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Pseudosarcomatous Myofibroblastic Proliferations of the Genitourinary Tract are Genetically Different from Nodular Fasciitis and Lack *USP6*, *ROS1*, and *ETV6* Gene Rearrangements

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Abstract

Aims: Pseudosarcomatous myofibroblastic proliferations of the genitourinary tract have a debatable relationship to inflammatory myofibroblastic tumour (generally lacking *ALK* rearrangement); however, they share several overlapping features with nodular fasciitis of soft tissue. Since rearrangement of the *USP6* gene has been recently recognized as a recurrent alteration in soft tissue nodular fasciitis, and several other alternative gene fusions have been recently recognized in inflammatory myofibroblastic tumour, we studied whether *USP6*, *ROS1,* or *ETV6* gene rearrangements were present in these lesions (12 cases).

Methods and Results: Fluorescence in-situ hybridization (FISH) analysis was performed using bacterial artificial chromosome (BAC) derived break-apart probes against *USP6*, *ROS1,* and *ETV6*. Two cases with adequate genetic material from recent paraffin tissue blocks were also tested by using a solid tumour gene fusion detection assay via next-generation sequencing, targeting over 50 known genes involved in recurrent fusions. None of the genitourinary pseudosarcomatous myofibroblastic proliferations was detected to harbour *USP6* (0/12), *ROS1* (0/8)*,* or *ETV6* (0/7) rearrangement and no gene fusions were detected in 2 cases studied by sequencing.

Conclusions: Despite overlap in histologic and immunohistochemical features between pseudosarcomatous myofibroblastic proliferation and nodular fasciitis, these tumours lack

the recently recognized *USP6* gene rearrangements that occur in nodular fasciitis, as well as alternative fusions found in *ALK*-negative inflammatory myofibroblastic tumours. At present, this diagnosis remains based primarily on clinical, histologic, and immunohistochemical features.

Introduction:

Pseudosarcomatous myofibroblastic tumours of the genitourinary tract,[1] whether procedure-related or spontaneous, can pose diagnostic challenge for pathologists, due to one or more worrisome features, such as high mitotic rate, infiltrative growth pattern, recurrence, and overlapping morphological and immunophenotypic features with other spindle cell tumours, such as low-grade leiomyosarcoma and sarcomatoid carcinoma. These tumours are also known by a variety of names, including fibromyxoid pseudotumor and postoperative spindle cell nodule, and it remains controversial whether this and inflammatory myofibroblastic tumour[2, 3] are the same or different entities.[4-7] Regardless of the terminology used, both pseudosarcomatous myofibroblastic proliferation and inflammatory myofibroblastic tumours of the urinary tract generally exhibit nonaggressive behaviour without metastasis, although some cases have persisted or recurred, requiring multiple surgical procedures. Therefore, recognition is important, as the differential diagnosis for spindle cell tumours of the urinary tract should almost always include consideration of sarcomatoid carcinoma and sarcoma, including leiomyosarcoma.

Since rearrangement of the ubiquitin-specific protease 6 (*USP6*) gene has been recently recognized as a recurrent alteration in soft tissue nodular fasciitis,[8] and since pseudosarcomatous myofibroblastic proliferation of the genitourinary tract shares a number of similarities with nodular fasciitis, including labelling for myofibroblastic marker smooth muscle actin, we aimed to evaluate for *USP6* rearrangement in pseudosarcomatous myofibroblastic tumours of the urinary tract, in order to determine whether this may be a confirmatory diagnostic test for this uncommon diagnosis, making it the genitourinary tract equivalent of nodular fasciitis. Additionally, we also studied *ROS1* and *ETV6* genes for rearrangement, as these have been recently implicated in a subset of

ALK rearrangement-negative inflammatory myofibroblastic tumours. [9, 10] Two representative cases with the most optimal genetic material were also tested with a gene fusion detection panel targeting over 50 genes involved in recurrent gene fusions using next-generation sequencing.

Material and methods:

After obtaining institutional review board approval from the Henry Ford Health System, we retrieved 17 cases of pseudosarcomatous myofibroblastic proliferation of the genitourinary tract from our pathology databases from 2002 to 2017, none of which were included in a prior study of inflammatory myofibroblastic tumors.[11] Of the 17 cases, 4 were reclassified upon histologic review. One occurred in the inguinal canal, which being a non-visceral location, was excluded. We studied the remaining 12 cases of pseudosarcomatous myofibroblastic proliferations, 10 of which occurred in the urinary bladder and 2 in renal pelvis. Histopathology and immunohistochemical staining results were reviewed by two of the authors (JASJ and SRW). We included pseudosarcomatous myofibroblastic proliferations with the following histological and immunohistochemical features: Lesions were required to be composed of spindle-shaped cells arranged in compact fascicles or loose tissue culture-like appearance. The spindle-shaped cells were required to be bipolar with eosinophilic cytoplasm, uniform ovoid or spindle-shaped nuclei with smooth nuclear contours. Stromal changes included myxoid change, areas of collagenization, haemorrhage, and scattered inflammatory cells. Analogous to nodular fasciitis, a specific cut-off of mitotic activity was not employed; however, absence of overt cytologic atypia, atypical mitosis, and necrosis was required. The expected immunohistochemical phenotype included positivity for smooth muscle actin or muscle specific actin, and negative staining for p63, GATA3, high molecular weight cytokeratin. Variable staining for desmin and broad spectrum cytokeratin was allowed, in light of previous reports showing these findings.[12 13] If not already performed at the time of original diagnosis, ALK immunohistochemical staining was performed using anti-ALK antibody (monoclonal mouse anti-human CD246, ALK protein, clone ALK-1, Dako, Carpinteria, CA). Since *ALK* rearrangement is rare in cases with negative immunohistochemical staining,[1, 11, 14] we did not perform *ALK* FISH.

Fluorescence in situ hybridization (FISH):

FISH analysis for *USP6*, *ROS1*, and *ETV6* genes were performed using bacterial artificial chromosome (BAC) derived break-apart probes. FISH for *USP6* was validated to be positive in four well-characterized cases of nodular fasciitis (**Figure 1 A-B**), one aneurysmal bone cyst, and negative in eight histologic mimics of nodular fasciitis. *ROS1* and *ETV6* was performed on all 12 cases but lacked adequate hybridization signals in 5 cases each for *ROS1* and *ETV6*, even after repeated hybridization. One case that was noninformative for *ROS1* by FISH, was informative using the next generation sequencing assay, bringing the total of *ROS1* and *ETV6* analysed cases to 8/12 and 7/12, respectively.

Next generation sequencing

A multiplex RNA fusion panel (Archer® FusionPlex® Solid Tumour Kit) was run on RNA extracted from representative formalin-fixed, paraffin-embedded tissue tumour block of 2 of the most recent cases regarded as having the most adequate genetic material for assessment (less than 2 years since tissue collection). Briefly, this assay is a targeted sequencing assay that uses anchored multiplex polymerase chain reaction to prepare target-enriched cDNA libraries from RNA to detect fusions and other mutations in over 50 genes linked to carcinomas, other solid tumours, known sarcoma and haematological malignancy-associated fusions, as well as prominent *BRAF* and *PDGFRA* mutations using next generation sequencing. This technology allows the detection of not only known recurrent fusions, but also previously unidentified fusions, so long as one of the fusion partners is included in the panel. Anchored multiplex polymerase chain reaction creates target enriched libraries by using a combination of unidirectional gene-specific primers and universal adapters to enrich for both known and unknown mutations. Adapters that contain both molecular barcode adapters and sample indices permit quantitative multiplex data analysis, read deduplication and accurate mutation calling. Molecular barcode adapters aid in error correction, sample identification, deduplication, and duplicate read binning for confident mutation detection. Genes targeted in this assay include: *AKT3*, *ALK*, *ARHGAP26*, *AXL*, *BRAF* (fusion and V600E mutation), *BRD3*, *BRD4*, *EGFR* (fusion and mutation), *ERG*, *ESR1*, *ETV1*, *ETV4*, *ETV5*, *ETV6*, *EWSR1*, *FGFR1*, *FGFR2*, *FGFR3*,

FGR, *INSR*, *MAML2*, *MAST1*, *MAST2*, *MET* (fusion and mutation), *MSMB*, *MUSK*, *MYB*, *NOTCH1*, *NOTCH2*, *NRG1*, *NTRK1*, *NTRK2*, *NTRK3*, *NUMBL*, *NUTM1*, *PDGFRA* (fusion and mutation), *PDGFRB*, *PIK3CA*, *PKN1*, *PPARG*, *PRKCA*, *PRKCB*, *RAF1*, *RELA*, *RET*, *ROS1*, *RSPO2*, *RSPO3*, *TERT*, *TFE3*, *TFE3*, *TFEB*, *THADA*, and *TMPRSS2*.

Results:

The age distribution of the 12 cases studied ranged from 26 to 87 (median 62.5), including 5 men and 7 women. Three of the 12 cases had recurrent or persistent tumour in more than one transurethral resection. One had multiple recurrences, ultimately managed with cystectomy. Three of 10 patients had known history of previous instrumentation in the bladder, and 1 had prior uterine surgery. The histological findings were similar in all cases, irrespective of the age, site, and the recurrence status, as described in the criteria and discussion. ALK immunohistochemical staining was negative in all cases. FISH analysis (**Figure 1 C-D**) for USP6 was negative in all 12 cases (0/12) including 10 large tumours (making up multiple tissue cassettes of lesional tissue, (**Figure 2**) and 2 small lesions (entirely examined in 1 or 2 tissue cassettes). FISH analysis for ROS1 and ETV6 was negative in 7 out of the 12 cases (0/7). The remaining cases lacked hybridization signals even after repeating. The next generation sequencing revealed no identifiable gene fusions from the studied panel of over 50 genes in 2 cases with adequate genetic material for evaluation, which included *ROS1* in one of the cases that failed FISH (total 0/8 for *ROS1*).

Discussion:

Pseudosarcomatous myofibroblastic proliferation is a descriptive term that encompasses the mucosal lesions post-operative spindle cell nodule, visceral fasciitis, pseudosarcomatous fibromyxoid tumour, pseudomalignant spindle cell proliferation, and inflammatory pseudotumor. [1] In general, the term post-operative spindle cell nodule is principally used in the setting of previous injury or biopsy, in contrast to other terms when the mass occurs idiopathically. These spindle-shaped cells show ultrastructural features of myofibroblasts intermediate between a fibroblast and smooth muscle cell. [15, 16] Although this has been reported in multiple mucosal sites, the urinary tract is among the most common locations. In addition to these several names, it also remains a subject of debate

whether this entity is part of the spectrum of inflammatory myofibroblastic tumour.[2-4] Both of these entities share a very similar morphology, with perhaps exception of a lesser inflammatory component in pseudosarcomatous myofibroblastic proliferation, and both share a myofibroblastic immunohistochemical phenotype. Although inflammatory myofibroblastic tumour of soft tissue is classified as a neoplasm of intermediate malignancy, both of these entities in the urinary tract are characteristically nonaggressive (without metastasis, although persistence or recurrence, requiring multiple surgeries, can occur). In general, our practice is to reserve the term inflammatory myofibroblastic tumour (in the genitourinary tract) predominantly for tumours with ALK immunohistochemical positivity or *ALK* gene rearrangement; however, even this is a subject of debate, as some studies have found that cases with positive ALK immunohistochemical staining do not necessarily always harbour rearrangement, making this distinction quite blurry. [1, 4, 5, 11]

Histologically, pseudosarcomatous myofibroblastic proliferations are composed of proliferations of spindle-shaped cells with loose myxoid stroma or tight fascicles. They have a variable degree of mitotic activity and inflammatory cell infiltrate. However, marked pleomorphism, atypical mitosis, and necrosis are absent. Immunohistochemically, these typically stain for muscle specific actin, smooth muscle actin (**Figure 2C**), sometimes desmin, and often cytokeratin (**Figure 2D**). Overall, these could be argued to resemble soft tissue nodular fasciitis in both histology and reactivity for smooth muscle actin; however, labelling for keratin is typically a point of distinction that is frequently present in bladder myofibroblastic tumours but lacking in nodular fasciitis.[17, 18] Despite that nodular fasciitis has long been considered an often self-limiting exuberant reactive lesion, clonal rearrangement of *USP6* locus at 17p13 resulting in *MYH9-USP6* gene fusion has been recently identified, suggesting that rather than a purely reactive process, this may in fact represent a transient form of neoplasia. Of note, nodular fasciitis also shares with pseudosarcomatous myofibroblastic proliferations of the urinary tract an incomplete association with preceding trauma. Therefore, in soft tissue tumour pathology, evaluation for *USP6* rearrangement may be a potentially helpful technique to resolve challenging diagnostic cases. [19] Interestingly, rearrangement of the *USP6* locus is also shared by aneurysmal bone cyst, [20] which is histologically quite different, although perhaps less so if one considers the bland spindle-shaped cell component of aneurysmal bone cyst to be the

true neoplastic cell population. Despite the histological, immunophenotypic and ultrastructural [16, 21, 22] similarity between pseudosarcomatous myofibroblastic proliferations of genitourinary tract and nodular fasciitis, we found these lesion to lack *USP6* rearrangement (**Figure 1D**), which refutes our hypothesis that this may be its visceral equivalent.

The staining for cytokeratin that is often present in pseudosarcomatous myofibroblastic proliferation may also raise consideration of sarcomatoid urothelial carcinoma; however, features than can be helpful in distinguishing these entities include reactivity for p63 or GATA3 in sarcomatoid carcinoma, and usual lack of reactivity for desmin and limited extent of labelling for smooth muscle actin in sarcomatoid carcinoma [23]. Although cytokeratin staining is often present in pseudosarcomatous myofibroblastic proliferation, staining for high molecular weight cytokeratin is characteristically absent, contrasting to urothelial carcinoma. [24]

A limitation of this study is that the FISH and sequencing testing employed was only targeted at known gene fusions and therefore was not designed to identify entirely novel gene fusions. Further study, such as with RNA sequencing (RNA-seq) or other techniques, may determine whether these pseudosarcomatous myofibroblastic proliferations of the urinary tract represent a benign neoplasm, like nodular fasciitis, yet with a so far unknown gene fusion, or whether they represent an exuberant reactive process, as nodular fasciitis with originally postulated to be. Based on our current data, this diagnosis remains principally based on morphologic and immunohistochemical features, without a known confirmatory molecular feature. If these rearrangements ever occur in pseudosarcomatous myofibroblastic proliferations of the urinary tract, their rate is likely sufficiently low that testing is not a robust diagnostic tool.

Author contributions:

Drafting the manuscript: Jebastin

Critical revision and final approval of the manuscript: all authors

Data collection, analysis, and interpretation: Jebastin, Carskadon, Chitale, Palanisamy, Williamson

Conception / design: Williamson

Figure Legends:

Figure 1: A: Histologic appearance of a case of *USP6* rearranged nodular fasciitis demonstrates fusiform cells with areas of myxoid oedematous stroma. B: Break-apart fluorescence in situ hybridization (FISH) shows rearrangement of *USP6*, with separation of one pair of green and red signals. C: Pseudosarcomatous myofibroblastic proliferation of the urinary bladder exhibits a very similar morphologic appearance. D: However, break-apart FISH negative is negative for *USP6* rearrangement.

Figure 2: A: Gross appearance of a partial cystectomy specimen from a case of pseudosarcomatous myofibroblastic proliferation of the urinary bladder demonstrates a solid, white mass in the wall of the urinary bladder. B: The same tumour demonstrates a nodular fasciitis-like histology. C: Immunohistochemical staining for smooth muscle actin demonstrates diffuse, strong staining, consistent with a myofibroblastic pattern. D: Keratin
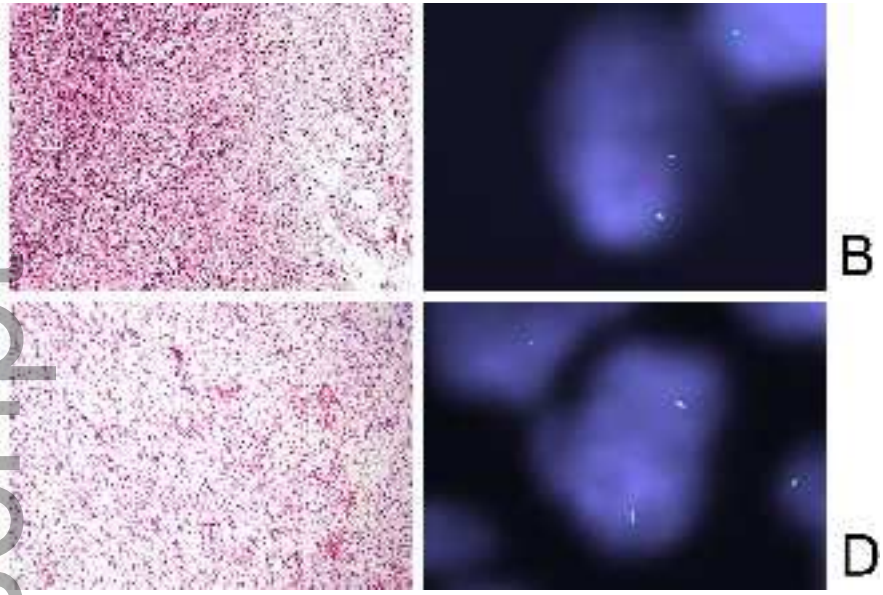
(broad spectrum cocktail) immunohistochemical staining demonstrates substantial labelling, in contrast to classic soft tissue nodular fasciitis.

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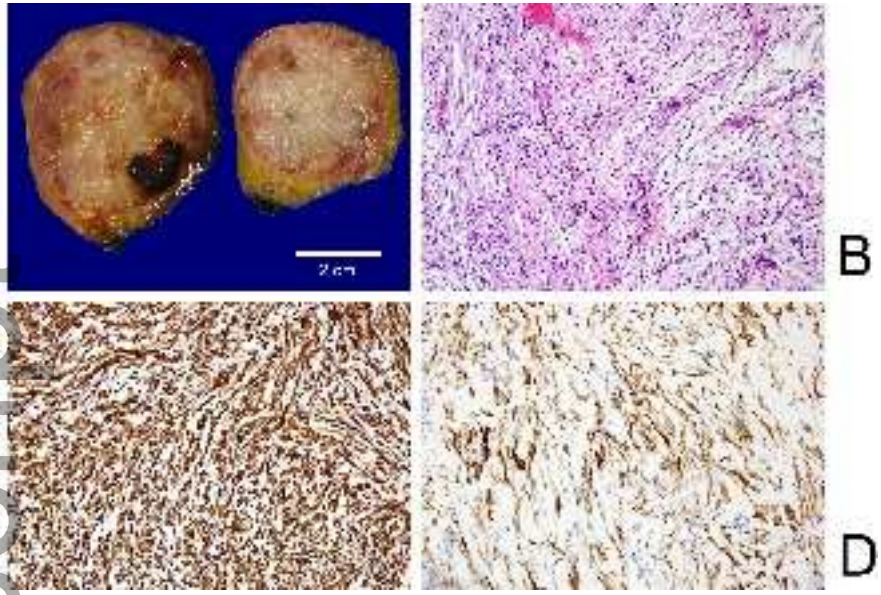
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