Published in final edited form as:

Am J Surg Pathol. 2010 June ; 34(6): 757–766. doi:10.1097/PAS.0b013e3181dd577e.

Dual-color, Break-apart FISH Assay on Paraffin-embedded Tissues as an Adjunct to Diagnosis of Xp11 Translocation Renal Cell Carcinoma and Alveolar Soft Part Sarcoma

Minghao Zhong, MD, PhD^{*}, Patricia De Angelo, MS[†], Lisa Osborne, MS[†], Megan Keane-Tarchichi, MD^{*}, Michael Goldfischer, MD[‡], Lisa Edelmann, PhD[§], Youfeng Yang, MS^{||}, W. Marston Linehan, MD^{||}, Maria J. Merino, MD[¶], Seena Aisner, MD^{*}, Meera Hameed, MD^{*}

^{*} Pathology and Laboratory Medicine

[†] Institute of Genomic Medicine, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark

[‡] Hackensack University Medical Center, Hackensack, NJ

[§] Mount Sinai School of Medicine New York, NY

^{||} Urologic Oncology Branch

[¶] Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, MD.

Abstract

Both Xp11.2 translocation renal cell carcinoma (RCC) and alveolar soft part sarcoma (ASPS) are characterized by various translocations disrupting chromosome Xp11.2, which result in gene fusions involving the TFE3 transcription factor gene. Diagnostic tools to detect translocations involving the TFE3 gene on chromosome X would be valuable in the evaluation of these tumors. We developed a dual-color, break-apart fluorescence in situ hybridization (FISH) assay to identify the chromosomal break point in paraffin-embedded tissue. This assay was validated using 4 cases of Xp11.2 RCC [proven by karyotype and/or reverse-transcriptase polymerase chain reaction (RT-PCR)], 2 cases of ASPS (proven by karyotype or RT-PCR), the UOK109 cell line carrying the inv(X) (p11;q12), and several negative controls (both neoplastic and non-neoplastic). This break-apart FISH assay is a relatively quick procedure for detecting Xp11.2 RCC and ASPS translocations and can be applied to archival paraffin-embedded tissue.

Keywords

FISH; Xp11 translocation renal cell carcinoma; alveolar soft part sarcoma

Renal cell carcinomas (RCCs) are heterogeneous in terms of histopathology, clinical features, and response to different forms of therapy.¹⁸ The common histopathologic subtypes include clear cell, papillary, chromophobe, and collecting duct carcinomas. These different

subtypes are associated with characteristic changes at the genetic and molecular level. Clear cell carcinomas, the most common subtype, constitute up to 80% to 85% of cases and exhibit frequent loss of the regions 3p21, 3p1214, and 3p25. In Addition, loss of heterozygosity and somatic mutation of the Von Hippel–Lindau (VHL) tumor suppressor gene located on chromosome 3p25 are common findings in sporadic clear cell carcinomas.¹⁴ Several lines of evidence suggest that the VHL-HIF2 α -signaling pathway may be critical in the development of these tumors and provide a rationale for treatment strategies targeting this single-gene pathway.¹⁹ The second most common subtype, papillary RCC, is characterized by trisomies (chromosomes 3q, 7, 12, 16, 17, and 20) and loss of the Y chromosome in male patients.¹⁶ Chromophobe and oncocytic RCCs have been associated with Birt-Hogg-Dube (BHD) syndrome.²¹ Kidney tumors occur in 15% to 25% of BHD patients and can be bilateral and multifocal. In the NCI series,²⁷ 33% of BHD kidney tumors were chromophobe renal carcinoma, 50% were oncocytic renal carcinoma, 9% were clear cell renal carcinoma, and 6% were oncocytoma.

Xp11 translocation RCC-bearing fusions involving the TFE3 transcription factor gene is a newly recognized subtype of RCC discussed in the 2004 renal tumor classification system of World Health Organization (WHO). These tumors most commonly have ASPL-TFE3 or PRCC-TFE3 gene fusion, resulting from the translocations t(X;17)(p11;q25)¹⁵ and t(X;1)(p11;q21),²³ respectively. Other reported translocations include t(X;1)(p11.2;p34), which fuses the PSF and TFE3 genes¹¹; inv(X)(p11;q12), which fuses the NonO (p54^{nrb}) and TFE3 genes¹¹; and t(X;17)(p11.2;q23), which fuses the CLTC and TFE3 genes.⁵ The swaps of stronger promoters by these translocations results in increased TFE3 fusion proteins in the nucleus. Aberrant nuclear immunoreactivity for TFE3 has been demonstrated specifically in this distinctive subtype of RCC.⁵ Several reports suggest that Xp11.2-associated carcinomas make up at least 20% to 40% of pediatric RCC^{2,28} and a much smaller proportion (perhaps 1%) of RCC in adults.¹⁰ These neoplasms are considered aggressive with early age of onset and pleomorphic histologic features including clear cell and/or “voluminous” eosinophilic morphologies and papillary and/or alveolar structures.³

Alveolar soft part sarcoma (ASPS), a rare soft tissue tumor, has ASPL-TFE3 gene fusion resulting from an unbalanced recurrent der¹⁷ t(X;17)(p11;q25)¹⁷ or occasionally a balanced t(X;17)(p11;q25).²⁶ The classic ASPS is characterized by large, round-to-oval tumor cells arranged in an alveolar pattern surrounded by fibrous septa with intracytoplasmic, periodic acid-Schiff (PAS)-positive, diastase-resistant, crystalloid inclusions.¹⁷

Clinical presentation, histopathologic features, and positive TFE3 immunohistochemical staining are helpful elements in the diagnosis of this family of tumors (RCC and ASPS). Identifying the translocation by genetic and molecular studies provides confirmation of the histologic diagnosis. At this time, karyotypic analysis and reverse transcriptase-polymerase chain reaction (RT-PCR) are the only available tools for identifying this translocation. Unfortunately, karyotypic analysis is limited by the availability of viable tumor cells and RT-PCR may be limited by RNA quality and the requirement to perform multiple PCRs to include the various partners. To overcome these limitations, we wanted to develop a fluorescence in situ hybridization (FISH) assay to serve as an alternative diagnostic tool

using the most accessible material in the lab, formalin-fixed, paraffin-embedded (FFPE) tissues, for the detection of TFE3 (Xp11.2) gene rearrangement.

MATERIALS AND METHODS

Tissue Samples

The study was performed on Xp11 translocation RCC and ASPS cases proven by karyotype and/or RT-PCR (Table 1), using both conventional whole-tissue sections and a tissue microarray (TMA). After Institutional Review Board approval (protocol 0120080157), neutral-buffered FFPE tissues were obtained from resection specimens from the files of the Pathology Department of New Jersey Medical School, University Hospital, Newark. All cases were reviewed by Meera Hameed and Minghao Zhong. Whole-tissue sections stained with hematoxylin and eosin were evaluated for tumor and appropriate areas were scored for TMA construction. Cases were arrayed in duplicate 1.0-mm cores from tumor and one 1.0-mm core from adjacent non-neoplastic tissue.

Immunohistochemistry

After initial deparaffinization, endogenous peroxidase activity was blocked with 0.3% H₂O₂. Deparaffinized sections were microwaved in 10 mmol/L citrate buffer (pH 6.0). The slides were then incubated for 1 h at room temperature using goat polyclonal antihuman TFE3 (1:300; Santa Cruz sc-5958), followed by biotinylated rabbit antigoat IgG (Vector Laboratories, Burlingame, CA) for 30 min, and finally ABCComplex (Vector Laboratories, Burlingame, CA). The bound complex was visualized with 0.125% amino-ethyl carbazole (AEC, Sigma, St Louis, MO) and 0.003% (vol/vol) H₂O₂. The sections were then counterstained in Mayer hematoxylin. For negative controls, the primary antibody was replaced with phosphate-buffered saline.

FISH Probe Design and Development

Bacterial artificial chromosome (BAC) clones were identified using the “CloneCentral human BAC Clone Locator” from EmpireGenomics (<http://www.empiregenomics.com/helixhq/clonecentral/search/human>). Four custom-labeled FISH probes flanking the TFE3 gene on chromosome X were generated by Empire Genomics. The BAC clones RP11–404P16 (458kb) and RP11–416B14 (182kb) located centromeric to the TFE3 were labeled with 5-TAMRA dUTP. The BAC clones RP11–528A24 (116kb) and RP11–58H17 (182kb) located telomeric to the TFE3 were labeled with 5(6)-rhodamine green dUTP (Fig. 3A).

Sample Preparation

After array construction, 4-μm sections were cut with the last section stained with hematoxylin and eosin to confirm the presence of tumor. The TMAs were then subjected to FISH assay. The slides were dewaxed and rehydrated, immersed successively in 2 baths of ethanol 100% (5 min at room temperature), dried and immersed in 0.2N of HCl for 20 min, washed twice in 2×sodium chloride/sodium citrate for 2 min, and then incubated with pretreatment solution (sodium thiocyanate 0.1 M) at 80°C for 15 min, washed twice in 2×sodium chloride/sodium citrate for 2 min, in distilled water for 1 min, in 2×SSC for 3 min, and finally distilled water for 1 min. Slides were then digested with protease for 20 min

at 37°C, washed in 1×phosphate-buffered saline for 5 min at room temperature, then twice in 2×SSC for 5 min, fixed in 4% formaldehyde for 10 min at room temperature, washed in 1×phosphate-buffered saline for 5 min at room temperature, washed twice in 2×sodium chloride/sodium citrate, and then dehydrated by immersing in 70%, 85%, and 100% ethanol for 1 min each at room temperature.

Hybridization and Detection

Aliquots of labeled DNA of each BAC probe were diluted to a final concentration of 1 ng/μL in 50-fold excess Human Cot 1 in hybridization solution, denatured at 90°C for 8 min, and annealed at 37°C for 30 min. Probe mixtures, 10 μL per section, were placed onto the denatured slides and covered with glass coverslips. The hybridization was carried out in a humidified chamber at 37°C overnight. Excess of the probe was washed in 2 × SSC/ 0.1% Tween (3 × 5 min at 37°C), 0.1 × SSC (3 × 5 min at 60°C) followed by Tris/NaCl/ 0.05% Tween, pH 7.5 for 5 min at room temperature. The slides were then washed in posthybridization wash buffer at 72°C. Subsequently, 10 μL of 4,6 diamino-2-phenylindole counterstain was placed on the slide, which was then mounted with Vectashield. After hybridization, all slides were maintained at – 20°C in the dark.

Evaluation

Slides were analyzed with an Olympus BX51 fluorescence microscope equipped with PL Fluotar 10 ×, 20×, 60 ×, and 100 × objectives and specific filters for fluorescein isothiocyanate, Texas Red, and 4,6 diamino-2-phenylindole and also double and triple band-pass filters. Images were captured using Cytovision FISH software (Applied Imaging Ltd). In each case, hybridization signals were examined in both tumor areas and non-neoplastic areas. Hundred nuclei were analyzed for each sample. The interpretation of intact and split signals was based on generally accepted guidelines recommended by Vysis and used for all other commercially available break-apart FISH assays in clinical laboratories performing testing using this method. Make sure the criteria of space between 2 signals greater than 1 signal width to be considered a split signal. Cells without the rearrangement have 1 or 2 sets of red and green fusion signals (depending on patient sex), indicating intact Xp11. In keeping with other FISH assays validated for paraffin-embedded tissue in our laboratory, a positive result was reported when greater than 10% of the tumor nuclei had evidence of Xp11 rearrangement. To avoid false positives resulting from nuclear truncation occurring in a subset of cells in paraffin-embedded samples, only tumor nuclei with all 4 signals (or 2 signals in male) were evaluated, and overlapping cells indistinguishable as separate nuclei were excluded from the analysis.

Cytogenetic Analysis

A fresh sample of the tumor was collected in Roswell Park Memorial Institute Medium tissue culture medium and was sent directly to the cytogenetics laboratory. The tumor tissue was carefully sliced into small pieces and further treated with collagenase (3 mg/mL Hanks Balanced Salt Solution, Roche/Boehringer, IN) for 2 h to yield the highest concentration of cells. These cells were cultured in Roswell Park Memorial Institute Medium 1640 solution for 3 to 5 days on a coverslip dish (MatTek Corporation, Ashland, MA). After adding 10 uL of colchicine (10 mg/mL Hanks Balanced Salt Solution) to each chamber for 45 min, the

cells were harvested. The slides were prepared using standard in situ techniques. The chromosomes on the slides were G-banded after trypsin pretreatment. The aberrations were designated according to the International System for Human Cytogenetic Nomenclature (1995).

RESULTS

Clinicopathologic Features

Searching our department files, we found 4 cases of Xp11 translocation RCCs, with 2 cases of t(X;1) (p11;p34) proven by karyotype and 2 cases of ASPL-TFE3 Type I fusion proven by RT-PCR. We also found 2 cases of ASPS, 1 case with a balanced t(X;17)(p11;q25) proven by karyotype²⁶ and 1 case with type I ASPL-TFE3 fusion proven by RT-PCR.¹ In addition, we used the UOK109 cell line¹¹ with the inv(X)(p11;q12) (Table 1). The detailed clinical information of each case is provided in Table 1.

On microscopic examination (Fig. 1), all the RCC cases (patients 1–4) had similar morphology. The tumors had solid, nested, pseudopapillary, and microcystic architecture with geographic necrosis. The tumor cells were large with discrete cell borders, granular eosinophilic to clear cytoplasm, vesicular nuclear chromatin and prominent nucleoli. There were no psammoma bodies or hyaline nodules. Although the tumors resembled clear cell and papillary RCC with clear cytoplasm and pseudopapillary architecture, they did not contain the typical delicate and uniform small vessels common in clear cell RCC or collections of foamy histiocytes common in papillary RCC. The ASPS cases (patients 5 and 6) showed classical alveolar growth pattern. The tumor cells were large and discohesive with granular eosinophilic to clear cytoplasm, vesicular nuclear chromatin, and prominent nucleoli. Intracytoplasmic diastase-PAS+ granules were sparse in both ASPS cases (images not shown). The TFE3 immunohistochemical stain showed strong nuclear staining in the Xp11 RCC and ASPS tumors (Fig. 1). Figure 2 shows the karyotype of patient 4 with der(X)t(X;1) (p11;p34) and additional chromosomal abnormalities including +3, -4, -9 -14, -21, -22, and -22. Being aware of the partial deletion of der(X) after translocation is important of the FISH results in this case.

Probes Development and Hybridization on Normal Cells

Karyotype (cytogenetic analysis), RT-PCR, and FISH can be used to detect chromosomal translocation. FISH can be used to visualize structural chromosomal abnormalities in archival tissue. There are 2 basic kinds of FISH probes: fusion probes and break-apart probes. Break-apart assays are well suited to detect translocations that involve multiple partner loci, such as in Xp11 translocation RCC where at least 5 gene partners from different chromosomes fuse with the X chromosome TFE3 gene. We designed 2 pairs of probes that flank the translocation break point of the X chromosome TFE3 loci (Fig. 3A, detailed information in Materials and Methods). The probes were first tested on metaphase spreads of normal female peripheral blood cells. Signal validation was verified by observing both metaphase and interphase cells. In metaphase, 2 pairs of green and red signals were colocalized to the expected X chromosome short arm (Fig. 3B). In interphase, 2 pairs of green and red fusion signals were present (Fig. 3C).

FISH Assay on Metaphase Spreads of Positive Control

We tested probes on metaphase spreads of 2 available Xp11 translocation RCC cases (patients 3 and 4). Both patients were female and had t(X;1) (p11;p34). Patient 4 had a der(X) with partial deletion (Fig. 2). Both specimens showed evidence of a TFE3 gene rearrangement (Figs. 4A, B). Figure 4A shows metaphase from patient 4. There is 1 fused signal on X chromosome and 1 green signal on chromosome 1. The second red signal which is centromeric is absent due to partial deletion der(X) after translocation. The karyotype as seen in Figure 2 confirms the translocation t(X;1) in this case. Figure 4B shows a single interphase nucleus of patient 3, with split red and green signals indicating the presence of a TFE3 gene rearrangement involving X chromosome. The second signal is fused indicating an intact Xp11 locus in this cell.

FISH Assay on FFPE Tissues

The next step was to test the FISH probes on FFPE tissues. We constructed a TMA, which included the specimens from patients 1 to 6 and several non-neoplastic kidney tissues from both male and female patients. The FISH results showed 1 fusion signal within a single nucleus of normal male tissue and 2 fusion signals within a single nucleus of normal female tissue (Figs. 5A, B). For the tumor specimens (except patient 4), there were both a fusion signal and a split red and green signal within a single nucleus (Fig. 5C). For patient 4, there was 1 fusion signal and 1 single green signal. The second red signal which is centromeric is absent due to partial deletion of der(X) after translocation. These results show that the probes can be used on FFPE tissue to detect TFE3 gene rearrangement.

FISH Assay to Detect inv(X)(p11;q12)

Up to this point, we had tested these probes on specimens that contained interchromosomal translocations (ASPL-TFE3 and PSF-TFE3). The NonO-TFE3 associated RCC contains an intrachromosomal translocation resulting from inv(X)(p11;q12). The next step was to test whether these probes can also be used to detect this intrachromosomal translocation. We employed the cell line, UOK109, which carries the inv(X)(p11;q12).¹¹ The TFE3 immunohistochemical (IHC) stain showed strong nuclear staining in this cell line (Fig. 6A). This result is consistent with the presence of Xp11 translocation in this cell line. The hybridization result in metaphase clearly shows that there was a green signal on the X chromosome short arm and a red signal on the X chromosome long arm (Fig. 6B). This result indicates chromosomal inversion involving the Xp11.2 region resulting in translocation of the TFE3 gene. In the interphase hybridization, there was a split of the red and green signal in the single nucleus (Fig. 6C). This experiment proved that these probes can be used to detect inv(X) (p11;q12).

Sensitivity and Specificity of the FISH Assay

Both Xp11 translocation RCC and ASPS are uncommon neoplasms. Cases proven by karyotype and/or RT-PCR were difficult to collect for this study. We were able to recruit an additional Xp11 translocation RCC case harboring a novel variant translocation, t(X;3) (p11;q23).⁷ This new case, along with the previous 4 Xp11 translocation RCC and 2 ASPS, were included in a new TMA. The new TMA also contained 32 nontranslocation renal

tumors: 20 cases of clear cell RCC, 5 cases of papillary RCC, 5 cases of chromophobe carcinomas, and 2 cases of oncocytoma. Among the nontranslocation renal tumors, 17 of the specimens with cytogenetic results showed intact X chromosome(s). In addition, all these nontranslocation tumors were negative for TFE3 IHC staining.

The FISH assay was applied to this TMA. The split signals were seen in all 5 Xp11 translocation RCC and 2 ASPS cases including the new case of RCC with t(X;3). No split signal was seen in the 25 nontranslocation tumors. The remaining 7 nontranslocation renal tumors failed to yield interpretable signal (due to poor FISH probes penetration, nuclear truncation, or cell overlapping). These results suggest that this FISH assay is highly sensitive and specific, if interpretable signals can be obtained.

DISCUSSION

Identification of specific genetic changes as a fingerprint in certain malignancies, such as lymphomas and sarcomas, has not only aided in diagnosis, but also has paved the way towards better therapeutic options. Even though solid tumors have lagged behind, in the last decade or so there has been a surge in the discovery of many consistent and reproducible genetic alterations in these tumors. Xp11.2 translocation carcinoma is recognized in the 2004 WHO renal tumor classification and is an uncommon tumor generally arising in children and young adults. The multiple fusions seen in these tumors include the more common ASPL-TFE3¹⁵ and PRCC-TFE3²³ and the less common PSF-TFE3, NonO-TFE3, and Clathrine-TFE3.^{5,11} Another rare group of renal carcinomas showing the translocation t(6; 11)(p21; q12) involving transcription factor EB (TFEB) has also been reported.^{4,6} Both TFE3 and TFEB belong to the microphthalmia transcription factor (MiTF) subfamily, which also includes MiTF and transcription factor EC. Argani and Ladanyi³ have proposed regrouping these neoplasms into the category of MiTF/TFE family translocation carcinomas. TFEB translocation RCC is beyond the scope of this study. The clinicopathologic features of the 4 cases of Xp11 translocation RCC in this study (Table 1) are consistent with the previous reported cases.^{7,10} The patients were 10 to 20 years younger than patients with clear cell and papillary RCC, the tumors were large (7.2–14 cm), and 3 out of the 4 patients presented with lymph node or distant metastases. Histologically, papillary, nested, and compact (solid) patterns of growth were seen, a mixture of large clear and eosinophilic cells was often present and by immunohistochemistry these tumors showed characteristically strong nuclear TFE3 staining.

A rare soft tissue tumor, ASPS, harbors the ASPL-TFE3 gene fusion. These tumors are most often seen in the deep soft tissues of the extremities and patients generally present with advanced disease. Histologically, the tumors show the classic alveolar growth pattern. ASPS tumors also display strong nuclear TFE3 IHC staining.¹² The cases studied here share these characteristic findings.

The diagnosis of Xp11 translocation carcinoma can be problematic in cases of atypical clinical presentation (such as an elderly patient), overlapping histopathology with clear cell and/or papillary RCC, or technical difficulty with TFE3 IHC staining. There are multiple reasons the TFE3 IHC stain can be technically difficult. First, the available antibody is

polyclonal and therefore shows some variation in quality from lot to lot. Second, the antibody has been shown to be fixation dependent.⁵ Last, the interpretation of the TFE3 IHC can be difficult as the difference between weak staining (negative result) and strong staining (positive result) is subjective. In these circumstances, cytogenetic examination of fresh tissue remains the gold standard if the tumor cells are dividing in the culture. When fresh tissue is unavailable, FISH and/or RT-PCR can be performed on FFPE tissues. Unfortunately, there can be problems associated with RT-PCR of archival tissue due to degradation of RNA, insufficient extraction efficiency and difficulty with the availability of adequate material in small biopsy samples. Additionally, Xp11 translocation RCC has at least 5 known fusion partners⁷ with TFE3 making RT-PCR time consuming and labor intensive. Potential false negative results are also possible due to potential unknown translocation(s) involving the TFE3 gene.⁷ In contrast to cytogenetics and RT-PCR, FISH represents a cost and time-efficient method that uses FFPE tissue. Aulmann et al⁹ has reported the feasibility of detecting the ASPS-TFE3 gene fusion in ASPS with both split and fusion probes. Their paper is the first development of a FISH assay for the detection of TFE3 gene translocation in paraffin-embedded tissues. The BAC clones for FISH probes in our study were similar but not identical to those used by Aulmann and colleagues. As we used the break-apart probe type FISH assay in this study, the fusion partner with TFE3 is not identified, but we can potentially detect all translocations involving TFE3. Currently, the clinical significance of different types of translocation Xp11 RCC is poorly characterized due to an insufficient number of published cases. The diagnosis of specific types of Xp11 translocation RCC, as opposed to simply the presence of TFE3 translocation, will not contribute to diagnosis or prognosis at the present time.

Because all the probes target the X chromosome, the patient's sex is important for FISH interpretation. The male patient should have 1 pair of signals (1 X chromosome) and the female patient should have 2 pairs of signals (2 X chromosomes). In rare situations, the additional gain or loss of an X chromosome (such as patient 4 with X chromosome loss) can occur. The FISH results in these cases would be extremely difficult to interpret if no karyotype is available.

The diagnosis of Xp11 translocation RCC and ASPS requires the incorporation of clinical information, histopathologic features, TFE3 IHC stain, cytogenetic, and molecular studies. We recommend that the FISH assay be reserved for use as a helpful ancillary technique in situations such as limited tissue for evaluation in cases of small biopsy/fine needle aspiration and cases of equivocal TFE3 IHC stain.

It has been known for sometime that tumors of different origins can harbor similar genetic events including translocations. In general, such neoplasms bear certain morphologic similarities; however, have varied biologic behavior depending upon the tissue of origin. Besides Xp11 translocation RCC and ASPS, perivascular epithelioid cell tumors (PEComas) have also shown immunoreactivity for TFE3. Folpe et al¹³ showed that 5 of 17 PEComas were TFE3 positive. Recently, a case of PEComa with PSF-TFE3 gene fusion proven by FISH and RT-PCR has been reported.²⁴ Interestingly, Argani et al⁸ reported a distinctive type of renal cancer with overlapping features of PEComa, Xp11 translocation carcinoma,

and melanoma. Our FISH assay will be an important tool to detect the translocation of Xp11 in this growing spectrum of tumors.

In our study, we found that TMA assembled from formalin-fixed tissue are a useful tool for evaluating immunohistochemical stains and for performing FISH. TMAs allow for evaluation of large numbers of cases and are cost-effective in that they require less probe and reagents, are less time consuming for the technologist, conserve tissue, and yield reproducible results. Since the incidence of Xp11 translocation RCC in adults remains unknown, we are now investigating larger numbers of cases by FISH and IHC on TMA to quantify the incidence of this unique subtype of RCC.

Many causal cancer genes have been identified through the discovery of recurrent chromosomal rearrangements and resulting gene fusions, and are characteristic in leukemias, lymphomas, and sarcomas.²⁰ The prototypic example is the rearrangement between chromosomes 9 and 22 that results in fusion of the break point cluster region gene, BCR, and the tyrosine kinase gene, ABL, and characterizes chronic myelogenous leukemia. This finding led to the development of imatinib (Gleevec), which inhibits the BCR-ABL gene fusion product and has revolutionized the treatment of chronic myelogenous leukemia.²² In contrast, only a few recurrent rearrangements have been identified in epithelial tumors (examples include TMPRSS2-ERG in prostatic adenocarcinoma and PAX8-PPARG in follicular thyroid carcinoma).

The biologic function of the TFE3 fusion protein is far from clear. Lardanyi et al²⁵ reported that TFE3 fusions activate MET signaling by transcriptional up-regulation, shedding light on the possibility of using the TFE3 fusion protein and/or signaling pathway as a therapeutic target for Xp11 translocation RCC and ASPS.

In this study, we developed a FISH assay to serve as a relatively quick test for detecting Xp11.2 translocations in cases of RCC and ASPS. This FISH assay can be used as an adjunct to morphology and immunohistochemistry to better identify TFE3-associated carcinomas and other neoplasms.

ACKNOWLEDGMENTS

The authors thank Frank Tian, Dana Settembre, and Taozhi Lin for technical supports.

Supported by NJMS pathology department resident research fund.

REFERENCES

1. Aisner SC, Beebe K, Blacksin M, et al. Primary alveolar soft part sarcoma of fibula demonstrating ASPL-TFE3 fusion: a case report and review of the literature. *Skeletal Radiol.* 2008;37:1047–1051. [PubMed: 18594808]
2. Altinok G, Kattar MM, Mohamed A, et al. Pediatric renal carcinoma associated with Xp11.2 translocations/TFE3 gene fusions and clinicopathologic associations. *Pediatr Dev Pathol.* 2005;8: 168–180. [PubMed: 15747097]
3. Argani P, Ladanyi M. Translocation carcinomas of the kidney. *Clin Lab Med.* 2005;25:363–378. [PubMed: 15848741]

4. 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]
5. Argani P, Lal P, Hutchinson B, et al. Aberrant nuclear immunoreactivity for TFE3 in neoplasms with TFE3 gene fusions: a sensitive and specific immunohistochemical assay. *Am J Surg Pathol.* 2003; 27:750–761. [PubMed: 12766578]
6. 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]
7. Argani P, Olgac S, Tickoo SK, et al. Xp11 translocation renal cell carcinoma in adults: expanded clinical, pathologic, and genetic spectrum. *Am J Surg Pathol.* 2007;31:1149–1160. [PubMed: 17667536]
8. Argani P, Aulmann S, Karanjawala Z, et al. Melanotic Xp11 translocation renal cancers: a distinctive neoplasm with overlapping features of PEComa, carcinoma, and melanoma. *Am J Surg Pathol.* 2009;33:609–619. [PubMed: 19065101]
9. Aulmann S, Longerich T, Schirmacher P, et al. Detection of the ASPSCR1-TFE3 gene fusion in paraffin-embedded alveolar soft part sarcomas. *Histopathology.* 2007;50:881–886. [PubMed: 17543078]
10. Camparo P, Vasiliu V, Molinie V, et al. Renal translocation carcinomas: clinicopathologic, immunohistochemical, and gene expression profiling analysis of 31 cases with a review of the literature. *Am J Surg Pathol.* 2008;32:656–670. [PubMed: 18344867]
11. Clark J, Lu YJ, Sidhar SK, et al. Fusion of splicing factor genes PSF and NonO (p54nrb) to the TFE3 gene in papillary renal cell carcinoma. *Oncogene.* 1997;15:2233–2239. [PubMed: 9393982]
12. Folpe AL, Deyrup AT. Alveolar soft-part sarcoma: a review and update. *J Clin Pathol.* 2006;59:1127–1132. [PubMed: 17071801]
13. Folpe AL, Mentzel T, Lehr HA, et al. Perivascular epithelioid cell neoplasms of soft tissue and gynecologic origin: a clinicopathologic study of 26 cases and review of the literature. *Am J Surg Pathol.* 2005;29:1558–1575. [PubMed: 16327428]
14. Gnarr JR, Duan DR, Weng Y, et al. Molecular cloning of the von Hippel-Lindau tumor suppressor gene and its role in renal carcinoma. *Biochim Biophys Acta.* 1996;1242:201–210. [PubMed: 8603073]
15. Heimann P, El Housni H, Ogur G, et al. Fusion of a novel gene, RCC17, to the TFE3 gene in t(X;17)(p11.2;q25.3)-bearing papillary renal cell carcinomas. *Cancer Res.* 2001;61:4130–4135. [PubMed: 11358836]
16. Kovacs G, Frisch S. Clonal chromosome abnormalities in tumor cells from patients with sporadic renal cell carcinomas. *Cancer Res.* 1989;49:651–659. [PubMed: 2910484]
17. Ladanyi M, Lui MY, Antonescu CR, et al. The der(17) t(X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. *Oncogene.* 2001;20:48–57. [PubMed: 11244503]
18. Linehan WM, Zbar B. Focus on kidney cancer. *Cancer Cell.* 2004; 6:223–228. [PubMed: 15380513]
19. Linehan WM, Pinto PA, Bratslavsky G, et al. Hereditary kidney cancer: unique opportunity for disease-based therapy. *Cancer.* 2009; 115:2252–2261. [PubMed: 19402075]
20. Mitelman F, Johansson B, Mertens F. The impact of translocations and gene fusions on cancer causation. *Nat Rev Cancer.* 2007;7: 233–245. [PubMed: 17361217]
21. Pavlovich CP, Walther MM, Eyler RA, et al. Renal tumors in the Birt-Hogg-Dube syndrome. *Am J Surg Pathol.* 2002;26: 1542–1552. [PubMed: 12459621]
22. Quintas-Cardama A, Kantarjian H, Cortes J. Imatinib and beyond-exploring the full potential of targeted therapy for CML. *Nat Rev Clin Oncol.* 2009;6:535–543. [PubMed: 19652654]
23. Shipley JM, Birdsall S, Clark J, et al. Mapping the X chromosome breakpoint in two papillary renal cell carcinoma cell lines with a t(X;1)(p11.2;q21.2) and the first report of a female case. *Cytogenet Cell Genet.* 1995;71:280–284. [PubMed: 7587394]

24. Tanaka M, Kato K, Gomi K, et al. Perivascular epithelioid cell tumor with SFPQ/PSF-TFE3 gene fusion in a patient with advanced neuroblastoma. *Am J Surg Pathol.* 2009;33:1416–1420. [PubMed: 19606011]
25. Tsuda M, Davis IJ, Argani P, et al. TFE3 fusions activate MET signaling by transcriptional up-regulation, defining another class of tumors as candidates for therapeutic MET inhibition. *Cancer Res.* 2007;67:919–929. [PubMed: 17283122]
26. Uppal S, Aviv H, Patterson F, et al. Alveolar soft part sarcoma–reciprocal translocation between chromosome 17q25 and Xp11. Report of a case with metastases at presentation and review of the literature. *Acta Orthop Belg.* 2003;69:182–187. [PubMed: 12769020]
27. Vocke CD, Yang Y, Pavlovich CP, et al. High frequency of somatic frameshift BHD gene mutations in Birt-Hogg-Dube-associated renal tumors. *J Natl Cancer Inst.* 2005;97:931–935. [PubMed: 15956655]
28. Winarti NW, Argani P, De Marzo AM, et al. Pediatric renal cell carcinoma associated with Xp11.2 translocation/TFE3 gene fusion. *Int J Surg Pathol.* 2008;16:66–72. [PubMed: 18203790]

FIGURE 1.

H&E of Xp11 translocation RCC cases 2 (A, B) and 4 (D, E). The tumors had solid, nested, pseudopapillary, and microcystic architecture with geographic necrosis. The tumor cells were large with discrete cell borders, granular eosinophilic to clear cytoplasm, vesicular nuclear chromatin, and prominent nucleoli. H&E of ASPS case 6 (G, H) showed classic alveolar growth pattern. The tumor cells were large and discohesive with granular eosinophilic to clear cytoplasm, vesicular nuclear chromatin, and prominent nucleoli. TFE3 IHC on case 2 (C), case 4 (F) and case 6 (I) showed strong nuclear staining. ASPS indicates alveolar soft part sarcoma; H&E, hematoxylin and eosin; IHC, immunohistochemistry; RCC, renal cell carcinoma.

FIGURE 2.

Karyotype from patient 4 showing the $\text{der(X)t(X;1)(p11;p34)}$ with additional chromosomal abnormalities including $+3, -4, -9, -14, -21, -22$, and -22 . The arrow points to t(X;1)(p11;p34) .

FIGURE 3.

A, The binding loci for the break-apart probes in relation to the TFE3 gene on chromosome X. Distances are measured in kilobases and are approximate. The FISH probes were tested on normal female peripheral blood cells. A, In the metaphase cell, 2 sets of green and red fusion signals are located on X chromosome. B, In the interphase cell, 2 sets of green and red fusion signals were detected. FISH indicates fluorescence in situ hybridization.

FIGURE 4.

FISH was performed on the same GTG-banded positive for Xp11 translocation metaphase preparation on metaphase from patient 4 (A), which showed 1 fused signal on X chromosome and 1 green signal on chromosome 1. The second red signal was lost due to der(X). Interphase from patient 3 (B), which showed 1 set of fusion signal and 1 set of green and red split signal. FISH indicates fluorescence in situ hybridization.

FIGURE 5.

FISH was performed on the formalin-fixed, paraffin-embedded (FFPE) tissues. A, Normal female's kidney tissue, 2 fusion signals present. B, Normal male's kidney tissue, 1 fusion signal present. C, Representative image from patient 2, 1 fusion signal, and 1 pair of green and red split signal present. D, Image from patient 4, which showed a fusion signal and a single green signal. The second red signal was lost due to der(X). FISH indicates fluorescence in situ hybridization.

FIGURE 6.

A, TFE3 IHC on UOK109 cell showed strong nuclear staining which is consistent with the presence of Xp11 translocation in the cells. FISH was performed on UOK109 cells. B, Metaphase showing X chromosome inversion, and C, interphase showing split green and red signal. FISH indicates fluorescence in situ hybridization; IHC, immunohistochemistry.

TABLE 1.

Clinicopathologic Information of Xp11 Translocation Renal Cell Carcinoma and Alveolar Soft Part Sarcoma

	Age/Sex	Location	Size (cm)	Initial Presentation	Metastases	Follow-up (mo)	Status	Cytogenetic or RT-PCR Result
1 RCC	38/F	Left kidney	14	Asymptomatic, picked up by CT due to gastric bypass work up	Abdomen omentum	21	Alive	+7, +12, +16, +20 <i>ASPL-TFE3</i> Type I
2 RCC	65/F	Right kidney	9.5	Asymptomatic	Periaortic lymph node	19	Alive	Cytogenetic N/A <i>ASPL-TFE3</i> Type I
3 RCC	42/F	Right kidney	11.5	Picked up by CT due to incarcerated ventral hernia	No	42	Alive	+7, t(X;1)(p11;p34)
4 RCC	45/F	Right kidney	7.2	Pathologic fracture	Bone, liver	5	Dead	der(X)t(X;1)(p11;p34) <i>PSF-TFE3</i>
5 ASPS	31/F	Right forearm	7.5	Mass	Bilateral lung, pancreas	> 50	Dead	t(X;17)(p11;q25)
6 ASPS	41/F	Left fibula	12	Pathologic fracture	No	11	Dead	<i>ASPL-TFE3</i> Type I
7 UOK109	39/M	N/A	N/A	N/A	Lymph node	N/A	N/A	inv(X)(p11.2;q12) <i>NonO-TFE3</i>

Cases 5²⁶ and 6¹ have been reported previously. The UOK109 cell line was derived from a male patient's renal cell carcinoma, which carries inv(X)(p11;q12).¹¹ ASPS indicates alveolar soft part carcinoma; CT, computed tomography; RCC, renal cell carcinoma.