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RBM10-TFE3 renal cell carcinoma: A potential diagnostic pitfall due to cryptic intrachromosomal Xp11.2 inversion resulting in false-negative *TFE3* FISH

Pedram Argani, MD[1,2], **Lei Zhang, MD**[3], **Victor E. Reuter, M.D**[3], **Satish K. Tickoo, MD**[3], and **Cristina R. Antonescu, MD**[3]

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

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

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

Abstract

Xp11 translocation renal cell carcinoma (RCC) are defined by chromosome translocations involving the Xp11 breakpoint which results in one of a variety of *TFE3* gene fusions. *TFE3* break-apart florescence in situ hybridization (FISH) assays are generally preferred to TFE3 immunohistochemistry as a means of confirming the diagnosis in archival material, as FISH is less sensitive to the variable fixation which can result in false positive or false negative immunohistochemistry. Prompted by a case report in the cytogenetics literature, we identify three cases of Xp11 translocation RCC characterized by a subtle chromosomal inversion involving the short arm of the X chromosome, resulting in an *RBM10-TFE3* gene fusion. *TFE3* rearrangement was not detected by conventional *TFE3* break-apart FISH, but was suggested by strong diffuse TFE3 immunoreactivity in a clean background. We then developed novel fosmid probes to detect the *RBM10-TFE3* gene fusion in archival material. These cases validate *RBM10-TFE3* as a recurrent gene fusion in Xp11 translocation RCC, illustrate a source of false negative *TFE3* break-apart FISH, and highlight the complementary role of TFE3 immunohistochemistry and *TFE3* FISH.

Keywords

Renal Neoplasm; TFE3; Translocation

Introduction

Xp11 translocation renal cell carcinoma (RCC) encompasses a variety of gene fusions involving *TFE3* on Xp11.2 with different gene partners, which triggers oncogenic activation

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

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of the TFE3 transcription factor. Known *TFE3* fusion partners include *ASPSCR1 (ASPL), PRCC, SFPQ1 (PSF), NONO, CLTC, PARP14, LUC7L3, DVL2,* and *KHSRP* [1, 2, 3, 4, 5, 6, 7]. Xp11 translocation RCC comprise the majority of pediatric RCC and approximately 1–4% of adult RCC [8–11]. While a wide range of morphologic appearances have been reported[12], the most common is that of an RCC with papillary architecture, clear cells, and psammoma bodies. By immunohistochemistry, these tumors underexpress cytokeratins, but often express melanocytic markers and the cysteine protease cathepsin k, which distinguishes them from more common RCC subtypes[13–15]. Overall, outcome is similar to that of clear cell RCC; increased age and advanced stage are poor prognostic factors[16, 17]. Immunohistochemistry to detect overexpressed TFE3 fusion proteins using an overnight incubation protocol was shown to be a highly sensitive and specific assay to confirm this diagnosis in formalin-fixed, paraffin embedded archival material[18]; however, variable fixation (particularly when automated immunostaining techniques are used) significantly reduces the specificity of TFE3 immunohistochemistry[19]. Break-apart fluorescence in situ hybridization (FISH) assays demonstrating *TFE3* gene rearrangement are less affected by variable fixation and now are generally considered the preferred method[11, 12, 20, 21].

Despite recent advances, some RCC displaying clinical, morphologic, and immunohistochemical profiles typical of Xp11 translocation RCC do not demonstrate *TFE3* gene rearrangements by FISH. A subset of these prove to be the related t(6;11) translocation RCC resulting in the *MALAT1 (Alpha)-TFEB* gene fusion, which are grouped together in the current 2016 World Health Organization Classification of Renal Tumors with Xp11 translocation RCC as MiT family translocation RCC[22,23]. These cases can be detected by TFEB immunohistochemistry or, preferably, by *TFEB* break-apart FISH[24]. Nonetheless, some cases with a translocation RCC phenotype remain negative by conventional *TFE3* and *TFEB* FISH assays. One possibility is that these cases demonstrate subtle cytogenetic alterations involving the *TFE3* gene at the Xp11.2 locus which are not detectable by FISH resolution. Along these lines, the *NONO-TFE3* gene fusion resulting from the intra-chromosomal X inversion, inv(X) (p11.2;q13.1), can be difficult to detect by *TFE3* break-apart FISH, often resulting in small constant gaps between the *TFE3* signals[7, 12].

Recently, a case report of an Xp11 translocation RCC with fusion between the *RBM10* gene at Xp11.23 and *TFE3* at Xp11.2 was described in the cytogenetics literature[25]. As *RBM10* is located only 1.8 mb apart from *TFE3* on the short arm of the X chromosome, the abnormality was difficult to identify by *TFE3* break-apart FISH. The gene fusion was confirmed by RNAseq and reverse transcriptase-polymerase chain reaction demonstrating the fusion of *RBM10* exon 17 with *TFE3* exon 5. This report suggests that *RBM10-TFE3* gene fusions could potentially represent the underlying genetic alteration of at least a subset of the group of RCC with a translocation phenotype but which appear to be negative by conventional *TFE3* and *TFEB* FISH[12].

We now report three further cases of *RBM10-TFE3* RCC. All 3 occurred in adults and were initially reported to be negative for *TFE3* rearrangement by FISH. However, based upon strong morphologic suspicion of Xp11 translocation RCC, strong cathepsin K and TFE3 immunoreactivity, custom fosmid probes were developed to demonstrate the *RBM10-TFE3* gene fusion.

MATERIALS AND METHODS

IRB Approval

This study was approved by the Institutional Review Boards at our institutions.

Case Selection and FISH analysis

The cases studied derive from the consultation files of the authors. Immunohistochemistry was performed as previously described[7].

FISH on interphase nuclei from paraffin-embedded 4-micron sections was performed applying custom probes using bacterial artificial chromosomes (BAC) and fosmids**,** covering and flanking genes of interest. Fosmids are similar to cosmids but utilize the bacterial F-plasmid to allow cleavage of DNA fragments in the range of 35–40kb. *TFE3* break-apart FISH was performed as previously described[12]. BAC clones and fosmid clones were chosen according to UCSC genome browser (http://genome.ucsc.edu), see Supplementary Table 1. The BAC and fosmid 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 and fosmids 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 described[26]. 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 split-apart or come-together signal, depending upon the type of assay applied, either break-apart or fusion assay, respectively. Nuclei with incomplete set of signals were omitted from the score.

Results

Clinical Features and Morphology

All three cases occurred in adults. Case one was a 61 year-old female who had a radical nephrectomy for a 3.8 cm pT3 RCC associated with a 3cm tumor deposit in perirenal adipose tissue, and then developed a peritoneal recurrence 1 year later. Case two was a 54 year-old male with a 2.5 cm pT1bNX organ-confined RCC treated by partial nephrectomy. Case 3 was a 45 year-old female with a 3.8 cm pT1NX organ-confined RCC treated by partial nephrectomy. As the latter two cases are recent, no meaningful clinical follow-up is available. All three cases demonstrated clear cells and papillary architecture. Case 1 also had prominent solid architecture. Case 2 had prominent psamomma bodies, while case 3 demonstrated focally a second population of smaller cells as has been described previously in both t(6;11) RCC and Xp11 translocation RCC[12, 23], especially those with the *SFPQ-TFE3* gene fusion [27]. Cases 1 and 3 demonstrated foci of subnuclear cytoplasmic vacuolization as often seen in the *SFPQ-TFE3* and *NONO-TFE3* RCCs[7]. Melanin pigment was not identified in any case. All three cases demonstrated diffuse immunoreactivity for cathepsin k and focal labeling for melan A (Figures 1–3). Cytokeratin 7 was negative in 2

cases in which was tested; in one of these cases, cytokeratin Cam5.2 and EMA were only focally positive. All three cases were initially reported as negative for *TFE3* FISH using previously published break-apart probes[12], and the two cases tested (cases 1 and 2) were negative for *TFEB* rearrangements by break-apart FISH[24]. However, all three cases demonstrated diffuse, strong TFE3 labeling by immunohistochemistry. Therefore, further evaluation was performed to detect the *RMB10-TFE3* gene fusion.

RBM10-TFE3 Fusion FISH

As a 1.8 mb chromosomal inversion that would result in an *RBM10-TFE3* fusion remains cryptic for the standard FISH resolution using the TFE3 BAC flanking probes, we applied a custom design using two fosmid clones covering the most likely breakpoint for each of the *RBM10* and *TFE3* genes. A positive inversion event was interpreted when each color signal (green for *RBM10* and red for *TFE3*) splits into half-sized pairs, while a negative result revealed non-split signals (Figure 4). Despite the smaller-sized signals detected using fosmids compared to the more common BACs, all three RCC demonstrated a *RBM10-TFE3* fusion using this novel assay (Figure 5). Adjacent normal cells did not show this alteration (Supplementary Figure 1).

Discussion

We report 3 cases of *RBM10-TFE3* RCC. Previously, a single case of an RCC with this gene fusion was reported in the cytogenetics literature[25]. The patient was a 32 year-old female with a 4.5cm pT1b RCC with solid, papillary, and trabecular architecture, and epithelioid cells with clear to eosinophilic cytoplasm. The neoplasm was strongly immunoreactive for TFE3 protein by immunohistochemistry, and the gene fusion was confirmed by RNAseq and reverse transcriptase-polymerase chain reaction demonstrating the fusion of *RBM10* exon 17 with *TFE3* exon 5. The *RMB10-TFE3* gene fusion appears to be a recurrent cytogenetic alteration, as two additional RCC cases harboring the *RMB10-TFE3* fusion transcript were identified in two large sequencing studies of cases characterized as clear cell[28] or papillary[29] RCC, though the details of the clinical, morphologic and immunohistochemical features of these cases were not provided. Our results confirm the recurrent nature of this cytogenetic alteration, and document that the *RBM10-TFE3* RCC have malignant potential, as one of our cases recurred.

Our results also highlight the novel utilization of fosmid probes to detect subtle intrachromosomal inversions that can be missed by standard FISH. Fosmid clones are much smaller (35–40 kb) compared to the typical BAC clones (159–200 kb) applied in the clinical lab setting, and thus interpretation of fosmid results is less robust, due to their weaker and subtle signals. However, despite the smaller-sized signals, all three RCC in our study definitively demonstrated the *RBM10-TFE3* gene fusion using this assay. To our knowledge, this technique has not previously been utilized to demonstrate cryptic chromosome inversions and rearrangements in cancer.

Furthermore, our findings illustrate an important pitfall when using *TFE3* FISH as the gold standard in diagnosis rather than TFE3 immunohistochemistry. While we generally find *TFE3* FISH to be more reliable in variably-fixed consultation material than TFE3

immunohistochemistry[12], and typically use it as the first-line test to evaluate for Xp11 translocation RCC, the *RBM10-TFE3* fusion may be missed using the conventional *TFE3* break-apart FISH assays. Hence, when we encounter an RCC with a phenotype strongly suggestive of Xp11 translocation RCC (clear cells and papillary architecture, cathepsin k and or melanocytic marker immunoreactivity, conventional *TFE3* and *TFEB* FISH negative), we now routinely perform TFE3 immunohistochemistry. Strong immunoreactivity for TFE3 in this setting using a properly calibrated assay suggests the possibility of a cryptic rearrangement involving *TFE3*, and prompts us to evaluate for the *RBM10-TFE3* gene fusion. This highlights the importance of utilizing a properly calibrated TFE3 immunohistochemical assay; strong nuclear TFE3 labeling in a clean background is highly suggestive of the rearrangement, while weak or moderate nuclear staining with high background is likely nonspecific as documented previously[19]. While young patient age makes the diagnosis of translocation RCC more likely, translocation RCC is numerically more common in adults than children due to the much greater overall incidence of RCC in adults[30], so adult age of the patient should not preclude further work-up. Indeed, all cases of *RBM10-TFE3* RCC reported to date (including the three in this study) have been in adults.

For pathologists who may not have immunohistochemistry or FISH for *TFE3* or *TFEB* available, utilization of other more common immunohistochemical markers can help suggest the diagnosis of MiT family translocation RCC. Underexpression of cytokeratins is a useful clue to the diagnosis[1,2], which can be substantiated by aberrant expression of cathepsin k and /or melanocytic markers. Absence of the latter two markers essentially excludes the t(6;11) RCC, but does not exclude Xp11 translocation RCC which will be cathepsin K negative in approximately 50% of cases depending upon fusion type[7,14,15]. As our study shows, even a negative conventional *TFE3* break-apart FISH study does not exclude the diagnosis of Xp11 translocation RCC, and such cases require more specialized testing for confirmation

Finally, we note that the *RBM10-TFE3* gene fusion does not account for all renal neoplasms previously described as having a translocation phenotype (i.e., RCC with papillary architecture and clear cells that label for cathepsin K by immunohistochemistry) but for which *TFE3* and *TFEB* FISH assays are negative[12]. We tested six additional renal neoplasms with this profile in the course of this study, and none of these six were *RBM10-TFE3* positive. Three of these six cases were strongly positive for TFE3 by IHC (Supplementary Table 2). It is possible that some of these cases harbor other cryptic rearrangements resulting in *TFE3* gene fusions that remain to be elucidated. Other such cases likely have different but related genes involved in their pathogenesis, and these genes also remain to be determined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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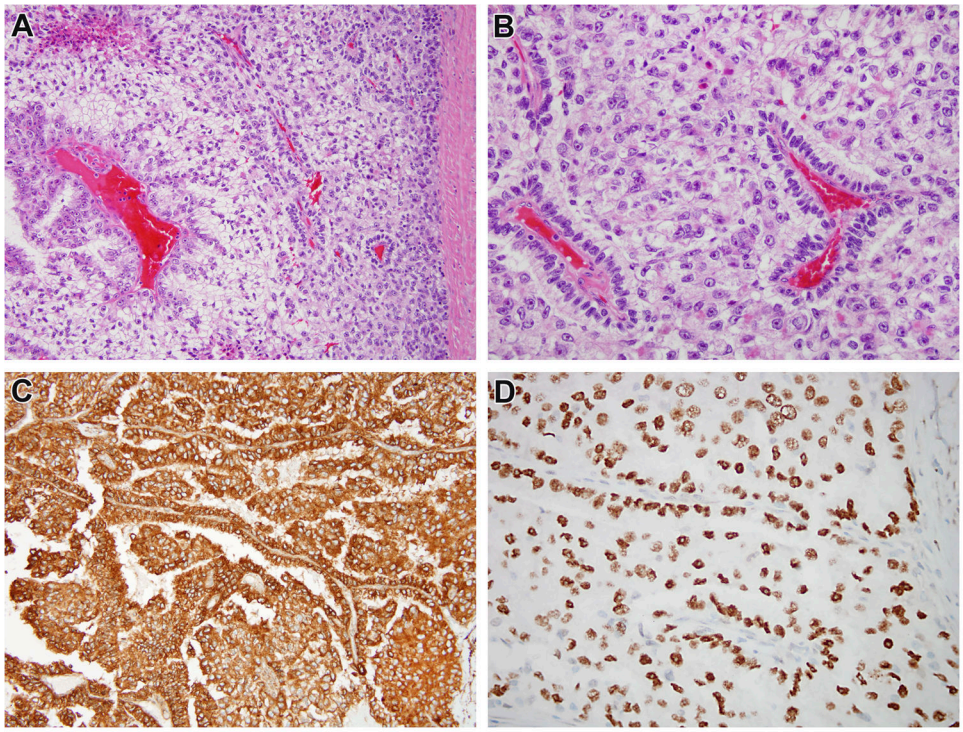

Figure 1.

Case 1. This recurrent renal cell carcinoma demonstrates solid papillary architecture and epithelioid cells with clear cytoplasm (A, B). The neoplastic cells are diffusely immunoreactive for cathepsin k (C). While conventional *TFE3* FISH did not demonstrate rearrangement, the neoplasm demonstrated diffuse, strong nuclear labeling for TFE3 protein with a clean background (D), prompting additional FISH fusion studies which demonstrated the *RBM10-TFE3* gene fusion.

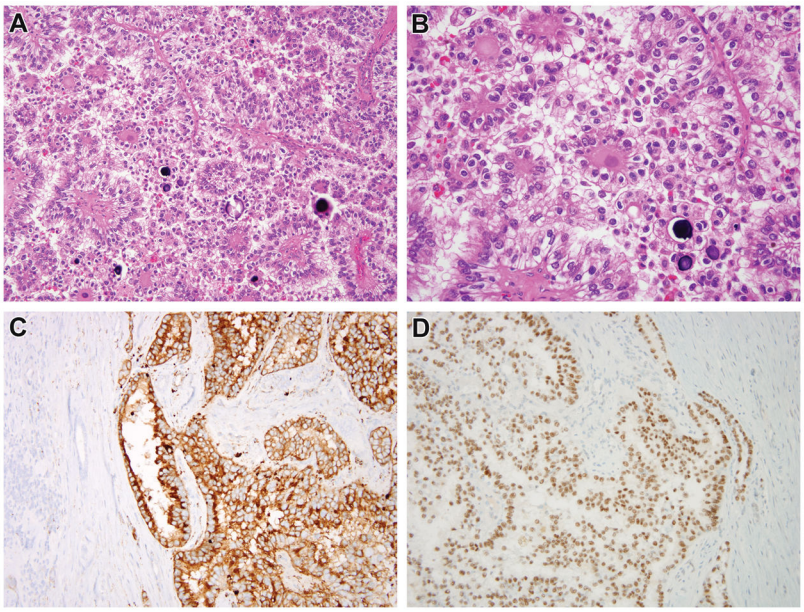

Figure 2.

Case 2. The renal cell carcinoma demonstrates papillary architecture, clear cells, and abundant psammoma bodies, highly suggestive of Xp11 translocation RCC (A, B). The neoplasm was diffusely immunoreactive for cathepsin k (C), with surrounding kidney being appropriately negative. While conventional *TFE3* FISH was negative for rearrangement, immunostain for TFE3 demonstrated diffuse strong nuclear staining in the neoplastic cells with absence of staining in the normal kidney (D), leading to additional FISH fusion studies which demonstrated the *RBM10-TFE3* gene fusion.

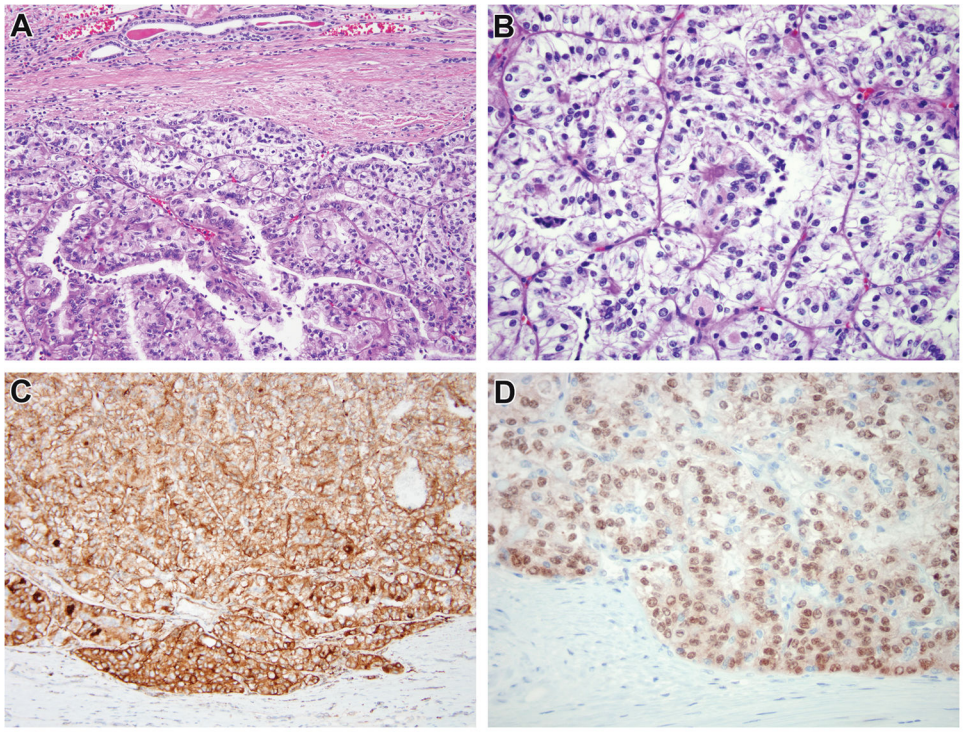

Figure 3.

Case 3. This renal cell carcinoma demonstrates nested and papillary architecture and epithelioid cells with clear to eosinophilic cytoplasm (A, B). A subpopulation of smaller cells is present within the lumens of acini. The neoplastic cells are diffusely immunoreactive for cathepsin k (C). While conventional *TFE3* FISH did not demonstrate rearrangement, the neoplasm demonstrated diffuse, strong nuclear labeling for TFE3 protein with a clean background (D), prompting additional FISH fusion studies which demonstrated the *RBM10-TFE3* gene fusion.

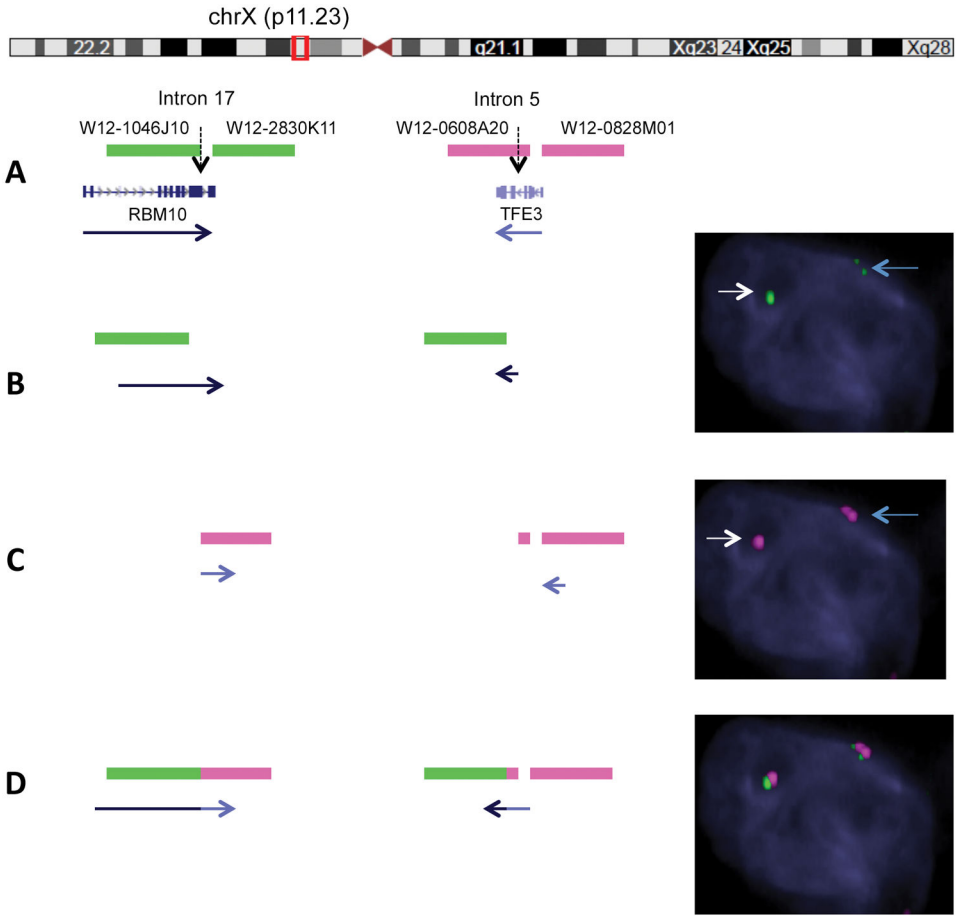

Figure 4.

Fosmid FISH design and novel application to detect *RBM10-TFE3* fusion. A. Diagrammatic representation of custom design of fosmid probes covering the likely breakpoints of *RBM10* (2 green probes flanking intron 17) and *TFE3* (2 red proves flanking intron 5) genes. Red box illustrates the near proximity (1.8 mb) of the 2 genes on chromosome X (Xp11.23 locus). B. Single color channel assay using the above design for *RBM10* in a tumor cell reveals one intact green signal (wild type *RBM10* allele, white arrow) and one split green signal into two half-sized fragments, in keeping with a *RMB10* break and inversion event (small gap corresponds to the 1.8 mb distance, blue arrow). C. Similar single color assay for *TFE3* showing one intact red probe (wild type *TFE3* allele, white arrow) and one split signal into two smaller red fragments pieces (TFE3 break/inversion, blue arrow). D. Two-color fusion assay FISH showing one normal sized fused green-red signal due to close proximity of wild type *RBM10* and *TFE3* genes, and two pairs of split-fused smaller signals *RBM10* (green) and *TFE3* (red) in keeping with the *RBM10-TFE3* fusion/inversion.

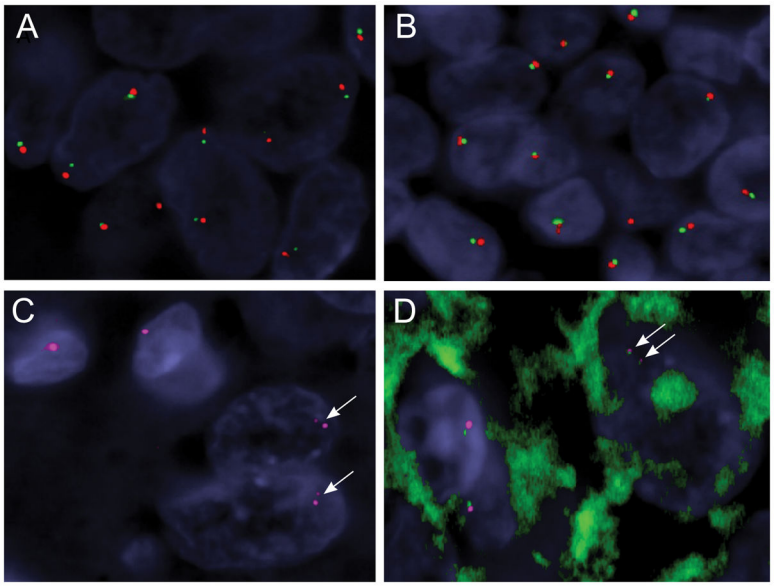

Figure 5.

Identification of *RMB10-TFE3* fusion/inversion by the custom fosmid FISH assay, which is not detected by the standard BAC FISH assay resolution. (Case 2)

A,B. Conventional break-apart assay using typical flanking *TFE3* probes (red, centromeric; green, telomeric) show small gaps in the tumor cells (A), compared to the normal lymphocytes adjacent to the tumor (B), but interpreted as a negative result in the clinical setting. However the small gaps may suggest the presence of a *TFE3* cryptic inversion in the setting of strong TFE3 immunoreactivity and should trigger additional custom FISH assays to investigate a potential *RMB10-TFE3* fusion (C, D).

C. Single color channel FISH using 2 fosmids flanking the *TFE3* breakpoint (red) shows two split smaller red signals in the 2 tumor cells (lower right, arrows), in contrast to 2 normal cells showing one intact red signal (upper left).

D. Two-color fusion assay using custom fosmids showing 2 pairs of smaller red-green signals in keeping with a *RBM10-TFE3* inversion in a tumor cell (upper right, arrows), compared to two normal cells showing only one pair of intact size red-green signals (lower left).