHC, immunohistochemistry; NED, no evidence of disease
Figure Legends

**Figure 1.** Genomic subtypes of Xp11.2 tRCC. TFE3 tRCC could have different RCC profiles consistent with ccRCC (n = 5), pRCC (n = 3), or novel RCC karyotypes (n = 5).

**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. C, Kaplan-Meier survival estimates for patients with and without 17q gain.

**Figure 3.** A, karyotype for the novel MDA-92 cell line, derived from patient T3, shows the 3p loss and 5 different translocations enumerated as follows: M1 = t(1;3), M2= t(9;17), M3= t(6;10), M4= t(18;21) and M5= different translocations involving t(X;17) B, spectral karyotyping results for the novel HCR-59 cell line derived from patient T1. C, classified karyotyping results for the novel HCR-59 cell line derived from patient T1. B and C show 6 different translocations enumerated as follows: t(1;3); t(5;17); t(17;22); t(18;21), t(6;10) and t(X;17).

**Figure 4.** Partial 17q gain and gene expression. GSEA of genes expressed differently between selected cases with partial 17q gain and without was performed by RNA-seq. A, significant correlation was observed between chromosomal 17q gain and expression of genes located in the 17q arm (e.g., 17q21 and 17q25). B) C6 oncogenic signature in the Molecular Signatures Database showing enrichment for genes down-regulated by TP53 in NCI-60 panel of cell lines with mutated TP53 as compared to those classified as normal. C) Ingenuity Pathway Analysis showing the network of genes consistent with TP53 inactivation. Overall there were 51 out of 89 genes which have expression direction consistent with inhibition of TP53.
Figure 5. Genomic aberrations and LINE-1 methylation. A, Spearman’s correlation between the number of chromosomal arms with gain or loss in tRCC samples and LINE-1 methylation ($n = 12$). B, LINE-1 methylation level is lower in tumors of adult as compared to young patients.
Figure 5

A

Spearman R = -0.6
p = 0.04

B

Methylation (%)

Normal  Tumor (<18 years)  Tumor (≥18 years)

ns  *
Clinical Cancer Research

Genomic Heterogeneity of Translocation Renal Cell Carcinoma

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