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USP6 genetic rearrangements in cellular fibroma of tendon sheath

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Fibroma of tendon sheath is a benign (myo)fibroblastic neoplasm of the tenosynovial soft tissues, typically affecting the distal extremities. It is classically described as a paucicellular, densely collagenized tumor; however, cellular variants have been described. A subset of cellular fibromas of tendon sheath shares similar histological features with nodular fasciitis. As nodular fasciitis very frequently harbors rearrangement of ubiquitin-specific peptidase 6 (*USP6*), we hypothesized that cellular fibromas of tendon sheath with nodular fasciitis-like features may also contain *USP6* rearrangements. Cases of fibroma of tendon sheath ($n=19$), including cellular ($n=9$) and classic ($n=10$) variants, were evaluated for *USP6* rearrangement by fluorescence *in situ* hybridization studies. A subset of cases was tested for *MYH9* rearrangements and *MYH9-USP6* and *CDH11-USP6* fusion products. Classic fibroma of tendon sheath occurred in 5 males and 5 females (median age 67 years, range 23–77 years) as soft tissue masses of the hand ($n=4$), finger ($n=3$), forearm ($n=1$) and foot ($n=2$). Cellular fibroma of tendon sheath occurred in 5 males and 4 females in a younger age group (median age 32 years, range 12–46 years) as small soft tissue masses of the finger ($n=5$), hand ($n=3$) and wrist ($n=1$). *USP6* rearrangements were detected in 6/9 cellular fibromas of tendon sheath. Among cellular fibromas of tendon sheath with *USP6* rearrangements, no *MYH9* rearrangements were detected. By RT-PCR, neither the *MYH9-USP6* or the *CDH11-USP6* fusion products were detected in any case. Neither *USP6* nor *MYH9* rearrangement were detected in any classic fibroma of tendon sheath. We report for the first time the presence of *USP6* rearrangements in a subset of cellular fibroma of tendon sheath. Based on the similar morphological and molecular genetic features, we suspect that a subset of cellular fibromas of tendon sheath are under-recognized examples of tenosynovial nodular fasciitis, driven by alternate *USP6* fusion genes. Further investigation will delineate how these lesions should best be classified.
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Originally described by Geschickter and Copeland,[1] fibroma of tendon sheath is a benign soft tissue tumor of the tenosynovial membranes, typically affecting the distal extremities. In 1979, in the largest clinicopathological series to date, Chung and Enzinger[2] reported that fibromas of tendon sheath occur most commonly in middle-aged adults, with the finger, hand and wrist as the most common sites of involvement.

Classically, fibromas of tendon sheath are paucicellular neoplasms, composed of bland stellate-to-spindled fibroblasts embedded in a dense fibrocollagenous stroma.[3] However, Chung and Enzinger[2] noted a subset of fibromas of tendon sheath with moderate-to-high cellularity. These cellular fibromas of tendon sheath were composed, in part, of loosely formed fascicles of spindled-to-stellate fibroblasts with microcystic change and erythrocyte extravasation. Remarkably, they reported that the histological features were strikingly similar to nodular fasciitis.

Unlike fibroma of tendon sheath, nodular fasciitis is a common, self-limited soft tissue lesion that typically occurs in young adults and often presents as a rapidly growing mass with a predilection for the superficial soft tissues of the arm, trunk and head and neck.[4,5] Typically, nodular fasciitis is composed of (myo)fibroblastic cells forming loosely arranged fascicles with a 'tissue culture-like' growth pattern. In 2011, Erickson-Johnson *et al*[6] demonstrated that nodular fasciitis frequently contains a recurring reciprocal translocation involving the *USP6* locus

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on chromosome 17. In their series, 92% of cases contained *USP6* rearrangements wherein 65% of those were partnered with *MYH9*, a non-muscle myosin expressed by fibroblasts and other cell types. In 2013, Amary *et al*[7] reported an identical frequency of *USP6* rearrangements in nodular fasciitis, with 92% of the cases in their series containing *USP6* rearrangements. *USP6* encodes a de-ubiquitinating enzyme, one member of a large family of proteases involved in intracellular protein trafficking and turnover.[8] *USP6* rearrangements also occur in a majority of aneurysmal bone cysts; however, the fusion partners differ from those seen in nodular fasciitis.[9–14] As a subset of cellular fibromas of tendon sheath has overlapping histological features with nodular fasciitis, we hypothesized that cellular fibroma of tendon sheath may be a tenosynovial variant of nodular fasciitis and harbor *USP6* genetic rearrangements. We tested a series of classic and cellular fibromas of tendon sheath for *USP6* and MYH9 rearrangements and *MYH9-USP6* and *CDH11-USP6* fusion products.

Materials and methods

The study was approved by the Mayo Clinic Institutional Review Board. Our institutional and consultation archives were searched for cases coded as fibroma of tendon sheath, yielding 19 cases with available formalin-fixed, paraffin-embedded tissue blocks. All available slides were re-reviewed by two of the authors (JMC, AMO) and showed the histological features of classic or cellular variants of fibroma of tendon sheath as previously described.[2] Designation as cellular variant of fibroma of tendon sheath required a minimum of 15% of the lesion to contain moderately to highly cellular areas. All cellular fibromas of tendon sheath also contained areas of classic fibroma of tendon sheath. Discrepant cases were resolved by consensus review.

Fluorescence *In Situ* Hybridization (FISH)

FISH studies for *USP6* and *MYH9* were performed in formalin-fixed, paraffin-embedded tissues using a break apart probe strategy and a probe set generated from commercially available BAC clones as previously described.[6] A minimum of 200 non-overlapping nuclei were scored and a rearranged locus was defined as one in which >15% of lesional cells showed a splitting of the spectrum orange and green signals.

RT-PCR

RT-PCR for the *MYH9-USP6* and *CDH11-USP6* fusion products was performed on RNA retrieved from archival formalin-fixed, paraffin-embedded tissues as previously described.[6] Briefly, 1 μg of RNA was reverse transcribed using the iScript Select cDNA Synthesis Kit using random primers per the manufacturer's instruction (Bio-Rad, Hercules, CA, USA). PCR was performed using specific primers as previously described.[6]

High-Throughput Sequencing

The Archer FusionPlex Sarcoma Panel was used to identify possible novel *USP6* fusion transcripts using the vendor's recommended protocols (http://archerdx.com/fusionplex-assays/sarcoma).

Results

The clinicopathological features of the fibromas of tendon sheath included in this study are summarized in Table 1. Following histological review, the 19 cases were classified as 10 classic and 9 cellular fibromas of tendon sheath. Classic fibromas of tendon sheath occurred predominantly in adults, median age 67 years (range 23–77 years), equally in males and females. They presented as small masses with an average size of 2.3 cm (range 0.7–6.5 cm), involving the soft tissues of the hand ($n=4$), finger ($n=3$), arm ($n=1$) and foot ($n=2$). Cellular fibromas of tendon sheath occurred in a younger age group than the classic variant (median 32 years, range 12–46 years), with an almost equal predilection for males and females. Similar to classic fibroma of tendon sheath, they presented as masses of the soft tissues of the finger ($n=5$), hand ($n=3$) and wrist ($n=1$), with a mean size of 1.1 cm (range 0.5–1.6 cm). Clinical follow-up data were available in 9 cases, including 3 cellular and 6 classic fibromas of tendon sheath, with a mean duration of 107 months (range

Table 1 Clinicopathological features and *USP6* and *MYH9* rearrangement status

Variant of fibroma of tendon sheath	*Age/sex*	*Site*	*Size (cm)*	*USP6*	*MYH9*
Cellular	24/M	Wrist	1.6	+	–
Cellular	32/F	Hand	NA	+	–
Cellular	33/F	Finger	NA	+	–
Cellular	42/F	Finger	NA	+	–
Cellular	12/M	Finger	NA	+	NA
Cellular	46/M	Hand	NA	+	–
Cellular	26/F	Finger	0.5	–	NA
Cellular	31/M	Thumb	NA	–	NA
Cellular	38/M	Hand	1.1	–	NA
Classic	23/F	Finger	NA	–	NA
Classic	43/F	Finger	0.7	–	NA
Classic	52/F	Forearm	NA	–	–
Classic	53/M	Foot	NA	–	–
Classic	65/F	Hand	1.2	–	NA
Classic	69/M	Hand	1.2	–	NA
Classic	70/M	Wrist	3.6	–	NA
Classic	71/F	Foot	2	–	NA
Classic	71/M	Finger	1	–	NA
Classic	77/M	Hand	6.5	–	NA

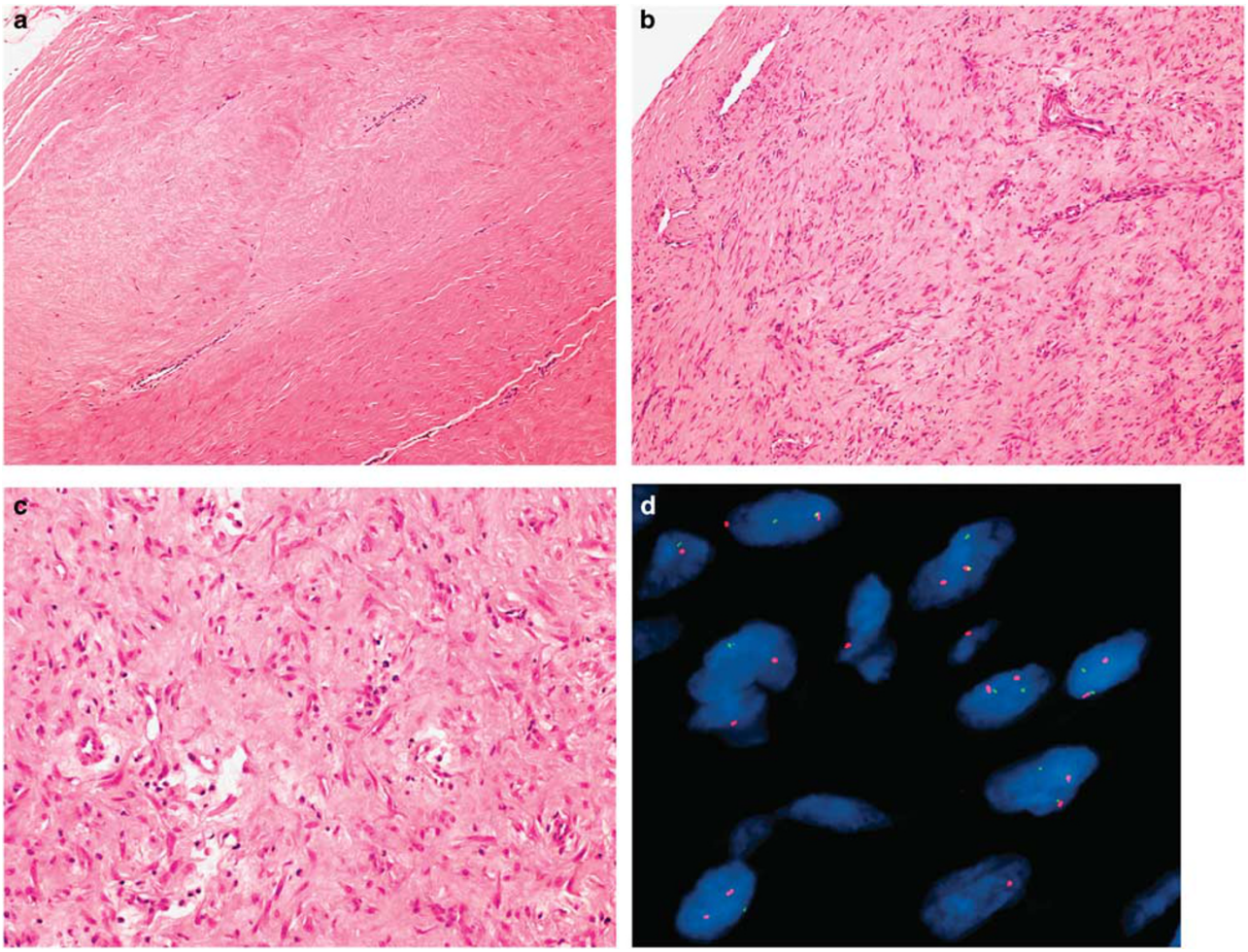

Figure 1 Classic and cellular variants of fibroma of tendon sheath. (**a**) Classic fibroma of tendon sheath is a paucicellular fibroblastic neoplasm with dense fibrocollagenous stroma. (**b**) Cellular fibroma of tendon sheath contains areas of increased cellularity composed of loosely formed fascicles of spindled-to-stellate fibroblasts. (**c**) Cellular fibroma of tendon sheath with areas of microcystic change and erythrocyte extravasation, histologically identical to those seen in nodular fasciitis. (**d**) Break apart *USP6* FISH strategy in a cellular fibroma of tendon sheath. Separation of the green and orange signals indicates rearrangement of the *USP6* locus.

60–192 months). All patients are currently alive with no evidence of disease.

The histological features of the classic and cellular fibromas of tendon sheath were similar to those previously described.[2] Briefly, both classic and cellular fibromas of tendon sheath were well-circumscribed, lobular masses with no infiltrative features and displayed a characteristic slit-like, compressed vasculature at the lesional periphery. Classic fibromas of tendon sheath were composed of cytologically bland, spindled fibroblasts in a dense fibrocollagenous stroma (Figure 1a). In contrast, cellular fibromas of tendon sheath had variable amounts of a fascicle-forming, moderately cellular component (Figure 1b). The spindled-to-stellate cells showed mild pleomorphism with open chromatin and variably prominent nucleoli. Mitoses were identified in some cases but atypical mitoses were not seen. There was no significant cytological atypia. Within the cellular component, areas of microcystic change, extravasation of erythrocytes and scattered chronic inflammatory cells, reminiscent of nodular fasciitis, were present (Figure 1c). In these cases, the cellular areas merged into paucicellular, densely fibrotic areas with the histological features of classic fibroma of tendon sheath. All 19 cases, including the cellular and classic subsets, were tested for *USP6* rearrangement by FISH as described in the Materials and Methods section. Among the classic fibromas of tendon sheath, none of the 10 cases had *USP6* rearrangements. Among the cellular fibromas of tendon sheath, 6 (of 9) cases showed rearrangement of the *USP6* locus in both the cellular and 'classic-appearing' components (Figure 1d). Although there was no difference in the clinical features between cellular fibromas of tendon sheath with and without *USP6* rearrangements, one case lacking *USP6* rearrangement did have a more distinctively storiform fascicular arrangement.

We further tested a subset ($n = 7$) of cases for *MYH9* rearrangements, *MYH9-USP6* and *CDH11-USP6* fusion products, and possible novel *USP6* fusion

transcripts using a high-throughput sequencing approach. Cases included four *USP6*-rearranged cellular fibromas of tendon sheath, one cellular fibroma of tendon sheath without *USP6* rearrangement and two classic fibromas of tendon sheath. None had *MYH9* rearrangements or the *MYH9-USP6* or *CDH11-USP6* fusion products. After multiple trials, we could not identify a novel *USP6* fusion transcript.

Discussion

Fibroma of tendon sheath is a rare benign soft tissue neoplasm, most commonly arising in the tenosynovial membranes of the distal extremities.[3] As originally described by Geschickter and Copeland,[1] fibromas of tendon sheath are typically paucicellular tumors, composed of scattered, stellate fibroblasts embedded within a fibrous stroma. Characteristically, they have 'slit-like' vessels at the lesional periphery. In 1979, Chung and Enzinger[2] described a cellular variant of fibroma of tendon sheath composed primarily of fascicle-forming spindle cells often associated with extravasated erythrocytes, a mucoid matrix and scattered mononuclear cells. Although the authors noted the similarity of this subtype of cellular fibroma of tendon sheath to nodular fasciitis, they observed that the cellular areas invariably transitioned to the conventional hypocellular, collagenous areas of classic fibroma of tendon sheath. In addition to nodular fasciitis-like cellular fibroma of tendon sheath, they reported a cellular variant with dense fascicles of spindled cells similar to that seen in what was previously designated malignant fibrous histiocytoma.[2]

Subsequently, a handful of small series of fibroma of tendon sheath have confirmed the benign behavior of these lesions and the (myo)fibroblastic nature of the constituent cells, both by ultrastructural and immunohistochemical analysis.[15–20] Although cases of cellular variants have been reported in almost all series, Pulitzer *et al*[21] and Smith *et al*[16] excluded nodular fasciitis-like examples from their respective series, arguing that such cases should be considered examples of tenosynovial nodular fasciitis rather than variants of fibroma of tendon sheath.

In our study, while six of the nine cellular fibromas of tendon sheath had *USP6* rearrangements, no *USP6* rearrangement was detected in any examples of classic fibroma of tendon sheath. Based on our data, we would also argue that at least a subset of nodular fasciitis-like cellular fibromas of tendon sheath represent under-recognized examples of tenosynovial nodular fasciitis. Although nodular fasciitis typically occurs in the subcutaneous soft tissues or rarely within skeletal muscle, it has been reported in unusual sites.[22] Intra-articular nodular fasciitis has also been described and is reported to contain prominent stromal hyalinization.[23] Although nodular fasciitis is typically composed of loosely arranged fascicles of (myo)fibroblastic cells with a 'tissue culture-like' growth pattern, Price *et al*[24] were the first to recognize three variants of nodular fasciitis: myxoid, cellular, and fibrous subtypes. The conventional view is that these variants likely represent chronological stages of evolving nodular fasciitis. Accordingly, perhaps nodular fasciitis-like cellular fibroma of tendon sheath represents an evolving cellular and fibrous nodular fasciitis of the tenosynovial membranes. Interestingly, prior to this study, the reported median age of patients presenting with fibroma of tendon sheath was within the third or fourth decades; however, these series did not discriminate between classic and cellular subtypes.[2,25] In our series, classic fibroma of tendon sheath occurred in a much older age group (median age 67 years) than cellular fibroma of tendon sheath (median age 32 years).

Classic fibroma of tendon sheath also appears to be genetically distinct from cellular fibroma of tendon sheath. Cytogenetic profiles have been reported in two cases of the classic type. In 1998, Dal Cin *et al*[26] reported a t(2;11)(q31-32;q12) in a classic fibroma of tendon sheath of the hand in a 60-year-old woman. Similar translocations have been reported in desmoplastic fibroblastoma, raising the question of whether these entities are related.[27–30] More recently, Nishio *et al*[31] reported a complex karyotype, including a t(9;11)(p24;q13-14) translocation, among other abnormalities, in a classic fibroma of tendon sheath of the hand in a 38-year-old woman. Additional studies are required to clarify the genetics of these lesions.

The demonstration of *USP6* rearrangements in nodular fasciitis was the first example of a self-limited lesion with a recurrent somatic gene fusion event.[6] *USP6* (also known as *TRE17* or *TRE2*) is located on chromosome 17p13 and encodes ubiquitin-specific peptidase 6, a de-ubiquitinating enzyme involved in protein trafficking, inflammatory signaling and cell transformation.[8] In nodular fasciitis, the most common chromosomal translocation breakpoints occur a t(17;22)(p13:q13) and result in a promoter swapping mechanism of the entire *USP6*-coding region with the promoter region of *MYH9*.

MYH9 is located on chromosome 22q12.3-q13 and encodes myosin heavy chain 9, a structural myosin classified within the non-muscle myosin class II family.[32] *MYH9* rearrangements are present in only 65% cases of nodular fasciitis, and alternative *USP6* fusion partners exist.[22] Presumably, similar to nodular fasciitis, *USP6*-rearranged cellular fibromas of tendon sheath have alternative, non-*MYH9* fusion partners.

In summary, to date, the USP-induced family of neoplasia includes nodular fasciitis and aneurysmal bone cyst, including the subset of aneurysmal bone cyst previously described as giant cell reparative granuloma of the hands and feet.[33,22] We report that cellular fibromas of tendon sheath frequently contain *USP6* genetic rearrangements. *USP6* rearrangements were not detected in classic fibromas of tendon sheath. Given the similar morphological and

molecular genetic features to nodular fasciitis, we propose that cellular fibromas of tendon sheath with histological features of nodular fasciitis are likely a tenosynovial subset of nodular fasciitis. Additional study should delineate the frequency of *USP6* rearrangements in these lesions and identify the *USP6* genetic fusion partners.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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