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CORRESPONDENCE

Superficial *ALK*-rearranged myxoid spindle cell neoplasm with a novel *FMR1-ALK* fusion gene

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TO THE EDITOR:

We read with great interest the recent article "Superficial *ALK*-rearranged myxoid spindle cell neoplasm: a cutaneous soft tissue tumor with distinctive morphology and immunophenotypic profile" by Dermawan et al., describing a series of six cases with *ALK* gene fusions, including *FLNA-ALK*, *MYH10-ALK*, and *HMBOX1-ALK*, and a peculiar histomorphology of spindle to ovoid cells forming whorls or cord-like arrangements in a background of myxoid to myxohyaline matrix.[1] Rather than fitting into well-established tumor entities with *ALK* gene fusions, such as inflammatory myofibroblastic tumor, epithelioid fibrous histiocytoma, or spitzoid melanocytic tumors, these tumors show a certain degree of overlap with mesenchymal tumors harboring *NTRK* or other tyrosine kinase gene fusion by expression of CD34 and/or S100 and the occasional findings of lipofibromatosis-like pattern, perivascular hyalinization, and intra-tumoral lymphocytes. Nevertheless, the unique histologic features of concentric whorls and cord-like arrangements in these tumors are unorthodox in typical *NTRK*-fused mesenchymal tumors.

With only limited cases reported in a single series to date, the histologic and molecular spectrum of these superficial *ALK*-rearranged myxoid spindle cell neoplasms remains to be explored. Here we report a large subcutaneous tumor with a predominantly reticular to cord-like pattern and a novel *FMR1-ALK* fusion.

Our patient was a 37-year-old female presenting with an enlarging subcutaneous tumor in the left buttock for more than 1 year. Ultrasound examination revealed a lobulated and hypoechoic mass with some hyperechoic streaks in the deep subcutaneous tissue abutting fascia. Grossly, it was a lobulated and relatively well-defined tumor measuring about 11×5 cm in dimensions (Fig. 1A). Microscopically, the tumor was composed of relatively uniform tumor cells with round to ovoid nuclei, arranged in reticular and cord-like patterns in a myxohyaline stroma (Fig. 1B, C). The tumor cells had fine chromatin, scant cytoplasm, and occasional intranuclear pseudoinclusions (Fig. 1D). Ectatic thin-walled vessels were present throughout the tumor, and perivascular hyalinization or edema was present in some of the vessels (Fig. 1E). As a minor component, small cellular aggregates with whorl-like arrangements of the tumor cells were also seen focally (Fig. 1F). No significant lymphocytic infiltrate or lipofibromatosis-like area was observed. Mitotic activity was very low, if any, and no tumor necrosis was found. Immunohistochemically, the tumor cells exhibited strong but patchy expression of CD34 (40%, Fig. 2A) and focal reactivity to S100 (10%, Fig. 2B), while CK (AE1/AE3), LMW-CK, EMA, SOX10, SMA, GFAP, claudin-1, MUC4, STAT6, BCOR, and pan-Trk were all negative. With the differential diagnosis of myoepithelial tumor, ossifying fibromyxoid tumor, and *OGT*-rearranged mesenchymal neoplasm in mind, fluorescence in situ hybridization (FISH) assays for *EWSR1*, *FUS*, *PHF1*, *BCOR*, *OGT*, and *FOXO1* rearrangements were performed and all showed negative results. RNA sequencing using TruSeq RNA Exome (Illumina) revealed a novel *FMR1-ALK* fusion joining exon 17 of *FMR1* (mid-exonic breakpoint) to exon 20 of *ALK*, which was subsequently validated by reverse transcription-polymerase chain reaction with Sanger sequencing and by break-apart FISH assays, demonstrating rearranged *ALK* and *FMR1*, respectively (Fig. 2C–E). The fusion transcript was predicted to be in-frame and, like most *ALK* gene fusions, preserved the sequence encoding the receptor tyrosine kinase domain of ALK. Immunohistochemical stain for ALK (D5F3) exhibited diffuse and strong ALK reactivity (Fig. 2F). The tumor was marginally excised with positive margins. The patient had no local recurrence 9 months after the surgery.

Compared to the previously reported case series, our case had the largest tumor size (11 cm) and relatively limited extent of S100 staining. The proportions of different histologic patterns are not specified in the previous study. Our case showed a predominantly reticular to cord-like arrangement and only focal presence of the distinct whorl-like pattern, hence morphologically mimicking myoepithelial tumor, ossifying fibromyxoid tumor, sclerosing epithelioid fibrosarcoma, and *OGT*-rearranged mesenchymal tumor. Aside from focal S100 expression, other myoepithelial markers, such as CK (AE1/AE3), EMA, and GFAP, were negative in our case. It also lacked the ossified matrix and *PHF1* or *BCOR* rearrangements characteristic of ossifying fibromyxoid tumor. Negative MUC4 expression and lack of *EWSR1* and *FUS* rearrangements did not support the diagnosis of sclerosing epithelioid fibrosarcoma. Similar to these superficial *ALK*-rearranged tumors, *OGT*-rearranged mesenchymal tumors also show a biphasic pattern with cord-like and whorled arrangements of tumor cells in a myxohyaline to fibromyxoid stroma, accompanied by perivascular hyalinization, and are immunoreactive to CD34.[2] However, *OGT*-rearranged mesenchymal tumors arise more commonly in acral regions, although only rare cases have been reported and exceptions exist.[3] Our case was negative for *OGT*

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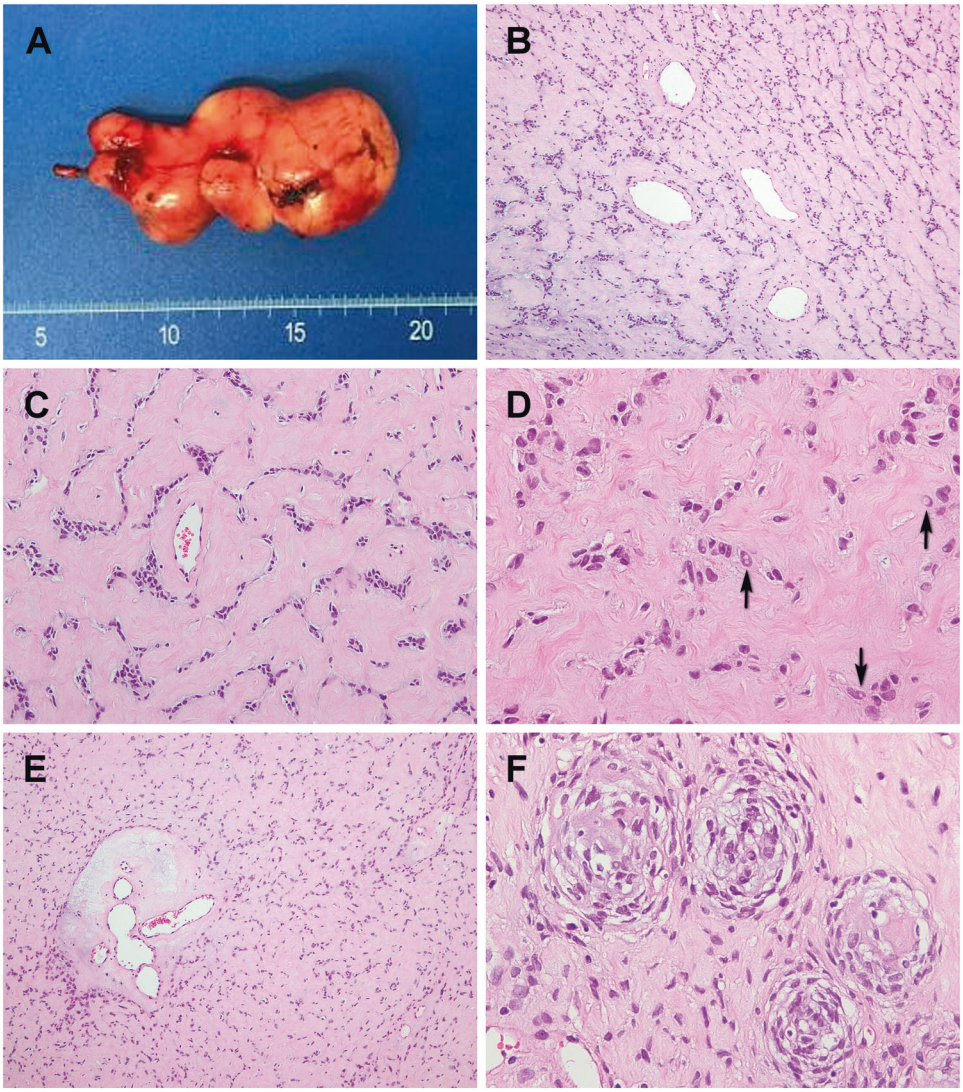

Fig. 1 Gross and microscopic features. A A lobulated subcutaneous tumor with rich vascularization on the outer surface. **B**, **C** Microscopically, the tumor shows a predominantly reticular (**B**) to cord-like (**C**) pattern in a hyalinized stroma, accompanied by thin-walled ectatic vessels. **D** The tumor cells have round to ovoid nuclei, fine chromatin, scant cytoplasm, and occasional intranuclear pseudoinclusions (arrows). **E** Some vessels show perivascular hyalinization or edematous change. **F** Focally, the tumor shows spindle cells arranged in whorl-like structures.

rearrangement by FISH study, disfavoring this diagnosis. Lastly, the minor whorl-like pattern component was reminiscent of perineurioma, but EMA and claudin-1 stains were negative.

Our case harbored a novel *ALK* fusion partner gene, *FMR1*. *FMR1* encodes an RNA-binding protein involved in multiple cellular processes including transcription, translation, RNA trafficking, cell cycle regulation, among others.[4] Expansion of a CGG trinucleotide repeat in the 5′-untranslated region of *FMR1* and the resultant loss of FMR protein (FMRP) are associated with fragile X syndrome. FMRP is ubiquitously expressed in various tissue types, especially in the central nervous system and testis.[5] Like many ALK fusion partners, FMRP forms dimers via its N-terminal domains, mainly Tudor2 domain (a.k.a NDF2).[5,6] The projected FMR1-ALK fusion protein in our case included almost the full length of FMRP (622 of 632 residues, NP_002015.1) and presumably retained the ability of dimerization as seen in other ALK fusion proteins. A recent study reporting a spindle cell sarcoma harboring *FMR1-RAF1* fusion in the buttock of a 4-year-old girl supports our finding of *FMR1* serving as the 5′ fusion partner gene in kinase gene fusions,[7] although the tumor carrying *FMR1-RAF1* fusion bore no histologic resemblance to our case.

In summary, our case demonstrated that superficial *ALK*-rearranged myxoid spindle cell neoplasm can exhibit a predominantly reticular to cord-like pattern with a minor but distinct whirling component and limited extent of S100 expression. *FMR1* was identified as a novel fusion partner in *ALK* gene fusions.

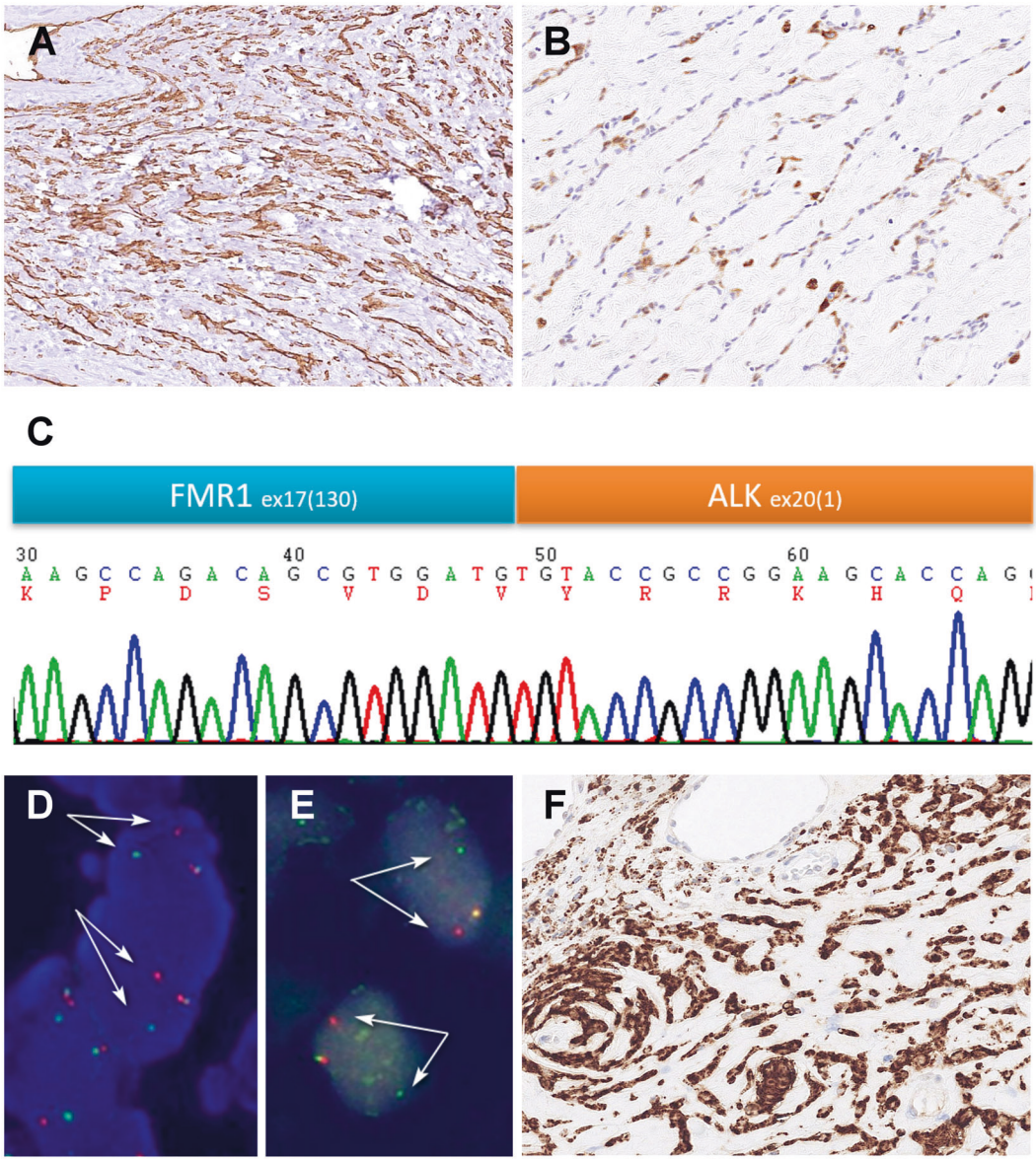

Fig. 2 Immunohistochemical and molecular findings. A, **B** The tumor cells show strong and patchy expression of CD34 (**A**) and focal S100 staining (**B**). **C** *FMR1-ALK* fusion transcript identified by RNA sequencing was confirmed by RT-PCR and Sanger sequencing, showing an in-frame fusion between exon 17 of *FMR1* and exon 20 of *ALK*. **D**, **E** Break-apart FISH assays showing *ALK* (**D**) and *FMR1* (**E**) gene rearrangements with split green and red signals (arrows). **F** The tumor cells show diffuse and strong ALK immunohistochemical staining.

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REFERENCES

1. Dermawan, J. K. et al. Superficial ALK-rearranged myxoid spindle cell neoplasm: a cutaneous soft tissue tumor with distinctive morphology and immunophenotypic profile. *Mod. Pathol.* **34**, 1710–1718 (2021).
2. Lee, J. C. et al. Myoepithelioma-like hyalinizing epithelioid tumors of the hand with novel OGT-FOXO3 fusions. *Am. J. Surg. Pathol.* **44**, 387–395 (2020).
3. Torrence, D., Zhang, L., Sung, Y. S., Dickson, B. C. & Antonescu, C. R. Hyalinizing epithelioid tumors with OGT-FOXO fusions. A case report of a non-acral soft tissue mass harboring a novel FOXO4 gene rearrangement. *Genes Chromosomes Cancer* **60**, 498–503 (2021).
4. Taha, M. S. et al. Novel FMRP interaction networks linked to cellular stress. *FEBS J.* **288**, 837–860 (2021).
5. Adinolfi, S. et al. The N-terminus of the fragile X mental retardation protein contains a novel domain involved in dimerization and RNA binding. *Biochemistry* **42**, 10437–10444 (2003).
6. Hu, Y. et al. The amino-terminal structure of human fragile X mental retardation protein obtained using precipitant-immobilized imprinted polymers. *Nat. Commun.* **6**, 6634 (2015).
7. Zhang, T., Wang, Q., Yi, X., Zhu, P. RAF1-rearranged spindle cell tumour: report of two additional cases with identification of a novel FMR1-RAF1 fusion. Virchows Arch. https://doi.org/10.1007/s00428-021-03178-3 (2021).

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

The institutional review board of Chang Gung Memorial Hospital approved this study (202002040B0).

ADDITIONAL INFORMATION

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