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Original contribution

Immunohistochemistry for TFE3 lacks specificity and sensitivity in the diagnosis of *TFE3*-rearranged neoplasms: a comparative, 2-laboratory study☆,☆☆

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Summary *TFE3* rearrangements are characteristic of alveolar soft part sarcomas (ASPS), Xp11.2 translocation renal cell carcinomas (Xp11-RCC), and other rare tumors. Immunohistochemistry for TFE3 protein has been considered by some to be a reliable surrogate for *TFE3* molecular studies, although others disagree. We compared 2 methods for TFE3 immunohistochemistry to determine if technical differences underlie these differences. Ninety-eight archival cases of mixed type, 19 ASPS, and 8 Xp11-RCC were stained for TFE3 at Laboratory A and Laboratory B using routine protocols. Positive controls were normal human testis (Laboratory A) and Xp11-RCC (Laboratory B). Nuclear staining was scored as "negative," "1+" (<10%), "2+" (10%-50%), and "3+" (>50%). Intensity was scored as "negative," "weak," "moderate," or "strong." Only moderate-strong, 2+ or 3+ staining was considered positive. Laboratory A results were as follows: archival cases (42 of 98, 43%), ASPS (16 of 19, 84%), and Xp11-RCC (7 of 8, 88%). Laboratory B results were as follows: archival cases (5 of 98, 5%), ASPS (14 of 19, 74%), and Xp11-RCC (5 of 8, 63%). *TFE3* fluorescence in situ hybridization was positive in all tested ASPS and Xp11-RCC. The overall sensitivity and specificity of TFE3 immunohistochemistry for *TFE3*-rearranged neoplasms were 85% (23/27) and 57% (56/98) at Laboratory A and 70% (19/27) and 95% (93/98) at Laboratory B. Technical differences, in particular, the type of control tissue, likely account for these different results. The results of our study and prior studies suggest that TFE3 immunohistochemistry should play only a minor role (if any) in the diagnosis of *TFE3*-rearranged tumors, with fluorescence in situ hybridization representing the preferred method.

1. Introduction

Rearrangements of the *TFE3* (transcription factor binding to IGHM enhancer 3) gene, a member of the microphthalmia-associated transcription factor family along with *TFEB*,

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Table 1 Immunohistochemical methods

	Laboratory A	Laboratory B
Positive control	Normal testis	Xpll-RCC
Clone	MRQ-37	MRQ-37
Source	Ventana Medical Systems, Tucson, AZ	Cell Marque, Rocklin, CA
Dilution	Predilute	1:200
Antibody incubation	16 min at 37°C	30 min at 37°C
Heat-induced epitope retrieval	32 min at 97°C with Tris buffer (pH 8-8.5)	30 min at 99°C with Tris buffer (pH 10)
Detection system	Ventana Optiview	Thermo Scientific Quanto

TFEC, and *MiTF* [1,2], have been implicated in the pathogenesis of a number of human neoplasms, including alveolar soft part sarcoma [3], Xp11.2 translocation renal cell carcinomas of conventional and melanotic type [4,5], subsets of perivascular epithelioid cell tumors (PEComas) [6-10], and a rare vascular tumor showing some features of epithelioid hemangioendothelioma [11]. *TFE3* rearrangements in these tumors are detectable by fluorescence in situ hybridization (FISH) in formalin-fixed, paraffin-embedded tissue sections [12].

However, as FISH for *TFE3* rearrangement is not available in all laboratories and may occasionally be compromised by specimen fixation, there has been continued interest in the application of immunohistochemistry for the detection of TFE3 protein overexpression as a surrogate for this genetic event. Initial studies of TFE3 immunohistochemistry suggested that it was a sensitive and specific substitute for FISH, with one early study by Argani et al noting strong nuclear TFE3 immunoreactivity in >95% of *TFE3*-rearranged alveolar soft part sarcomas and renal cell carcinomas as compared with only <0.05% of other neoplasms [13]. More recently, however, a number of studies have noted discordance between *TFE3* FISH and TFE3 immunohistochemical results, including a study of epithelioid hemangioendotheliomas showing TFE3 immunoreactivity in 21 of 24 cases, only 2 of which harbored *TFE3* rearrangements [14]. Anecdotally, we have also noted an imperfect correlation between TFE3 FISH and immunohistochemistry in our own practices.

We hypothesized that technical differences might underlie the disparate TFE3 results reported by different investigators. For this reason, we evaluated TFE3 expression in a series of tumors, both *TFE3*-rearranged and not, in 2 different laboratories that used different methodologies.

2. Materials and methods

2.1. Case selection

Approval for this study was granted by the Mayo Clinic Institutional Review Board. One hundred consecutive cases having readily available unstained slides were retrieved from the consultation archives of one of the authors (A. L. F.). These cases included a variety of benign and malignant mesenchymal and nonmesenchymal tumors, although mesenchymal tumors comprised the majority owing to the nature of this consultation practice. Following immunohistochemistry study (see below), 2 cases were felt to lack sufficient evaluable tumor and were excluded. Thus, this group ultimately consisted of 98 cases. We also searched our institutional and consultation archives for the period of 1995-2016 for tumors with known *TFE3* rearrangements and identified 19 cases of alveolar soft part sarcoma (11 internal, 8 from outside institutions) and 8 examples of Xp11–renal cell carcinoma with available unstained slides and/or tissue blocks.

2.2. Immunohistochemistry

Immunohistochemistry for TFE3 was performed on formalin-fixed, paraffin-embedded tissue sections using

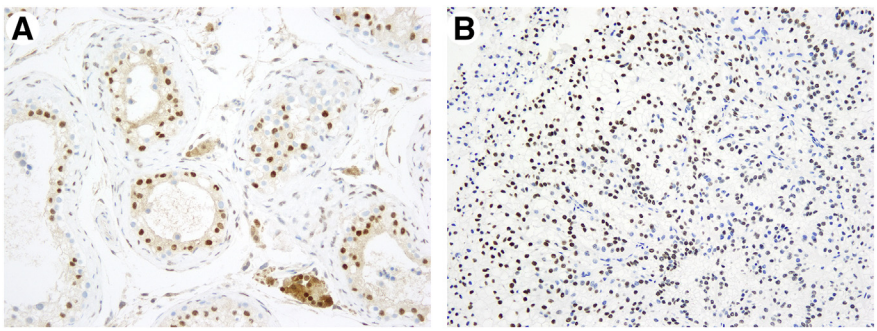

Fig. 1 The positive controls for TFE3 were normal human testis at Laboratory A (A) and an Xp11–renal cell carcinoma at Laboratory B (B) (A and B: hematoxylin and eosin [H&E], original magnification ×200).

Table 2 TFE3 immunohistochemical results

Tumor type	n	Laboratory A	Laboratory B
Epithelial tumors			
Adenocarcinoma (lung, gastrointestinal/pancreas, gynecologic, and breast origins)	8	3 (all 2+)	1 (2+)
Myoepithelial carcinoma	2	1 (3+)	0
Neuroendocrine carcinoma	1	0	0
Sarcomatoid mammary carcinoma	1	0 (1+: 1)	0
Squamous cell carcinoma	1	0	0
Urothelial carcinoma	1	1 (2+)	0
Mesenchymal tumors			
Angiomatoid fibrous histiocytoma	1	1 (3+)	1 (2+)
Angiosarcoma	1	0 (1+[a])	0
Atypical fibroxanthoma	2	2 (both 2+)	0
Benign fibrous histiocytoma	2	0	0
Capillary hemangioma	1	0	0
Cellular angiofibroma	2	1 (2+)	0
Cellular neurothekeoma	2	1 (3+)	0
Chondroma	2	0	0
Clear cell sarcoma	1	0	0
Dedifferentiated liposarcoma	1	1 (2+)	0
Desmoplastic small round cell tumor	1	0	0
Desmoid-type fibromatosis	1	0	0
Epithelioid hemangioma	1	0	0
Epithelioid hemangioendothelioma	1	1 (2+)	0
Epithelioid sarcoma	2	1 (2+)	0
Ewing sarcoma	2	0	0
Extraskeletal myxoid chondrosarcoma	1	0	0
Epithelioid sarcoma-like/pseudomyogenic hemangioendothelioma	1	0	0
Fibrosarcoma arising in dermatofibrosarcoma protruberans	2	1 (2+)	0
Gastrointestinal stromal tumor	1	1 (2+)	0
Granular cell tumor	1	1 (3+)	1 (2+)
Leiomyosarcoma	1	1 (3+)	1 (2+)
Low-grade fibromyxoid sarcoma	1	0 (1+[a])	0
Malignant glomus tumor	1	0	0
Malignant peripheral nerve sheath tumor, epithelioid type	1	1 (2+)	0
Malignant PEComa	1	1 (2+)	0
Malignant solitary fibrous tumor	1	1 (3+)	0
Myofibroblastic sarcoma	2	2 (3+: 1; 2+: 1)	0
Myxofibrosarcoma	3	2 (both 3+)	0
Mammary-type myofibroblastoma	1	1 (3+)	1 (2+)
Myofibroma	1	0 (1+[a])	0
Osteosarcoma	1	0	0
Pleomorphic liposarcoma	1	0	0
PEComa	1	0	0
Perineurioma	1	0	0
Pleomorphic hyalinizing angiectatic tumor	2	0 (1+:1)	0
Rhabdomyosarcoma	3	1 (3+)	0
Synovial sarcoma	1	0	0
Solitary fibrous tumor	4	0 (1+: 1[a])	0
Spindle cell/pleomorphic lipoma	1	1 (3+)	0
Superficial acral fibromyxoma	1	0	0
Tenosynovial giant cell tumor	1	1 (3+)	0
Undifferentiated pleomorphic sarcoma	12	10 (3+: 4; 2+: 6; 1+: 1)	0
Well-differentiated liposarcoma	1	0	0
Miscellaneous			
Diffuse large B-cell lymphoma	1	0 (1+[a])	0
Langerhans cell histiocytosis	2	0	0
Malignant melanoma	6	2 (3+: 1; 2+:1)	0
Ovarian fibroma	1	1 (3+)	0

(*continued on next page*)

Table 2 (*continued*)

Tumor type	n	Laboratory A	Laboratory B
Spindle epithelial tumor with thymus-like differentiation	1	0	0
Thymoma	1	1 (3+)	0

[a] Cases showing only "1+" staining were scored as "negative." All positive cases showed moderate or strong staining intensity.

commercially available antibodies at 2 different reference laboratories (referred to hereafter as *Laboratory A* and *Laboratory B*). Table 1 summarizes the clones, dilutions, epitope retrieval methods, detection systems, and control tissues used at both laboratories. At Laboratory A, the TFE3 antibody had originally been titrated on non-neoplastic human testis, which was used as the positive control (Fig. 1A). At Laboratory B, an Xp11–renal cell carcinoma was used for the initial titration and as a positive control (Fig. 1B).

The immunohistochemistry studies were evaluated and graded by 2 independent reviewers (R. F. S. and A. L. F.), and any disagreements were resolved by consensus review. The percentage of positive tumor cell nuclei was initially scored as "negative," "1+" (<10% positive cells), "2+" (10%-50% positive cells), and "3+" (>50% positive cells). For final analysis, however, only cases showing 2+ or 3+ immunoreactivity were considered to be positive. Similarly, the intensity of nuclear staining was graded as "negative," "weak," "moderate," or "strong" for all cases; however, only moderate and strong staining intensity was considered to be positive in keeping with the original scoring system of Argani et al [13]. Moderate and strong nuclear immunoreactivity was readily

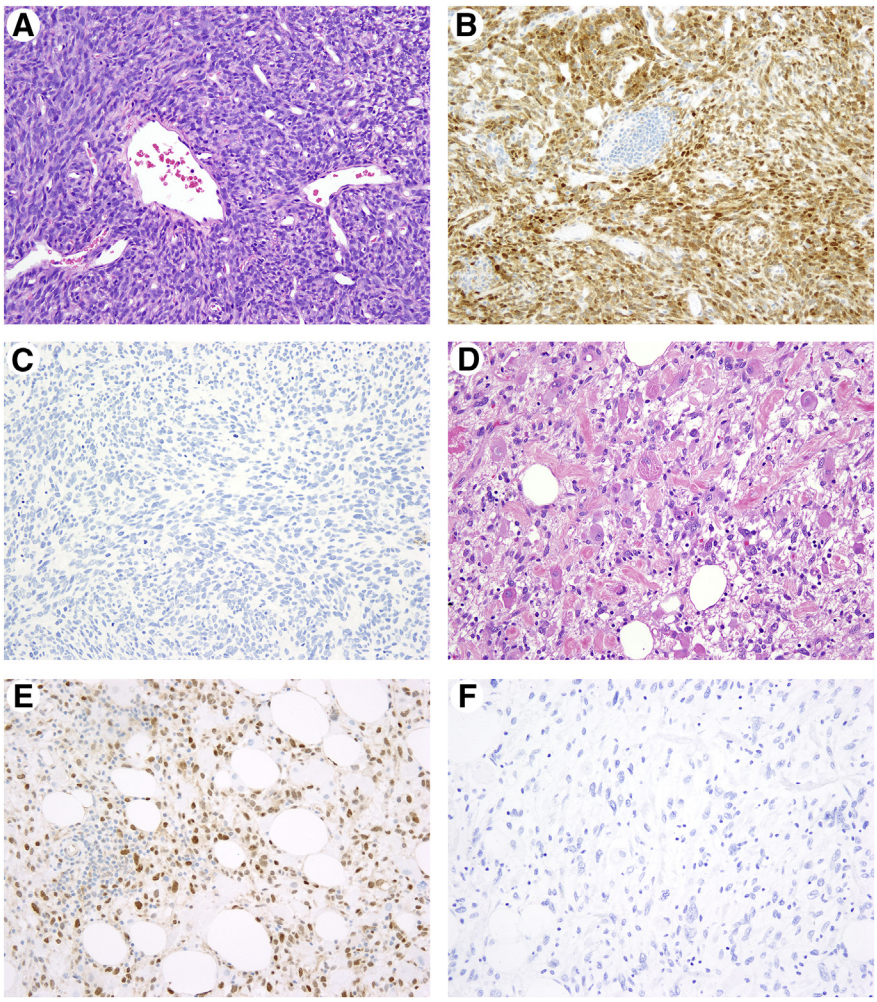

Fig. 2 A, Solitary fibrous tumor, strongly positive for TFE3 by immunohistochemistry in Laboratory A (B) but negative in Laboratory B (C). Although some nonspecific cytoplasmic staining is present in the tumor, non-neoplastic internal controls (lymphocytes and blood vessels) are entirely negative. D, This embryonal rhabdomyosarcoma was also strongly positive for TFE3 in a nuclear pattern in Laboratory A (E) but negative in Laboratory B (F) (A and D: H&E ×200; B, C, E, and F: immunohistochemistry [IHC] ×200).

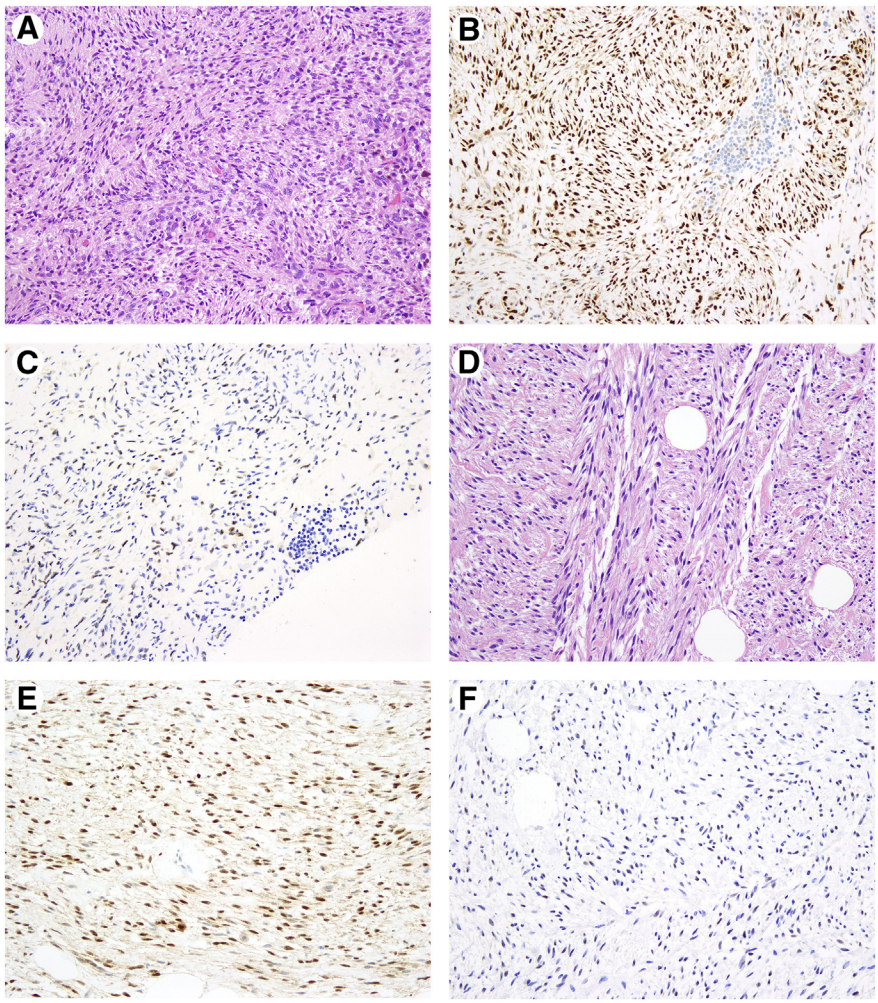

Fig. 3 Five cases were positive for TFE3 in both laboratories, including this angiomatoid fibrous histiocytoma (A) and mammary-type myofibroblastoma (D). Nuclear immunoreactivity for TFE3 was typically more intense in cases stained in Laboratory A (B and E) but was also present to a lesser degree in Laboratory B (C and F) (A and D: H&E ×200; B, C, E, and F: IHC ×200).

apparent at low-power magnification (4×), whereas weak staining intensity required higher-power magnification to be detected. Only nuclear immunoreactivity was considered "positive." Cytoplasmic immunoreactivity was disregarded unless nuclear immunoreactivity in the same cells was more intense.

2.3. Fluorescence in situ hybridization

FISH analysis for *TFE3* rearrangement was performed on unstained slides from 4 alveolar soft part sarcomas and 8 Xp11–renal cell carcinoma using an institutionally developed and validated breakapart probe assay and previously published methods [15,16]. Briefly, direct-labeled probes designed to flank the *TFE3* gene (at Xp11.2) were developed using bacterial artificial chromosomes which were selected using the University of California Santa Cruz Biotechnology Genome Browser and Database (http://genome.ucsc.edu, genome assembly hg18) and then obtained from Invitrogen (Carlsbad, CA). The *TFE3* hybridization set consisted of a 3′ telomeric probe detected by RP11-416B14 and RP11-1137J13 and labeled by SpectrumGreen (Abbott Molecular, Des Plaines, IL, 60018) and a 5′ centromeric probe detected by RP11-1037C20 and RP11-315L18 and labeled by SpectrumOrange (Abbott Molecular). A commercially available chromosome X centromere probe (DXZ1), labeled in SpectrumAqua (Abbott Molecular), was also included as a control. In a female cell, a pattern of 2 yellow fusion signals was indicative of an intact *TFE3* gene on both X chromosomes, and in normal male cells, only 1 yellow fusion signal was present. In contrast, although a variety of signal patterns are assessed for, a typical translocation of 1 *TFE3* locus would disrupt the fusion signal, resulting in spatially separated red/orange and green signals [16]. Male cases were scored as positive if at least 20% of 100 scored nuclei showed a split signal pattern, and female cases were considered positive if at least 11% of 100 scored nuclei showed a split signal pattern. All FISH pattern cutoffs incorporate cut artifact inherent to paraffin tissue section analysis.

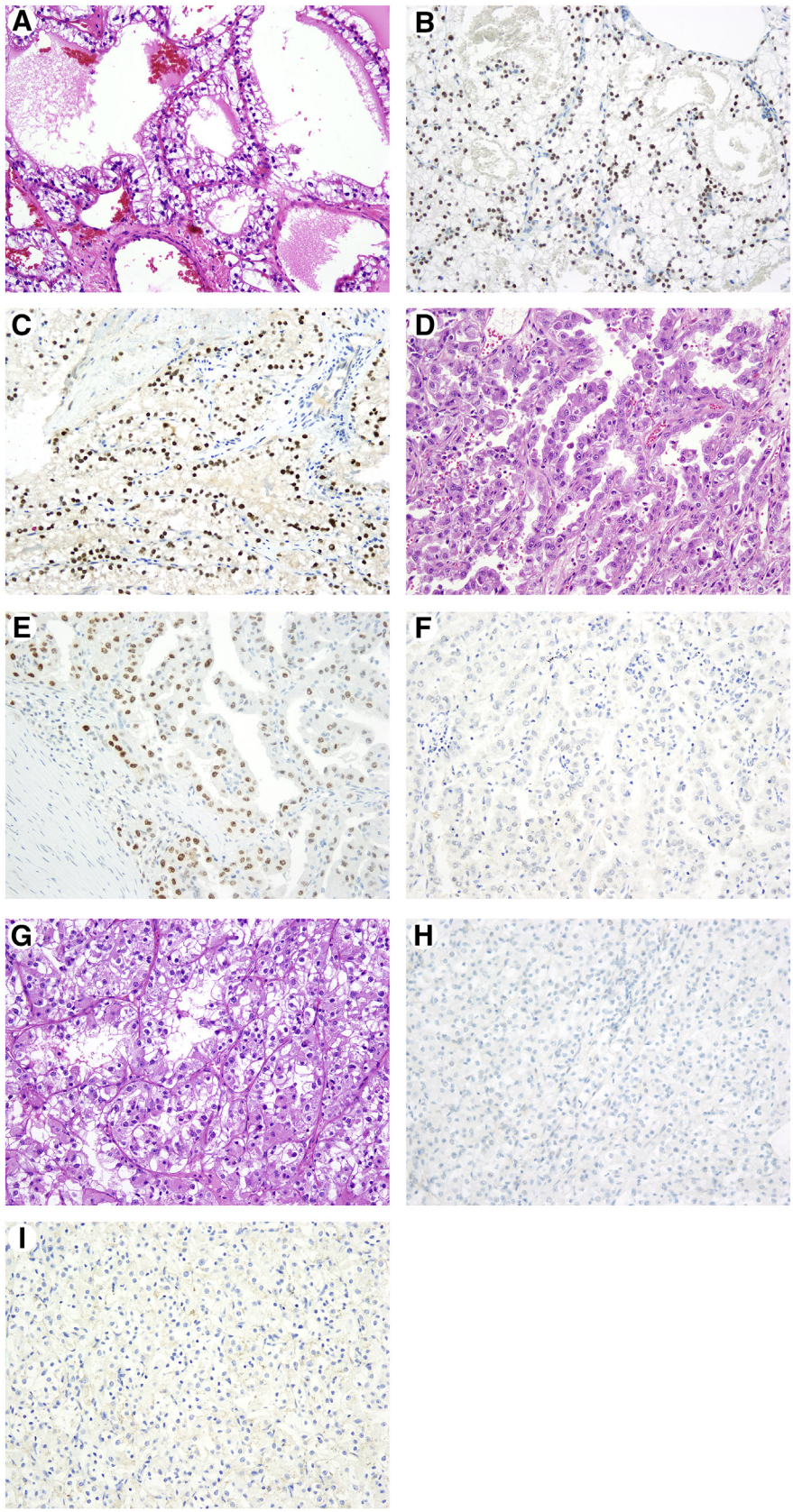

Fig. 4 A-C, The majority of molecularly confirmed Xp11–renal cell carcinomas were positive for TFE3 in both laboratories, as would be expected. D, However, 1 Xp11–renal cell carcinoma was positive only in Laboratory A (E) but not in Laboratory B (F). G, One other Xp11–renal cell carcinoma was negative in both laboratories (H and I); this case contained the lowest percentage of *TFE3*-rearranged nuclei (27%) among these tumors (A, D, and G: H&E ×200; B, C, E, F, H, and I: IHC ×200).

3. Results

3.1. Immunohistochemistry

Table 2 summarizes the immunohistochemistry results from both laboratories.

3.1.1. Tumors without known *TFE3* rearrangements

At Laboratory A, 42 of 98 (43%) cases were TFE3-positive (2+: 22; 3+: 20; moderate intensity: 21; strong intensity: 21) (Fig. 2A, B, D, and E). Eight cases showed 1+ staining but were scored as "negative." In contrast, only 5 of 98 (5%) cases were TFE3-positive at Laboratory B (2+: 5; moderate intensity: 4; strong intensity: 1) (Fig. 2C and F). These 5 cases were also positive at Laboratory A and included examples of granular cell tumor, angiomatoid fibrous histiocytoma (Fig. 3A-C), leiomyosarcoma, pancreatic adenocarcinoma, and mammary-type myofibroblastoma (Fig. 3D-F).

3.1.2. Tumors with known *TFE3* rearrangements

Sixteen of 19 alveolar soft part sarcomas (84%) were TFE3-positive at Laboratory A (2+: 1; 3+: 15; moderate intensity: 4; strong intensity: 12), and 14 of 19 cases (74%) were TFE3-positive at Laboratory B (2+: 4; 3+: 10; moderate intensity: 6; strong intensity: 8) (Fig. 4). The TFE3-negative cases were all consultation cases, suggesting that these may have represented false negatives secondary to fixation or processing.

Seven of 8 (88%) Xp11–renal cell carcinoma cases were TFE3-positive at Laboratory A (2+: 1; 3+: 6; moderate intensity: 2; strong intensity: 5); 5 of 8 cases (63%) were positive at Laboratory B (2+: 1; 3+: 4; moderate intensity: 2; strong intensity: 3) (Fig. 4A-F). One Xp11–renal cell carcinoma was TFE3-negative at both laboratories (Fig. 4G-I); this case was also originally a consultation case.

The overall sensitivity and specificity of TFE3 immunohistochemistry for *TFE3*-rearranged neoplasms were 85% (23/27) and 57% (56/98) at Laboratory A and 70% (19/27) and 95% (93/98) at Laboratory B.

3.2. Fluorescence in situ hybridization

The presence of *TFE3* rearrangements was confirmed in 4 of 4 tested alveolar soft part sarcomas and 8 of 8 tested Xp11–renal cell carcinomas. All but 1e of these cases (an Xp11–renal cell carcinoma) was strongly TFE3-positive by immunohistochemistry. Interestingly, this negative case contained the lowest percentage of *TFE3*-rearranged nuclei (27%) among the Xp11–renal cell carcinomas.

4. Discussion

TFE3 is a constitutively and ubiquitously expressed transcription factor that is a member of the *MiTF/TFE* subfamily

Table 3 Previous studies of TFE3 immunohistochemistry in tumor types not known to be associated with *TFE3* rearrangement

Study	Tumor type(s)	TFE3 immunohistochemistry	*TFE3* molecular genetic studies
Argani et al [13]	Adrenal cortical carcinoma	2/60	N/A
	Distal bile duct carcinoma	1/15	N/A
	Granular cell tumor	2/8	N/A
	High-grade myxofibrosarcoma	1/2	N/A
Williams et al [51]	Granular cell tumor	4/4	0/4 RT-PCR [a]
	Adrenal cortical carcinoma	1/1	0/1 RT-PCR [a]
Chamberlain et al [34]	Granular cell tumor	10/11	N/A
Schoolmeester and Lastra [52]	Granular cell tumor	6/6	0/6 FISH
Rekhi et al [53]	Paraganglioma	3/4	N/A
	Adrenocortical carcinoma	1/3	N/A
	Granular cell tumor	1/3	N/A
Tsuji et al [54]	Granular cell tumor	2/5	0/5 RT-PCR [a]
Dickson et al [36]	Melanoma	10/16	N/A
	Clear cell sarcoma	6/9	N/A
Park and Kim [37]	Ovarian sclerosing stromal tumor	7/9	N/A
Harrison et al [32]	Solid-pseudopapillary neoplasm of pancreas	30/31	0/17 FISH
	Pancreatic neuroendocrine tumor	3/26	N/A
Foo et al [38]	Solid-pseudopapillary neoplasm of pancreas	9/13	N/A
Jiang et al [33]	Solid-pseudopapillary neoplasm of pancreas	71/75	N/A
	Pancreatic neuroendocrine tumor	4/17	N/A
	Pancreatic ductal adenocarcinoma	2/14	N/A
	Pancreatic neuroendocrine carcinoma	1/4	N/A

Abbreviations: N/A, not applicable; RT-PCR, reverse-transcription polymerase chain reaction.
[a] Only *ASPSCR1-TFE3* fusion transcripts were evaluated in the included studies.

of transcription factors, along with *MiTF*, *TFEB*, and *TFEC*. This family of closely related helix-loop-helix leucine zipper transcription factors binds DNA as homo- and heterodimers that promote the expression of a wide array of genes involved in cell growth and proliferation [1,2,13,17,18]. *TFE3* and *TFEB* are involved in the gene fusions present in "MiT family translocation renal cell carcinoma", with *TFE3* rearrangements in Xp11–renal cell carcinoma and *TFEB* rearrangement in t(6;11) renal cell carcinoma [19,20]. Multiple *TFE3* fusion partner genes have been identified in Xp11–renal cell carcinoma, including *ASPSCR1*, *PRCC*, *SFPQ, NonO*, *CLTC*, *PARP14*, *LUC7L3*, *DVL2*, *KHSRP*, and *RBM10* [21-30]. The *ASPSCR1-TFE3* fusion is an unbalanced translocation which is also found in alveolar soft part sarcoma [24].

The TFE3 antibody used in this and other studies is directed against a C-terminus binding site believed to be retained in all known TFE3 fusion proteins in Xp11–renal cell carcinoma and alveolar soft part sarcoma [13,19,28,31]. Although this antibody was originally believed to be very sensitive and specific for *TFE3*-rearranged neoplasms [13,26], it has become increasingly clear over time that this is not the case [6,12,14,32-43]. As detailed in Table 3, TFE3 immunoreactivity has been reported by others in a variety of tumor types not known to harbor *TFE3* rearrangements.

Although TFE3 immunoreactivity in various epithelial and mesenchymal tumors not known to harbor *TFE3* rearrangements is arguably of questionable significance, the specificity of TFE3 immunohistochemistry is of greater importance in the evaluation of tumor types where *TFE3*-rearrangement defines specific variants of potential clinical significance (eg, Xp11–renal cell carcinoma, *YAP1-TFE3* epithelioid hemangioendothelioma, *TFE3*-rearranged perivascular epithelioid cell neoplasms). Even among these tumor types, evidence would seem to suggest that TFE3 immunohistochemistry is a less than perfect surrogate for molecular techniques. For example, TFE3 expression has been shown to be present in a considerable percentage of perivascular epithelioid cell neoplasms of various types, the great majority of which lack *TFE3* rearrangements by molecular methods [6,36,39,44-46]. Perhaps the best of these studies is that of Argani et al, who showed TFE3 immunoreactivity in 8 (manual staining with overnight incubation) and 13 (automated staining) of 28 PEComa cases, only 4 of which demonstrated *TFE3* rearrangement at the molecular level [6]. Similarly, Flucke and colleagues have shown many conventional epithelioid hemangioendotheliomas (*WWTR1-CAMTA1* type) to be TFE3-positive by immunohistochemistry, obviously complicating their distinction from the much rarer *YAP1-TFE3*–harboring hemangioendotheliomas [14]. Finally, TFE3 expression by immunohistochemistry has been demonstrated in many renal cell carcinomas lacking *TFE3* rearrangements, strongly suggesting that TFE3 immunohistochemistry is not of value in the distinction of conventional tumors from Xp11–renal cell carcinoma [35,41,47,48]. It has also been suggested that TFE3 immunohistochemistry is an imperfectly *sensitive* assay, with negative, weak/equivocal, or very limited staining in molecularly proven Xp11–renal cell carcinomas [12,40,42,43].

The results of the present study confirm and expand upon these earlier studies, with TFE3 immunoreactivity found in 43% of tested cases in one laboratory (specificity 57%) and 5% of tested cases in the other (specificity 95%). As TFE3 is ubiquitously expressed, it is perhaps not unexpected that TFE3 immunoreactivity might be found in neoplasms lacking *TFE3* rearrangements. Although it is possible that technical differences (eg, epitope retrieval techniques, differences in antibody concentration, or differences in detection systems) might explain the strikingly different findings in these 2 laboratories, the techniques used in both laboratories were generally quite similar, as shown in Table 1. It has been suggested by Argani and colleagues that technical differences may significantly impact TFE3 immunostains, with automated assays having greater sensitivity and lesser specificity than manual overnight incubation, likely reflecting enhanced detection of native TFE3 protein [6].

We strongly suspect that the critical laboratory-dependent difference in the present study is the use of different positive control tissues for the initial titrations of the antibodies, with non-neoplastic testicular germ cells and Leydig cells serving as the positive control in Laboratory A as compared to an Xp11–renal cell carcinoma in Laboratory B (Fig. 1). We hypothesize that this Xp11–renal cell carcinoma expresses higher levels of those epitopes recognized by the MRQ-37 clone than do normal tissues such that a lower antibody concentration is required to detect TFE3 in these tumors. This has been shown to be in true in *TFEB*-rearranged renal cell carcinoma, where Kuiper and colleagues have demonstrated TFEB protein levels to be dramatically increased as compared with normal kidney tissues [49]. Assuming an analogous situation exists in *TFE3*-rearranged carcinomas, it would be expected that use of such tumors as controls would result in an assay with greater specificity for *TFE3* rearrangement but lesser sensitivity for the presence of native TFE3 protein. Indeed, although the specificity of *TFE3* immunohistochemistry for *TFE3*-rearranged tumors at Laboratory B (95%) was very similar to that previously reported by Argani et al [13], the sensitivity (70%) was considerably lower. It is also possible that variable specimen fixation had some impact on the sensitivity of our assays, as most of these tissues were from consultation cases. Regrettably, we cannot directly compare the antibody concentrations between the 2 laboratories, as prediluted MRQ-37 antibody was used in Laboratory A.

Given the findings of the present and previous studies, one might justifiably ask whether there is any real role for TFE3 immunohistochemistry in the diagnosis of tumors with known *TFE3* rearrangements or in the potential identification of new tumor types showing this molecular event. Arguably, if one follows the "Laboratory B protocol" (ie, use of Xp11–renal cell carcinoma for antibody titration), TFE3 immunohistochemistry alone could be sufficient for the confirmation of tumor types showing appropriate morphology for a known *TFE3*-rearranged neoplasm, such as alveolar soft part

sarcoma. However, as should be obvious, use of the "Laboratory B protocol" almost certainly presupposes the availability of molecular techniques to prove *TFE3* rearrangement (and access to tissue from very rare tumor types to use as controls). This again raises the question of whether TFE3 immunohistochemistry is needed at all, especially given the ever-increasing availability of various molecular techniques even in community practice settings and the declining cost of these tests. FISH has been previously shown to be a highly sensitive and specific test for *TFE3* rearrangements [12,16,35,42,50], with only exceedingly rare cases of Xp11–renal cell carcinoma associated with the cryptic intrachromosomal Xp11.2 inversion (*RBM10-TFE3* gene fusion) resulting in false-negative tests [28]. TFE3 immunohistochemistry using the "Laboratory A protocol" would seem to be of little value in any setting, as it lacks sufficient specificity.

In conclusion, the current study, in combination with the previous literature, strongly suggests that TFE3 immunohistochemistry is neither a reliable screening test nor a dependable surrogate for molecular genetic studies to demonstrate*TFE3* gene rearrangement. Although there might potentially be some role for TFE3 immunohistochemistry in laboratories having access to known Xp11–renal cell carcinoma as control tissues, it is difficult to see any significant advantages to this test over FISH for *TFE3* rearrangement. Certainly, we would urge molecular genetic confirmation of most if not all TFE3-positive cases before making a definitive diagnosis of a *TFE3*-rearranged neoplasm, especially when the clinical setting or morphologic features are in any way atypical.

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