Published in final edited form as:

Am J Surg Pathol. 2016 November ; 40(11): 1484–1495. doi:10.1097/PAS.0000000000000720.

***TFEB*-Amplified Renal Cell Carcinomas: An Aggressive Molecular Subset Demonstrating Variable Melanocytic Marker Expression and Morphologic Heterogeneity**

Pedram Argani, MD^{1,2,*}, Victor E. Reuter, M.D.³, Lei Zhang, MD³, Yun-Shao Sung, MS³, Yi Ning, PhD¹, Jonathan I. Epstein, M.D.^{1,2}, George J. Netto, MD^{1,2}, and Cristina R. Antonescu, MD³

¹Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, MD, USA

²Department of Oncology, The Johns Hopkins Medical Institutions, Baltimore, MD, USA

³Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY

Abstract

Renal cell carcinomas (RCCs) with the t(6;11)(p21;q12) chromosome translocation are low grade RCC which often occur in young patients. They typically feature an unusual biphasic morphology characterized by nests of larger epithelioid cells surrounding intraluminal collections of smaller cells clustered around basement membrane material. The t(6;11)(p21;q12) translocation fuses the *Alpha* (*MALAT1*) gene with the *TFEB* transcription factor gene, resulting in upregulated expression of intact native TFEB that drives the aberrant expression of melanocytic markers which is a hallmark of this distinctive neoplasm. We now report 8 cases of RCC which demonstrate *TFEB* gene amplification (6 without *TFEB* rearrangement, 2 with concurrent *TFEB* rearrangement) and demonstrate downstream consequences of TFEB overexpression. Like the unamplified t(6;11) RCC, all *TFEB*-amplified RCC were associated with aberrant melanocytic marker expression. However, several differences between *TFEB*-amplified RCC and the usual unamplified t(6;11) RCC are evident. First, *TFEB*-amplified RCC occurred in older patients (median age 64.5 years) compared with unamplified t(6;11) RCC (median age 31 years). Second, the morphology of *TFEB*-amplified RCC is not entirely distinctive, frequently featuring nests of high grade epithelioid cells with eosinophilic cytoplasm associated with pseudopapillary formation and necrosis, or true papillary formations. These patterns raise the differential diagnosis of high grade clear cell and papillary RCC. Third, TFEB and melanocytic marker expression was more variable within the *TFEB*-amplified RCC. TFEB protein expression by immunohistochemistry was detectable in 6 of 8 cases. While all 8 cases expressed melan-A, only 5 of 8 expressed cathepsin K and only 3 of 8 expressed HMB45. Fourth, the *TFEB*-amplified RCC were associated with a more aggressive clinical course; 3 of 8 cases presented with advanced stage or metastatic disease, 2 subsequently developed metastatic disease, while the other 3 cases had minimal/no follow-up. Our results are corroborated by scant data reported on 6 *TFEB*-amplified RCC in the literature, gleaned from 1 case report, 1 abstract, and 4 individual cases identified within two

Correspondence: Pedram Argani, M.D., The Johns Hopkins Hospital, Surgical Pathology, Weinberg Building, Room 2242, 401 N. Broadway, Baltimore, Maryland 21231-2410, pargani@jhmi.edu, Tel# (410) 614-2428, Fax# (410) 955-0115, Cristina R. Antonescu, MD, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10021, antonesc@mskcc.org.

genomic studies of large cohorts of RCC. In summary, *TFEB*-amplified RCC represent a distinct molecular subtype of high grade adult RCC associated with aggressive clinical behavior, variable morphology, and aberrant melanocytic marker expression.

Keywords

Renal Neoplasm; TFEB; Translocation; Amplification

INTRODUCTION

Renal cell carcinomas (RCCs) with the t(6;11)(p21;q12) chromosome translocation were initially described in 2001¹. In the initial description of two cases, the neoplasms were noted to be composed of nests of epithelioid cells with clear to eosinophilic cytoplasm associated with a subpopulation of smaller cells surrounding hyaline basement membrane material. Despite the epithelioid morphology that suggested carcinoma, these neoplasms did not label for epithelial immunohistochemical markers but instead demonstrated focal labeling for melanocytic markers HMB45 and melan-A, which raised the question of whether these neoplasms represented RCC or variants of epithelioid angiomyolipoma. With greater clinical experience and broader immunohistochemical analysis, it has become clearer that these neoplasms represent RCCs which clinically, morphologically, and immunohistochemically overlap with the Xp11 translocation RCC^{2,3,4,5,6}. Though both may occur in adults, both the Xp11 translocation RCC and the t(6;11) RCC disproportionately affect young patients. Overall, the t(6;11) RCC are more indolent and metastasize less frequently than the Xp11 RCC, but both have the capacity to metastasize many years after diagnosis. Both can share common morphologic patterns including papillary architecture with clear cells, or a smaller cell population clustered around basement membrane material. Both typically express renal tubular markers like PAX8, but they underexpress cytokeratins and often express melanocytic markers and the serine protease cathepsin K^{7,8,9,10,11}. Genetic evidence of a relationship between the t(6;11) RCC and the Xp11 translocation RCC was provided when the t(6;11)(p21;q12) translocation was found to fuse the *Alpha (MALAT1)* gene with the *TFEB* transcription factor gene, a gene which belongs to the same sub-family of transcription factors as *TFE3* and has overlapping functional activity^{12,13}. Therefore, based upon clinical, morphologic, immunohistochemical, and genetic overlap, both the 2013 Vancouver Classification of Renal Neoplasia and 2016 World Health Organization (WHO) classification group the Xp11 translocation RCC and the t(6;11) RCC under the family of MiT family translocation RCC^{14,15}.

The expected consequence of the t(6;11)(p21;q12) translocation is overexpression of intact TFEB protein, since the breakpoint in *TFEB* is within intron 1, which is upstream of the ATG initiation codon. Indeed, overexpressed intact TFEB protein can be detected by immunohistochemistry as a marker for these RCC, since the native protein is expressed at low levels that are not detected². Logically, other mechanisms of increased *TFEB* gene expression (such as gene amplification, aberrant methylation, aberrant micro-RNA expression, etc) could in theory create a similar phenotype to the t(6;11) RCC; however, such cases have not been well described in the literature.

In this report, we describe 8 adult RCCs which demonstrate *TFEB* amplification. In 6 cases, amplification occurred without evidence of *TFEB* rearrangement; while in the other two, amplification and rearrangement occurred concurrently. These neoplasms demonstrated variable morphology, variable degrees of expression of melanocytic markers melan-A and HMB45 as well as cathepsin K, and more aggressive clinical behavior relative to the usual unamplified t(6;11) RCC.

MATERIALS AND METHODS

Cases

The eight cases reported herein derive from the consultation files of the authors (PA, VER). In 5 cases, the renal neoplasms were sent specifically for assessment of *TFE3* and *TFEB* rearrangements by fluorescence *in situ* hybridization (FISH) after extensive work-ups of otherwise unclassified RCC revealed aberrant labeling for melan-A. In 3 other cases, the renal neoplasms were sent for classification without specific suspicion of *TFE3* or *TFEB* alterations, but *TFEB* FISH was performed after we found the neoplasm labelled for melan-A. *TFEB* amplification was detected as increased numbers of fused or split signals in the routine *TFEB* FISH assay used on all tumors (see below). The referring pathologists were contacted for additional clinical follow up information.

Immunohistochemistry (IHC)

Immunohistochemistry (IHC) was performed using previously described methods¹⁶ for PAX8, cathepsin K, carbonic anhydrase 9 (CA-IX), HMB45, and melan-A. *TFEB* IHC was performed and scored as previously described². IHC for fumarate hydratase was performed using the Santa Cruz antibody, catalog #100743, clone J-13, at 1:200 dilution with steam retrieval.

FISH Methodology

FISH on interphase nuclei from paraffin-embedded 4-micron sections was performed applying custom probes using bacterial artificial chromosomes (BAC), covering and flanking *TFEB* gene. *TFEB* break-apart FISH was performed as previously described¹⁷. BAC clones were chosen according to UCSC genome browser (<http://genome.ucsc.edu>), see Supplementary Table 1. The BAC clones were obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (CHORI)(Oakland, CA)(<http://bacpac.chori.org>). DNA from individual BACs was isolated according to the manufacturer's instructions, labeled with different fluorochromes in a nick translation reaction, denatured, and hybridized to pretreated slides. Slides were then incubated, washed, and mounted with DAPI in an antifade solution, as previously described¹⁷. The genomic location of each BAC set was verified by hybridizing them to normal metaphase chromosomes. Two hundred successive nuclei were examined using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany), controlled by Isis 5 software (Metasystems, Newton, MA). A positive score was interpreted when at least 20% of the nuclei showed a break-apart or amplified signal. Nuclei with incomplete set of signals were omitted from the score. An amplified signal was defined as >10:1 ratio of *TFEB* signal to reference.

RESULTS

Cases

All of the patients in this cohort were adults, ranging in age from 23 years to 77 years (mean 62.5 years, median 64.5 years) (Table 1). There were five females and three males. Cases 1-6 demonstrated *TFEB* amplification alone, while cases 7 and 8 demonstrated both *TFEB* amplification and rearrangement. While the case numbers are small, we did not appreciate significant clinicopathologic differences between these two groups (see below).

Three cases (cases 1, 6 and 7) presented at advanced stage. Case 1 was an 11 cm pT3b neoplasm which presented with renal vein involvement and regional lymph node metastases. Case 6 was a 12 cm pT3b neoplasm which presented with renal vein involvement which extended to the vein margin. Case 7 was a 19 cm pT4 neoplasm which presented with vaginal metastasis. These cases had minimal clinical follow-up, with case 1 remaining free of disease at 28 months. Two other seemingly localized neoplasms metastasized within one year of diagnoses. Case 2 was a small (3 cm) neoplasm treated by partial nephrectomy with microscopic capsular invasion and a microscopically positive parenchymal margin, which recurred as para-aortic lymph node metastasis after three months. Case 4 was a 7 cm organ-confined neoplasm which recurred as a vaginal metastasis at 8 months follow-up. Three other recent cases (cases 3, 5 and 8) were small pT1 neoplasms (1.9, 2.7 and 4.0 cm.) treated by nephrectomy on which there is no available follow-up. No patient revealed a significant past medical history, such as prior exposure to chemotherapy.

The morphologic features of these cases were variable (Figures 1-5). All cases were high grade: 6 were ISUP nucleolar grade 3, and 2 were ISUP nucleolar grade 4. Six of the cases were predominantly characterized by nests of polygonal eosinophilic cells with prominent nucleoli, similar to the type commonly seen in high grade clear cell RCC. All six of these cases were associated with tumor necrosis, and demonstrated pseudopapillary formations (Figure 1A). In two of these cases, nucleoli were extremely prominent and associated with a clear perinuclear halo, raising the differential diagnosis of hereditary leiomyomatosis RCC (HLRCC)-associated RCC (Figure 1B, 3B). Two of these cases (cases 4 and 6) also demonstrated focal papillary areas with focal clustering of small cells in acinar lumens as is commonly seen in the typical non-amplified t(6;11) RCC, though no basement membrane material was evident. Two other cases had different morphologies. Case 2 was an RCC with predominant clear cell and papillary features and abundant psammoma bodies, which strongly suggested the possibility of Xp11 translocation RCC. Focal areas contained eosinophilic cells (Figure 2A-D). Case 8 was an unusual biphasic neoplasm, with acini formed by smaller epithelioid cells with pale cytoplasm associated with clusters of larger polygonal cells with eosinophilic cytoplasm, the latter often present within the lumen of acini (Figure 5A-B). Importantly, no case showed the well-developed biphasic appearance of the typical unamplified t(6;11) RCC throughout, with larger epithelioid cells forming acini and smaller cells surrounding basement membrane material in their lumen.

Immunohistochemistry

All 8 neoplasms demonstrated nuclear labeling for PAX8 protein. All 8 cases demonstrated immunoreactivity for melan-A, 3 diffusely and 5 in a focal or patchy pattern (Figure 1F, 2F, 3E, 4D, 5E). In six of eight cases, nuclear labeling for TFEB protein was appreciable (Figure 1E, 3D, 4C, 5D). In four cases, labeling was moderate (2+) which is considered positive; in the other 2, labeling was weak/equivocal (1+) which is scored as negative. This level of labeling (1-2+) is less than the strong positive (3+) TFEB labeling which is usually seen in the non-amplified t(6;11) RCC². Five of eight cases demonstrated immunoreactivity for cathepsin K (1 diffuse, 4 patchy), which contrasts with consistently diffuse labeling seen in usual t(6;11) RCC (Figure 3F, 4E, 5F). In three of eight cases, individual neoplastic cells labeled for HMB45, which is the typical pattern seen in the t(6;11) RCC (Figure 4F). All cases were at least focally immunoreactive for cytokeratin (Figure 1D). Fumarate hydratase was intact in all 8 cases, while no case demonstrated diffuse CA-IX expression.

FISH Results

All cases included in the study showed high level amplification of *TFEB*, defined as greater than a 10:1 ratio of *TFEB* signals relative to that of surrounding normal cells (Figure 6). In reality, all the cases showed hundreds of copies of *TFEB* in the form of homogeneous staining region (hsr) or multifocal amplicons. Cases 1-6 demonstrated *TFEB* amplification alone, while cases 7 and 8 demonstrated both *TFEB* amplification and rearrangement.

Case 4 was also studied by targeted sequencing of 350 cancer-related genes using the clinical MSKCC IMPACT-assay, which confirmed the presence of *TFEB* gene amplification and demonstrated no evidence of mutation within *TFEB*.

DISCUSSION

In this report, we describe 6 RCCs which demonstrate *TFEB* gene amplification. There is limited information regarding neoplasms with this genetic alteration in the literature (Table 1). Three cases with putative *TFEB* gene amplification based on the GISTIC algorithm for copy number alterations were reported within the TCGA sequencing study of 291 cases originally classified as papillary RCC. One case was a 28 year-old white female who presented with locally advanced (Stage III, pT3N1) disease and showed no evidence of recurrence in only limited follow-up (4 months). The second was a 61 year-old black female who also presented with locally advanced (pT3aNX) disease and recurred at 14 months follow-up¹⁸. The third was a 59 year-old black male who presented with locally-advanced disease (Stage III, pT3N1), recurred at 75 months follow-up and died at 86 months. While one TCGA case demonstrated a fumarate hydratase mutation, other genetic alterations of papillary RCC (i.e., trisomy 7 or 17) or other defined subtypes of renal cell carcinoma are not evident from the published data. A single case demonstrating *TFEB* amplification was reported by Durinck *et al.* as a low grade renal carcinoma with oncocytic and papillary features, but illustrations were not provided and further features were not described¹⁹. Peckova *et al.* reported a case in a 77 year-old woman which demonstrated *TFEB* rearrangement and amplification that metastasized to the adrenal gland and lung, causing patient death 2.5 months after diagnosis.²⁰ This neoplasm had nested/alveolar/

pseudopapillary architecture, and was predominantly composed of high grade polygonal eosinophilic cells similar to the majority of cases described herein. Williamson *et al.* recently reported in abstract form a single case of a pT3a RCC in a 57 year-old male associated with chromosome 6p amplification including *TFEB*, and referenced 2 of the TCGA cases noted above.²¹ The provided illustration demonstrates nested architecture, polygonal eosinophilic cells and vascular invasion. Overall, *TFEB* amplification appears to be a relatively uncommon event; the 8 cases of *TFEB*-amplified RCC in this study represent less than 10% of RCC associated with *TFEB* alterations in our files. Moreover, *TFEB* amplification was seen in only 1% of cases in the TCGA papillary RCC study referenced above.¹⁸

All of the cases described herein showed some evidence of aberrant melanocytic marker expression which is a hallmark of the usual unamplified t(6;11) RCC, though this was variable and overall of a lesser degree. Overall, all 8 cases expressed melan-A, but only 5 of 8 cases expressed cathepsin K and only 3 of 8 cases expressed HMB45. One simple explanation for this variability would be that the level of *TFEB* gene amplification correlates with aberrant melanocytic marker expression. However, all cases showed hundreds of *TFEB* signals on FISH analysis, so this direct correlation could not be made. We do note that the 2 cases in our series in which *TFEB* immunoreactivity could not be detected (cases 2 and 7) were the ones with the least amount of melanocytic marker expression (melan-A labeling only), so this mechanism remains possible. The lesser melanocytic marker immunoreactivity in the two cases with both rearrangement and amplification relative to usual unamplified t(6;11) RCC remains difficult to explain without investigating the *TFEB* fusion partner and the level of *TFEB* mRNA expression. Along these lines, two of the three cases showing *TFEB* amplification in the TCGA papillary RCC study had abnormal *TFEB* mRNA levels suggesting that there is potential heterogeneity of *TFEB* expression within this family of neoplasms.

However, many differences exist between the *TFEB*-amplified RCC reported herein and the usual unamplified t(6;11) RCC. First, the *TFEB*-amplified RCC occurred in a significantly older age group (median 64.5 years, mean 62.5 years) than the usual t(6;11) RCC (median age 31 years, mean age 32.8 years based on review of the literature and unpublished cases in our files) (two-sample t test=0.0003). These findings are consistent with the median age of the few *TFEB*-amplified RCCs reported in the literature (59 years). Second, our cases were associated with more aggressive clinical behavior than the usual t(6;11) RCC, which generally has an indolent course. Three of our 8 cases presented with advanced disease, two subsequently developed metastatic disease, while the other three were small, localized pT1 lesions with minimal follow up. Our results again corroborate prior reports, as all 3 *TFEB*-amplified cases included within the TCGA papillary RCC study presented with locally-advanced disease (two of which metastasized in limited follow-up), while the case reported by Peckova *et al.*²⁰ presented as a large tumor that metastasized and caused patient death. In contrast, non-amplified t(6;11) RCC have generally had an indolent clinical course, with only 5 metastases in approximately 60 cases (including published cases and unpublished cases in our files). Third, none of our cases demonstrated the typical morphology of the t(6;11) RCC (larger cells surrounding smaller cells clustered around basement membrane material) throughout, though case 8 did show a different biphasic pattern and cases 5 and 8

showed focal areas of small and large cells. This is again consistent with the previously described cases, in which only the amplified and rearranged case reported by Peckova *et al.*²⁰ was noted to show typical t(6;11) biphasic morphology, and this was noted to be less than typically seen in unamplified t(6;11) RCC. Instead, the morphology of the *TFEB*-amplified cases was more variable though consistently of high nucleolar grade. Most (6 of 8) cases demonstrated nested/pseudopapillary architecture and were composed of polygonal, epithelioid cells with prominent eosinophilic cytoplasm. Two cases demonstrated striking prominent nucleoli similar to that of the fumarate hydratase deficient HLRCC-associated RCC.²² Other cases showed clear cells and papillary architecture (cases 2 and 4) and the distinctive biphasic pattern noted above (case 8). It seems likely that additional genetic alterations within these neoplasms which may not be shared (perhaps including variable other genes co-amplified with *TFEB* in different cases) are responsible for the variable morphology and immunohistochemical profiles of these neoplasms.

It should be emphasized that the cases reported herein show high levels of *TFEB* amplification (i.e., greater than 100 copies present). This should be distinguished from cases with low levels of increased *TFEB* copy number. The latter may represent chromosome 6 polysomy, which is nonspecific and seen in a variety of cancers.

The association of *TFEB* amplification with more aggressive clinical course is intriguing given prior studies of the effects of gene amplification in MiT family cancers. Previously, Macher-Goeppinger et al demonstrated that *TFE3* amplification is another mechanism of TFE3 overexpression in RCC lacking *TFE3* rearrangement, and found that such cases were associated with a worse prognosis.²³ In melanoma, amplification of *MiTF* has been associated with poor prognosis.^{24,25} These results are intriguing given the aggressive behavior noted in *TFEB*-amplified RCC relative to the typical indolent course of the unamplified t(6;11) RCC. Given that TFEB immunoreactivity is less in *TFEB*-amplified RCC than unamplified t(6;11) RCC, it seems unlikely that an increased cellular level of TFEB in the *TFEB*-amplified RCC relative to that in the usual t(6;11) RCC is the key reason for the aggressive behavior. Instead, it seems likely that additional concurrent genetic alterations which are as yet unknown (such as genomic instability) drive the aggressive clinical behavior.

The differential diagnosis for these *TFEB*-amplified RCC is broad. The nested alveolar architecture combined with eosinophilic cytoplasm and prominent nucleoli raises the differential diagnosis of the most common RCC, conventional clear cell RCC, which frequently has eosinophilic cytoplasm when it is high grade. The absence of diffuse CA-IX immunoreactivity in any of the *TFEB*-amplified RCC argues against this differential diagnosis, as does the melanocytic marker expression noted. Given that the *TFEB*-amplified RCC appear to behave aggressively, it is likely that such cases might be encountered in the limited material of a biopsy of a metastatic site in a patient with a renal mass. Particularly in this setting, such cases could easily be misclassified as “consistent with high grade clear cell RCC”, given that CA-IX labeling tends to be diminished in high grade clear cell RCC and melanocytic markers are usually not performed when such material is limited. The pseudopapillary growth pattern noted in 6 cases and the papillary architecture noted in 2 cases raises the differential diagnosis of high grade papillary RCC. Indeed, as noted

previously, 3 apparent *TFEB*-amplified RCC were included in the TCGA study of papillary RCC¹⁸, further highlighting the genetic heterogeneity of RCC with papillary architecture and high grade nuclei. As above, small samples of such RCC in metastatic sites could easily be interpreted as “consistent with high grade papillary RCC” unless a melan-A stain were performed. The prominent nucleoli raise the possibility HLRCC-associated RCC, but the intact fumarate hydratase and aberrant melan-A expression argues against this possibility.

In summary, we report herein 8 adult RCC associated with *TFEB* amplification. While the morphology is variable, the tumors were all high grade and all showed some evidence of aberrant melanocytic marker expression similar to that typically seen in unamplified t(6;11) RCC, though this was more variable and inconsistent among these tumors. *TFEB*-amplified RCC overall have a more aggressive clinical course in this limited series than do the usual unamplified t(6;11) RCC, which overexpress *TFEB* only as a result of a chromosome translocation. Nonetheless, as all of these were consultation cases (which creates potential bias compared to unselected cases) and the numbers are relatively small, further studies of these unusual cancers are needed to better define their clinical and pathologic features.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Norman Barker MA, MS, RBP for expert photographic assistance.

Disclosures: Supported in part by: P50 CA 140146-01 (CRA), Cycle for Survival (CRA), Kristin Ann Carr Foundation (CRA), Dahan Translocation Carcinoma Fund and Joey's Wings (PA)

References

1. Argani P, Hawkins A, Griffin CA, et al. A distinctive pediatric renal neoplasm characterized by epithelioid morphology, basement membrane production, focal HMB45 immunoreactivity, and t(6;11)(p21.1;q12) chromosome translocation. *Am J Pathol.* 2001; 158:2089–2096. [PubMed: 11395386]
2. Argani P, Lae M, Hutchinson B, et al. Renal carcinomas with the t(6;11)(p21;q12): clinicopathologic features and demonstration of the specific *Alpha-TFEB* gene fusion by immunohistochemistry, RT-PCR, and DNA PCR. *Am J Surg Pathol.* 2005; 29:230–240. [PubMed: 15644781]
3. Smith N, Illei P, Allaf M, Gonzalez N, Morris K, Hicks J, De Marzo A, Reuter VE, Amin MB, Epstein JI, Netto G, Argani P. t(6;11) renal cell carcinoma (RCC): Expanded immunohistochemical profile emphasizing novel RCC markers and report of ten new genetically-confirmed cases. *Am J Surg Pathol.* 2014; 38:604–14. [PubMed: 24618616]
4. Argani P, Yonescu R, Morsberger L, et al. Molecular confirmation of t(6;11)(p21;q12) renal cell carcinoma in archival paraffin-embedded material using a break-apart *TFEB* FISH assay expands its clinicopathologic spectrum. *Am J Surg Pathol.* 2012; 36:1516–1526. [PubMed: 22892601]
5. Rao Q, Liu B, Cheng L, et al. Renal cell carcinomas with t(6;11)(p21;q12): A clinicopathologic study emphasizing unusual morphology, novel *Alpha-TFEB* gene fusion point, immunobiomarkers, and ultrastructural features, as well as detection of the gene fusion by fluorescence in situ hybridization. *Am J Surg Pathol.* 2012; 36:1327–38. [PubMed: 22895266]
6. Kuroda N, Tanaka A, Sasaki N, Ishihara A, Matsuura K, Moriyama M, Nagashima Y, Inoue K, Petersson F, Martignoni G, Michal M, Hes O. Review of renal carcinoma with t(6;11)(p21;q12)

- with focus on clinical and pathobiological aspects. *Histol Histopathol.* 2013; 28:685–90. [PubMed: 23426439]
7. Argani P, Antonescu CR, Illei PB, Lui MY, Timmons CF, Newbury R, Reuter VE, Garvin AJ, Perez-Atayde AR, Fletcher JA, Beckwith JB, Bridge JA, Ladanyi M. Primary renal neoplasms with the ASPL-TFE3 gene fusion of alveolar soft part sarcoma: A distinctive tumor entity previously included among renal cell carcinomas of children and adolescents. *Am J Pathol.* 2001; 159:179–92. [PubMed: 11438465]
 8. Argani P, Antonescu CR, Couturier J, Fournet J, Sciot R, Debiec-Rychter M, Hutchinson B, Reuter VE, Boccon-Gibod L, Timmons C, Hafez N, Ladanyi M. PRCC-TFE3 renal carcinomas: Morphologic, immunohistochemical, ultrastructural, and molecular analysis of an entity associated with the t(X;1)(p11.2;q21). *Am J Surg Pathol.* 2002; 26:1553–66. [PubMed: 12459622]
 9. Argani P. MiT family translocation renal cell carcinoma. *Semin Diagn Pathol.* 201; 32:103–13. [PubMed: 25758327]
 10. Martignoni G, Pea M, Gobbo S, Brunelli M, Bonetti F, Segala D, Pan CC, Netto G, Doglioni C, Hes O, Argani P, Chilosi M. Cathepsin-K immunoreactivity distinguishes MiTF/TFE family renal translocation carcinomas from other renal carcinomas. *Mod Pathol.* 2009; 22:1016–22. [PubMed: 19396149]
 11. Martignoni G, Gobbo S, Camparo P, Brunelli M, Munari E, Segala D, Pea M, Bonetti F, Illei PB, Netto G, Ladanyi M, Chilosi M, Argani P. Differential expression of cathepsin-K in neoplasms harbouring TFE3 gene fusions. *Mod Pathol.* 2011; 24:1313–1319. [PubMed: 21602817]
 12. Davis IJ, Hsi BL, Arroyo JD, et al. Cloning of an *Alpha-TFEB* fusion in renal tumors harboring the t(6;11)(p21;q13) chromosome translocation. *Proc Natl Acad Sci U S A.* 2003; 100:6051–6056. [PubMed: 12719541]
 13. Kuiper RP, Schepens M, Thijssen J, et al. Upregulation of the transcription factor *TFEB* in t(6;11)(p21;q13)-positive renal cell carcinomas due to promoter substitution. *Hum Mol Genet.* 2003; 12:1661–1669. [PubMed: 12837690]
 14. Srigley JR, Delahunt B, Eble JN, et al. The International Society of Urological Pathology (ISUP) Vancouver Classification of Renal Neoplasia. *Am J Surg Pathol.* 2013; 37:1469–1489. [PubMed: 24025519]
 15. Argani, P.; Cheville, J.; Ladanyi, M. MiT family translocation renal cell carcinoma. In: Moch, H.; Humphrey, PA.; Ulbright, TM.; Reuter, VE., editors. *World Health Organization Classification of Tumors: Pathology and Genetics of Tumors of the Urinary System and Male Genital Organs.* 4. IARC Press; Lyon: 2016. p. 33-34.
 16. Argani P, Hicks J, DeMarzo A, Albadine R, Illei P, Ladanyi M, Reuter VE, Netto G. Xp11 Translocation Renal Cell Carcinoma (RCC): Extended Immunohistochemical (IHC) Profile Emphasizing Novel RCC Markers. *Am J Surg Pathol.* 2010; 34:1295–1303. [PubMed: 20679884]
 17. Italiano A, Thomas R, Breen M, Zhang L, Crago AM, Singer S, Khanin R, Maki RG, Mihailovic A, Hafner M, Tuschl T, Antonescu CR. The miR-17-92 cluster and its target THBS1 are differentially expressed in angiosarcomas dependent on MYC amplification. *Genes Chromosomes Cancer.* 2012; 51:569–78. [PubMed: 22383169]
 18. Cancer Genome Atlas Research Network. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. *N Engl J Med.* 2016; 374:135–45. [PubMed: 26536169]
 19. Durinck S, Stawiski EW, Pavía-Jiménez A, Modrusan Z, Kapur P, Jaiswal BS, Zhang N, Toffessi-Tcheuyap V, Nguyen TT, Pahuja KB, Chen YJ, Saleem S, Chaudhuri S, Heldens S, Jackson M, Peña-Llopis S, Guillory J, Toy K, Ha C, Harris CJ, Holloman E, Hill HM, Stinson J, Rivers CS, Janakiraman V, Wang W, Kinch LN, Grishin NV, Haverty PM, Chow B, Gehring JS, Reeder J, Pau G, Wu TD, Margulis V, Lotan Y, Sagalowsky A, Pedrosa I, de Sauvage FJ, Brugarolas J, Seshagiri S. Spectrum of diverse genomic alterations define non-clear cell renal carcinoma subtypes. *Nat Genet.* 2015; 47:13–21. [PubMed: 25401301]
 20. Peckova K, Vanecek T, Martinek P, Spagnolo D, Kuroda N, Brunelli M, Vranic S, Djuricic S, Rotterova P, Daum O, Kokoskova B, Vesela P, Pivovarcikova K, Bauleth K, Dubova M, Kalusova K, Hora M, Michal M, Hes O. Aggressive and nonaggressive translocation t(6;11) renal cell carcinoma: comparative study of 6 cases and review of the literature. *Ann Diagn Pathol.* 2014; 18:351–7. [PubMed: 25438924]

21. Williamson ST, Grignon DJ, Favazza L, Gupta NS, Chitale DA, Palanisamy N. Chromosome 6p amplification including the TFEB gene: A novel mechanism of renal cell carcinoma pathogenesis. *Mod Pathol*. 2016; 29(S2):270A–271A.
22. Merino MJ, Torres-Cabala C, Pinto P, Linehan WM. The morphologic spectrum of kidney tumors in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome. *Am J Surg Pathol*. 2007; 31:1578–85. [PubMed: 17895761]
23. Macher-Goeppinger S, Roth W, Wagener N, Hohenfellner M, Penzel R, Haferkamp A, Schirmacher P, Aulmann S. Molecular heterogeneity of TFE3 activation in renal cell carcinomas. *Mod Pathol*. 2012; 25:308–15. [PubMed: 22037260]
24. Ugurel S, Houben R, Schrama D, Voigt H, Zapatka M, Schadendorf D, Bröcker EB, Becker JC. Microphthalmia-associated transcription factor gene amplification in metastatic melanoma is a prognostic marker for patient survival, but not a predictive marker for chemosensitivity and chemotherapy response. *Clin Cancer Res*. 2007; 13:6344–50. [PubMed: 17975146]
25. Garraway LA, Widlund HR, Rubin MA, Getz G, Berger AJ, Ramaswamy S, Beroukhi R, Milner DA, Granter SR, Du J, Lee C, Wagner SN, Li C, Golub TR, Rimm DL, Meyerson ML, Fisher DE, Sellers WR. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature*. 2005; 436:117–22. [PubMed: 16001072]

Figure 1.

(case 1). The tumor shows a solid to nested architecture with areas of pseudopapillary growth (A). The neoplastic cells show prominent eosinophilic nucleoli surrounded by a clear halo (B). The neoplastic cells demonstrate diffuse nuclear immunoreactivity for PAX8 (C) and patchy immunoreactivity for cytokeratin Cam5.2 (D). The tumors cells show moderate nuclear labeling for TFEB protein (E) and diffuse immunoreactivity for melan-A (F).

Figure 2.
(case 2). This neoplasm demonstrates papillary (A) and nested (B) architecture. The papillary areas are associated with abundant psammoma bodies (C). The cytoplasm is pale and nuclei have coarse chromatin (D). The neoplastic cells are diffusely immunoreactive for PAX8 (E) and multifocally immunoreactive for melan-A (F).

Figure 3. (case 3). The neoplasm has a solid nested architecture (A). The neoplastic cells have eosinophilic cytoplasm and prominent eosinophilic nucleoli surrounded by a clear halo (B). The neoplastic cells label strongly for PAX8 (C). The neoplastic cells demonstrate moderate immunoreactivity for TFEB (D). The neoplastic cells demonstrate diffuse immunoreactivity for melan-A (E) and cathepsin K (F).

Figure 4. (case 4). This neoplasm had predominantly solid nested architecture (A) with smaller areas of acinar architecture with small abortive papillae (B). The neoplasm showed weak nuclear labeling for TFEB (C). The neoplasm demonstrated patchy immunoreactivity for both melan-A (D) and cathepsin K (E), and multifocal labeling for HMB45 in the form of dispersed individual positive cells (F).

Figure 5.

(case 8). This neoplasm has an unusual biphasic appearance, with smaller paler epithelioid cells forming acinar structures and larger epithelioid cells with more abundant eosinophilic cytoplasm often clustered within the lumen of these acini (A, B). The neoplastic cells are diffusely immunoreactive for PAX8 (C). They show moderate nuclear labeling for TFEB (D). The neoplasm demonstrates patchy immunoreactivity for melan-A (E) and cathepsin K (F), with immunoreactivity preferentially seen in the larger epithelioid cells with eosinophilic cytoplasm.

Figure 6.

TFEB FISH analysis. (A) Case 1: High level of *TFEB* gene amplification showing innumerable red (centromeric) and green (telomeric) signals of *TFEB*, some condensed in larger blobs. For comparison a normal cell depicted at 12 o'clock for comparison with 2 copies each. B, C (Case 2). High level of *TFEB* amplification (typically defined as >10) consisting of both centromeric and telomeric parts of *TFEB* (no gene break-apart). D (Case 8). Unbalanced *TFEB* break-apart with subsequent amplification of the centromeric part

(red) and deletion of the telomeric (green) signal (remaining of 1X normal red-green pair of *TFEB* allele).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

TFEB-Amplified RCC in this Study and the Literature

Case	Age/Sex	Size/Stage	TFEB IHC	Melan A	HMB45	Cathepsin K	Morphology	Outcome
1	64/F	10.9cm; pT3bN2 (renal vein thrombus)	++	+	-	-	Nested/pseudopapillary with eosinophilic cells; nucleoli++; grade 3 with necrosis	NED 28 months
2	71/M	3cm; pT3aNXMX (partial nephrectomy, capsular invasion, margin +)	-	+	-	-	Papillary with clear and eosinophilic cells, psammoma bodies; grade 3 with focal necrosis	Periaortic lymph node metastasis 3 months later
3	65/M	1.9cm; pT1NXMX (partial nephrectomy)	++	+	-	+	Nested/pseudopapillary with eosinophilic cells; nucleoli++; grade 3 with focal necrosis	NA
4	23/F	7cm; pT2NXMX	+	+	rare cells +	+	Nested/pseudopapillary with eosinophilic cells; Other areas papillary pattern; grade 4 with necrosis	Vaginal metastasis 8 months later
5	77/F	4cm; pT1NX	++	+	rare cells +	+	Nested/pseudopapillary with eosinophilic cells; grade 3 with necrosis	
6	78/F	12cm; pT3bNX (renal vein thrombus)	+	+	-	+	Nested/pseudopapillary with eosinophilic cells; grade 3 with necrosis	NA
7 *	61/F	19cm; pT4NOM1 (vaginal metastasis)	-	+		-	Nested/pseudopapillary with eosinophilic cells; grade 4 with necrosis	NA
8 *	61/M	2.7cm pT1NX (partial nephrectomy) incidental discovery (proteinuria)	++	+	rare cells +	+	Unusual biphasic morphology; grade 3 with no necrosis	NA
Peckova et al. ²⁰ Case 6 *	77/F	12cm	NA	+	+	+	Nested/pseudopapillary with eosinophilic cells; grade 4 with necrosis	DOD 2.5 months (lung, adrenal metastases)
Durinck et al. ¹⁹ "Tumor 12/16T"	NA	NA	NA	NA	NA	NA	"Low grade papillary and oncocytic"	NA
Williamson et al. ²¹ **	57/M	pT3a	NA	NA	NA	NA	"tubulopapillary"	NA
TCGA- GL-7966 ²⁰	28/F	pT3N1	NA	NA	NA	NA	NA	NED 4 months
TCGA-Q2- A5QZ ²⁰	61/F	pT3	NA	NA	NA	NA	NA	Recurred at 14 months
TCGA-UZ-A9PQ ²⁰	59/M	9.2cm; pT3N1	NA	NA	NA	NA	NA	Recurred at 75 months; died at 86 months

NA=not available; NED=no evidence of disease; DOD=dead of disease

* Amplification and rearrangement

Williamson *et al.* reported that one unspecified case was patchy positive for Melan A and Cathepsin K among references 20 and 21, another was HMB45 and Melan A negative.

##

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript