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Novel *EWSR1-SMAD3* Gene Fusions in a Group of Acral Fibroblastic Spindle Cell Neoplasms

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汇报 卫美辰

指导 韩 铭

2018.05.09

Fibroblastic / Myofibroblastic Tumours

Nodular fasciitis

Proliferative fasciitis and proliferative myositis

Myositis ossificans and fibro-osseous pseudotumour of digits

Ischaemic fasciitis

Elastofibroma

Fibrous hamartoma of infancy

Fibromatosis colli

Juvenile hyaline fibromatosis

Inclusion body fibromatosis

Fibroma of tendon sheath

Desmoplastic fibroblastoma

Mammary-type myofibroblastoma

Calcifying aponeurotic fibroma

Angiomyofibroblastoma

Cellular angiofibroma

Nuchal-type fibroma

Gardner fibroma

Calcifying fibrous tumour

Palmar/plantar fibromatosis

Desmoid-type fibromatosis

Lipofibromatosis

Giant cell fibroblastoma

Dermatofibrosarcoma protuberans

Extrapleural solitary fibrous tumour

Inflammatory myofibroblastic tumour

Low-grade myofibroblastic sarcoma

Myxoinflammatory fibroblastic sarcoma

Infantile fibrosarcoma

Adult fibrosarcoma

Myxofibrosarcoma

Low-grade fibromyxoid sarcoma

Sclerosing epithelioid fibrosarcoma

INTRODUCTION

- Benign/low-grade fibroblastic tumors are a diverse group of tumors with overlapping morphologies and clinical presentations that can pose diagnostic challenge due to their rarity and lack of a specific immunoprofile

- A challenging congenital fibroblastic lesion, which did not fit in any of the known pathologic entities, we have applied whole transcriptome sequencing for further genomic characterization.

- Thus a novel EWSR1-SMAD3 fusion was identified

MATERIALS AND METHODS

1. Index Case and Patient Selection

- Index Case
  - a 1-year-old boy presenting with a skin-colored papule since birth on the left heel
  - negative for SMA, desmin, caldesmon,HHF35, S100, CD34, panCK, and EMA
  - initially diagnosed as lipofibromatosis
  - RNAseq identificate the fusion candidate of the index case（EWSR1）, we performed fluorescence in situ hybridization (FISH) to validate the gene fusion

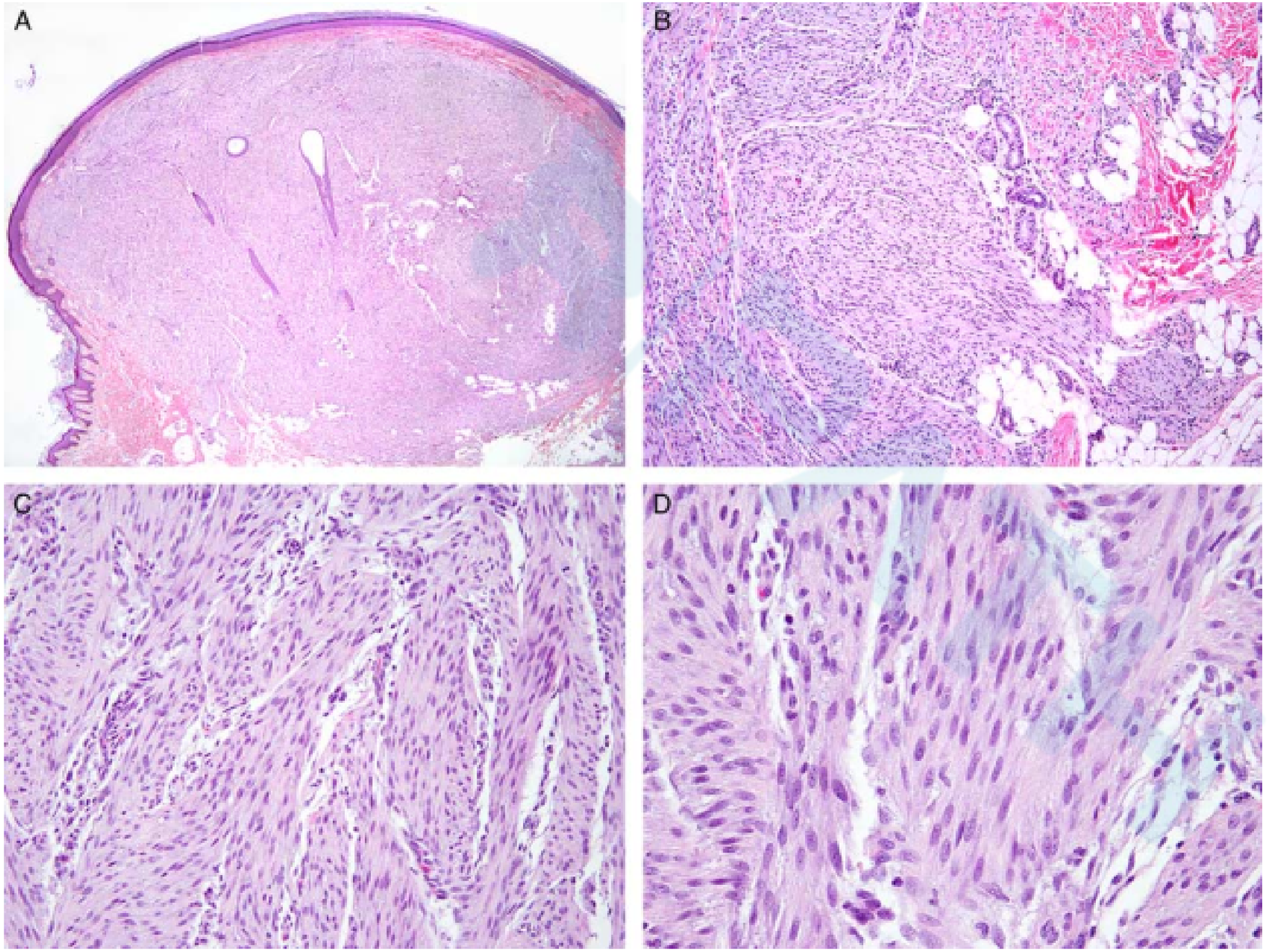


FIGURE 1. Histologic features of the index case, a heel tumor in a 1-year-old boy.

A and B, The tumor presented as a bulging nodule involving the dermis and subcutaneous tissues with infiltrative border.

C and D, It is composed of intersecting fascicles of uniform plump spindle cells with fibrillary cytoplasm and bland fusiform nuclei.

MATERIALS AND METHODS

1. Index Case and Patient Selection

- Following the identification of the index case, we screened 2 previously unclassified spindle cell neoplasms
  - bland and uniform cytomorphology
  - lack of a specific line of differentiation by immunohistochemistry
  - the presence of an EWSR1 gene rearrangement but unknown fusion partner

MATERIALS AND METHODS

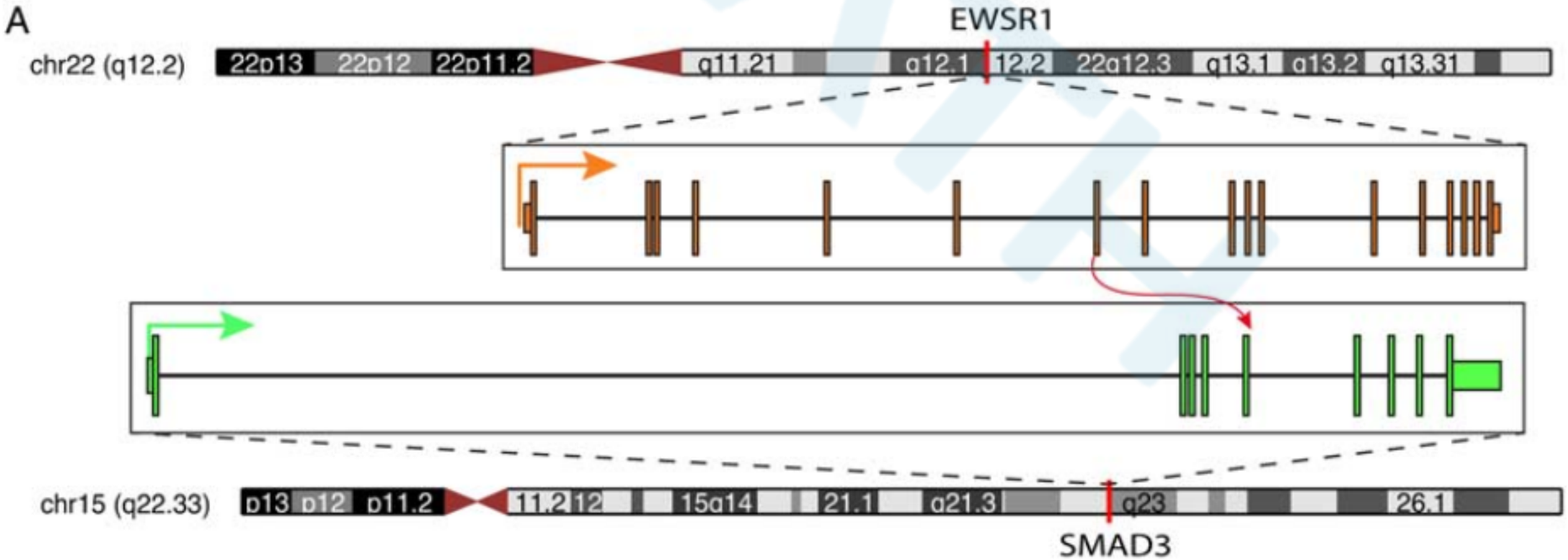
2. **Whole Transcriptome Sequencing and Analysis**

3. **Fluorescence in Situ Hybridization**

   – To validate the fusion candidates found by RNAseq, we performed FISH for EWSR1 and SMAD3 break apart.

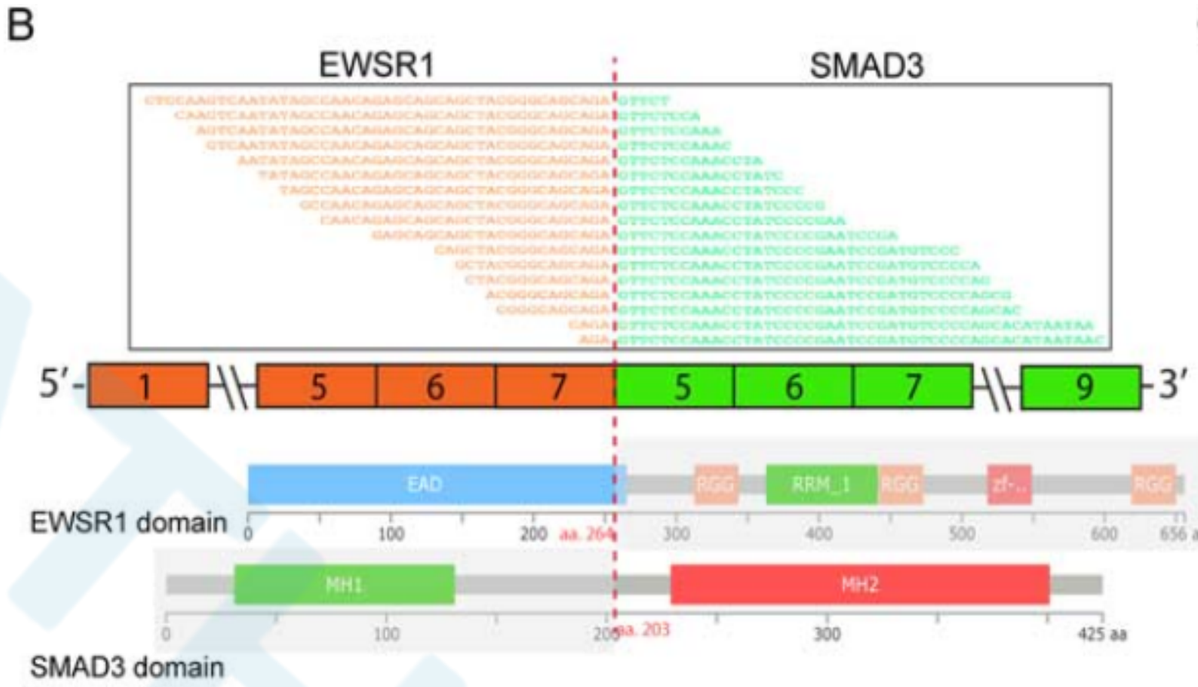
RESULTS

1. **Novel EWSR1-SMAD3 Fusion Identified in a Congenital Acral Spindle Cell Tumor**
   - exon 7 of EWSR1(22q12.2) fused to exon 5 of SMAD3 (15q22.33) ，resulting from a t(15;22)(q22.33;q12.2) was identified in the index case by whole transcriptome sequencing



RESULTS

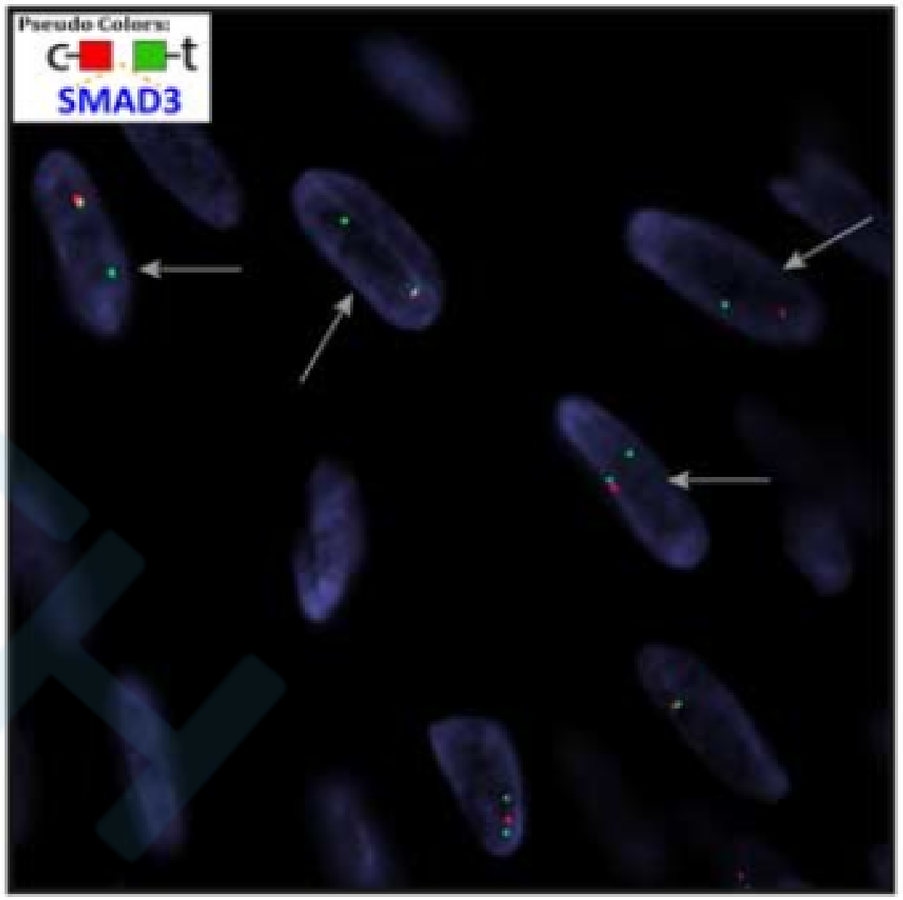
1. **Novel EWSR1-SMAD3 Fusion Identified in a Congenital Acral Spindle Cell Tumor**
   - The predicted chimeric amino acid sequence was in-frame and contained the N-terminal transcriptional activation domain of EWSR1 and part of the linker region and the entire MH2 (MAD homology 2) domain of SMAD3.



RESULTS

1. **Novel EWSR1-SMAD3 Fusion Identified in a Congenital Acral Spindle Cell Tumor**
   - The gene fusion was further confirmed in the index case by break-apart FISH assay of both genes. FISH showed unbalanced rearrangements, with telomeric deletion of EWSR1 (3′) and centromeric deletion of SMAD3 (5′)

C



RESULTS

2. **Unsupervised Clustering and Gene Expression Analysis are in Keeping With a Fibroblastic Lineage**
   - our index case clustered closely to other pediatric fibroblastic lesions available on the array

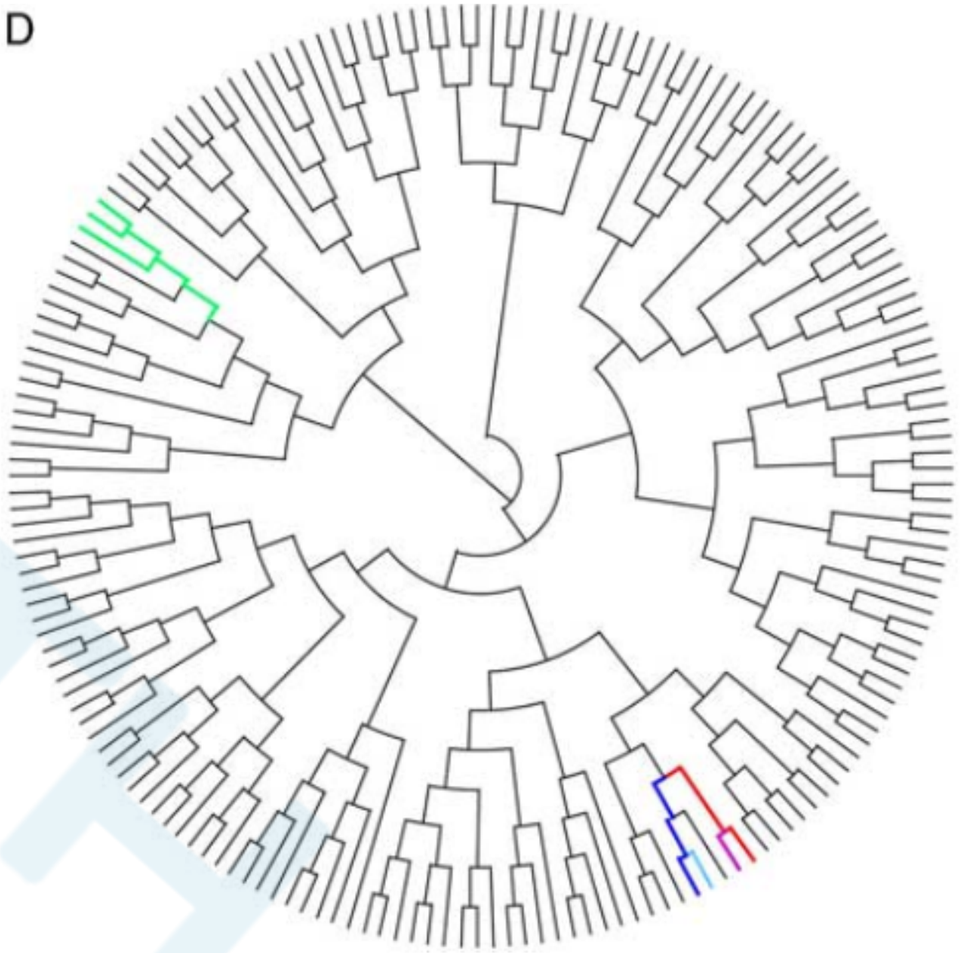


FIGURE 2. D.Unsupervised clustering of RNAseq data shows the index case (red) clusters with a calcifying aponeurotic fibroma with FN1-EGF fusion (purple), a lipofibromatosis (light blue), and a lipofibromatosis-like neural tumor with TPR-NTRK1 fusion (dark blue), among other soft tissue tumors

RESULTS

2. **Unsupervised Clustering and Gene Expression Analysis are in Keeping With a Fibroblastic Lineage**
   - the index case showed up-regulation of FN1 mRNA expression, similar to the calcifying aponeurotic fibroma harboring an FN1-EGF fusion and the lipofibromatosis-like neural tumor with TPR-NTRK1 fusion

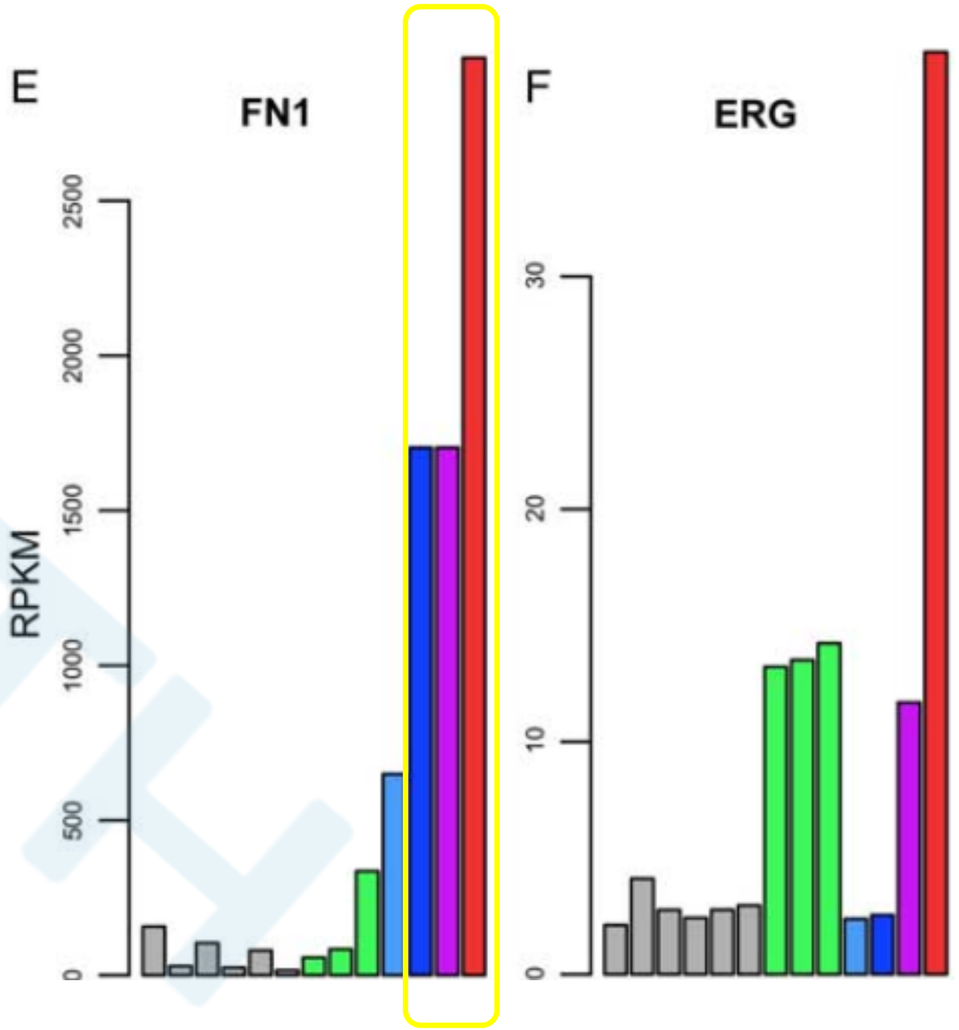
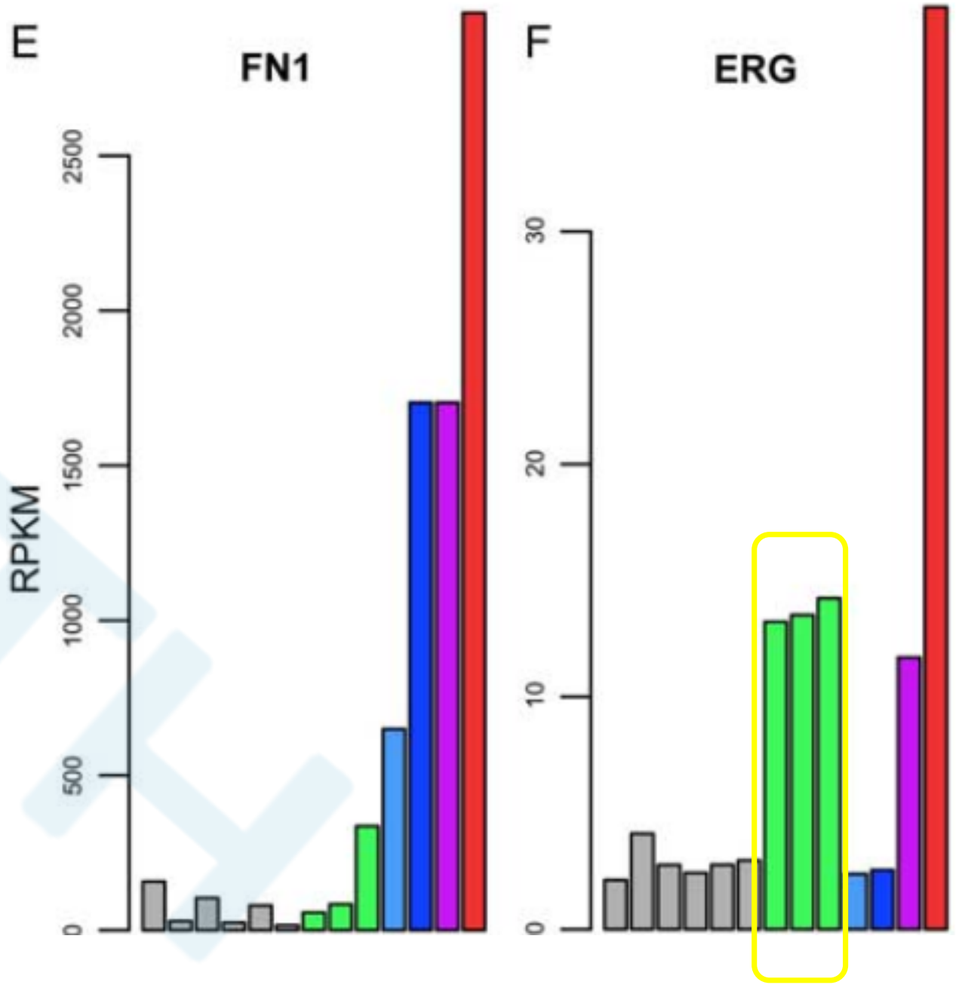


FIGURE 2. E, Left panel: high FN1 mRNA expression in the index case (red), calcifying aponeurotic fibroma (purple), and lipofibromatosis-like neural tumor (dark blue). Right panel ERG mRNA level is significantly upregulated in the index case (red), even at higher levels than 3 angiosarcomas in the same data set (green bars in D, E).

RESULTS

2. **Unsupervised Clustering and Gene Expression Analysis are in Keeping With a Fibroblastic Lineage**
   - ERG mRNA expression was significantly up-regulated in the index case, at even higher levels than 3 vascular neoplasms (2 head and neck and 1 breast angiosarcomas), which may explain the ERG positivity seen IHC



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RESULTS

3. Recurrent EWSR1-SMAD3 Fusions Were Detected in 2 Additional Acral Tumors in Adult Patients

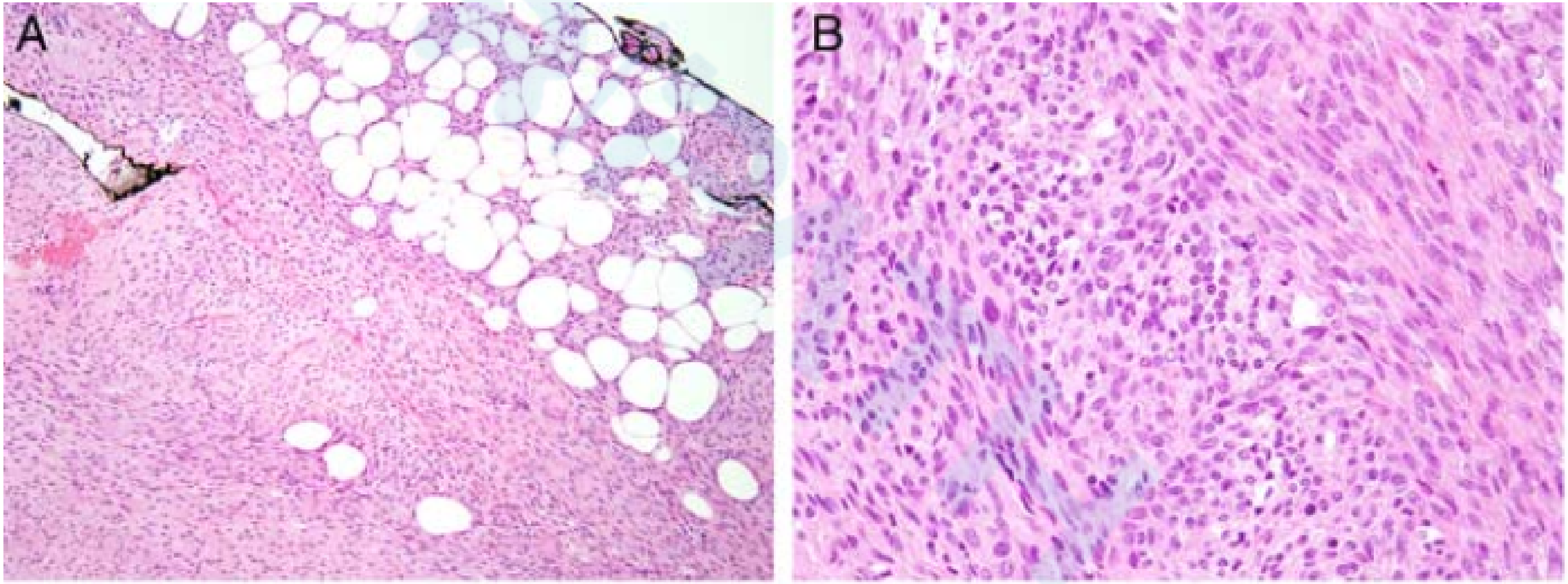
- Both cases # 2 and #3 were found to have EWSR1 gene rearrangements by FISH during a molecular work-up for a possible diagnosis of soft tissue myoepithelial tumor.
- However, a myoepithelial line of differentiation could not be confirmed by immunohistochemical studies.

TABLE 1. Clinicopathologic Features of Cases With *EWSR1-SMAD3* Fusions

					Immunohistochemistry				
Case #	Age (y)/Sex	Location	Depth	Size (cm)	ERG	CD34	SMA	S100	Follow-up
1	1/M	Heel	Dermis and subcutis	1.0	+	−	−	−	LR (14 mo)
2	61/F	Foot	Subcutis	2.0	+	−	−	−	NA
3	58/F	Toe	Dermis and subcutis	1.1	+	−	−	−	LR (5 mo)

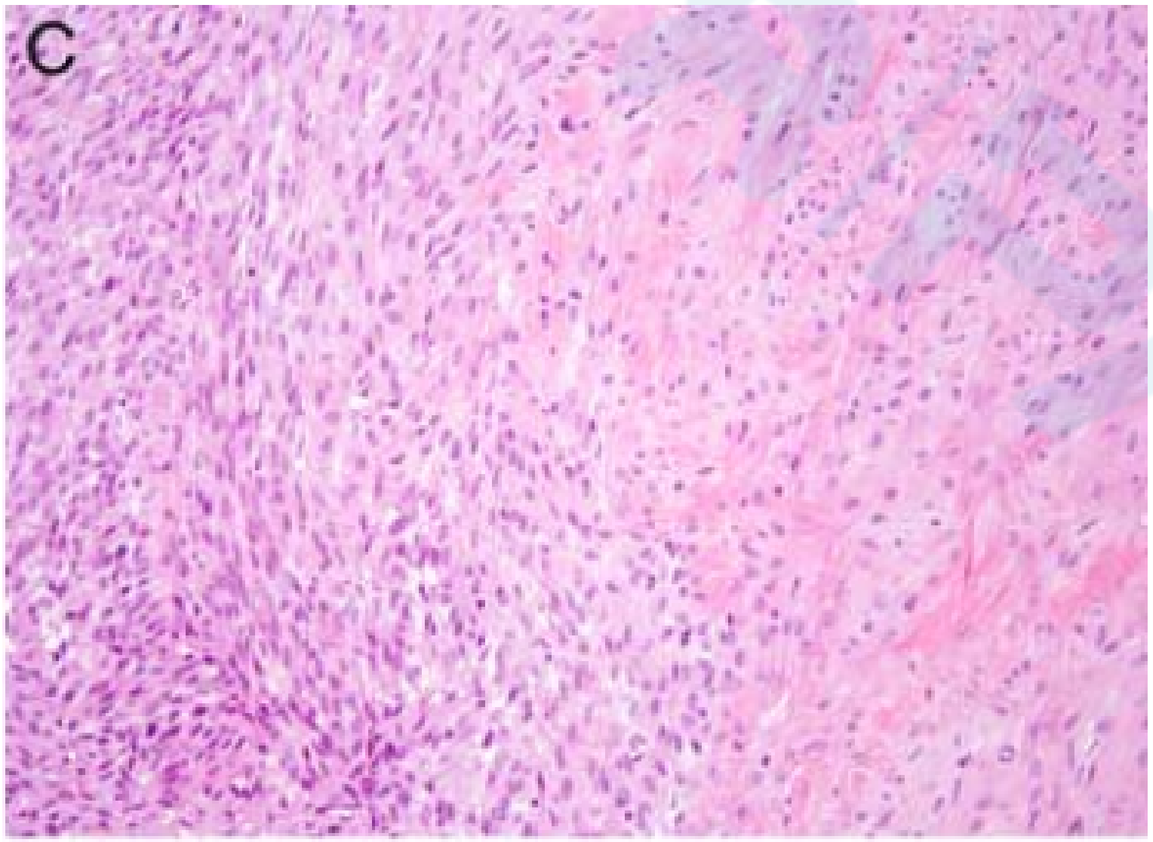
F indicates female; LR, local recurrence; M, male.

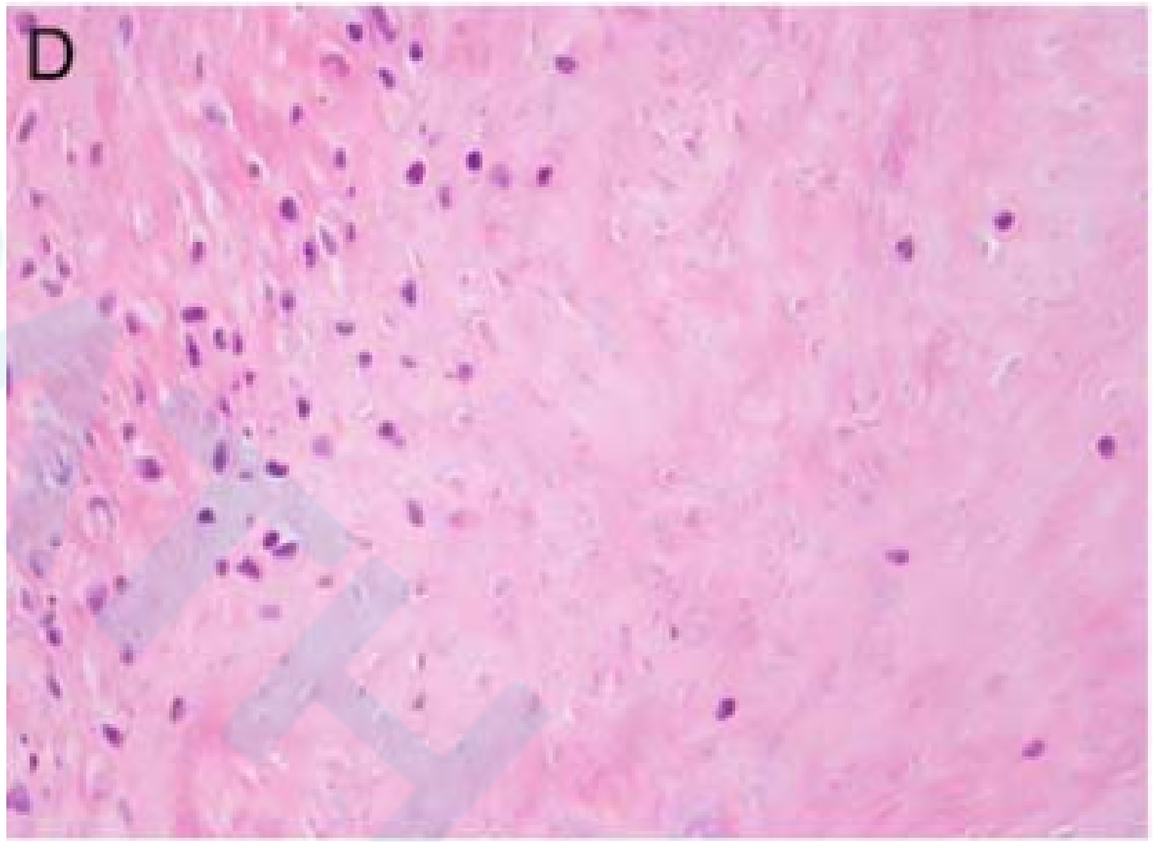
RESULTS



Case #2, a 61-year-old female;foot; a subcutaneous nodule showing an infiltrative border (A) and a distinctive zonation pattern with hypercellular periphery (B)

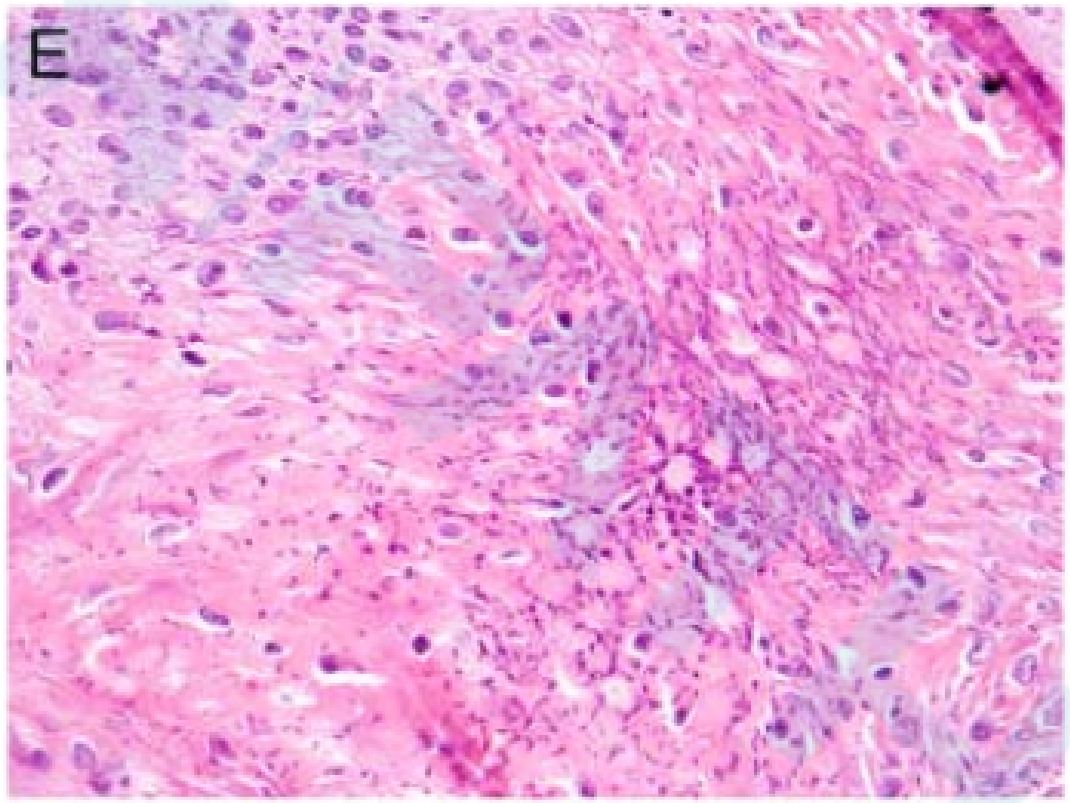
RESULTS





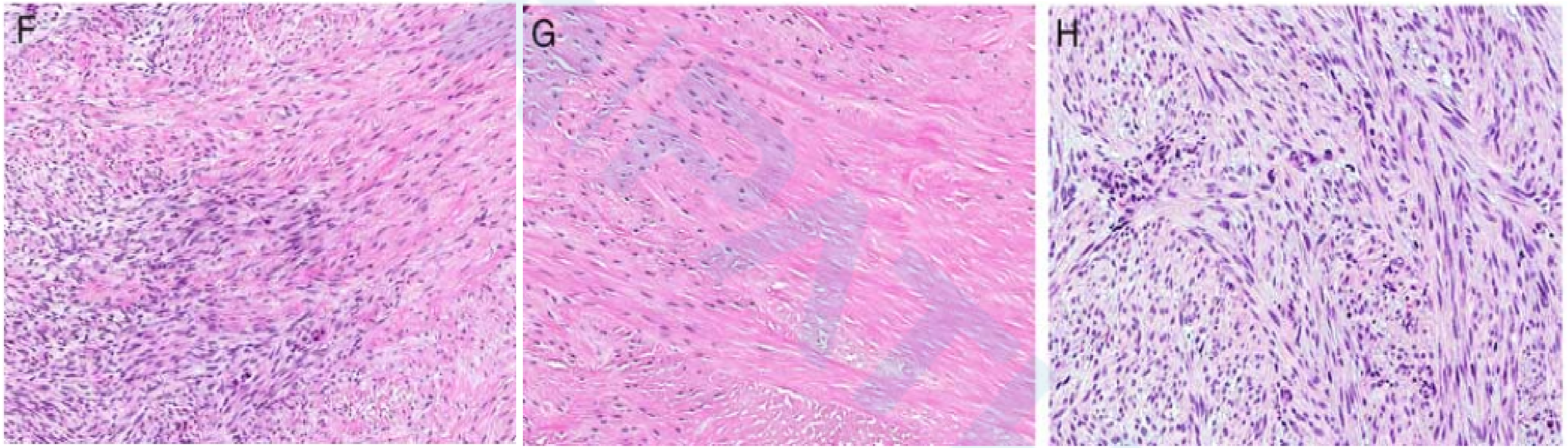
Case #2, transitioning to hypocellular collagenous area (C), and an acellular central zone (D)

RESULTS



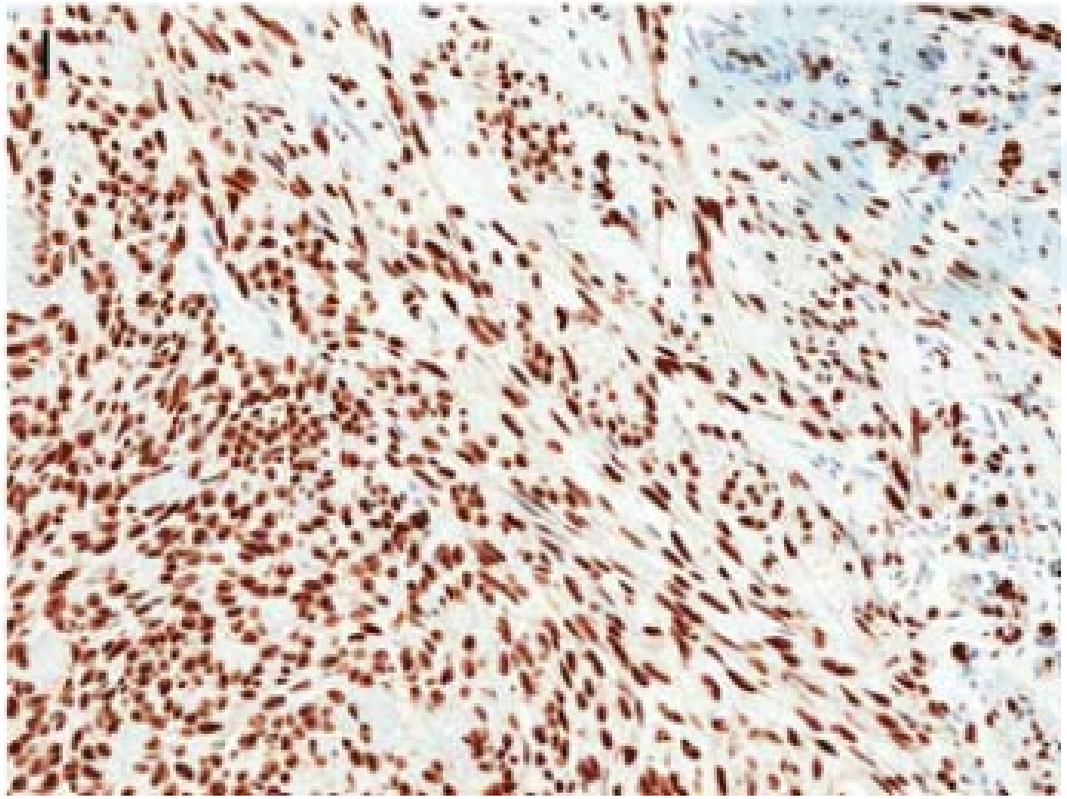
Case #2, E, Focal fine calcifications were also present

RESULTS



Case #3, a 58-year-old female, toe, displayed a similar zonation pattern in the primary lesion (F, G) and the cellular component only in the local recurrent lesion 5 months later (H).

RESULTS



The tumors lacked a specific line of differentiation, being negative for SMA, desmin, caldesmon, S100, and CD34.

Both cases showed diffuse and strong ERG nuclear staining similar to the index case (I)

Despite strong ERG immunoreactivity and ERG mRNA overexpression in the index case, **FISH showed no ERG break apart or other copy number abnormalities** in case #3.

RESULTS

4. EWSR1-SMAD3 Fusion Positive Tumors are Prone for Local Recurrence When Incompletely Excised

The tumors in all 3 cases were intralesionally or marginally excised with positive margins.

–Case #1 recurred as a 0.5 cm tumor at the junction of dermis and subcutis 14 months after the initial excision.

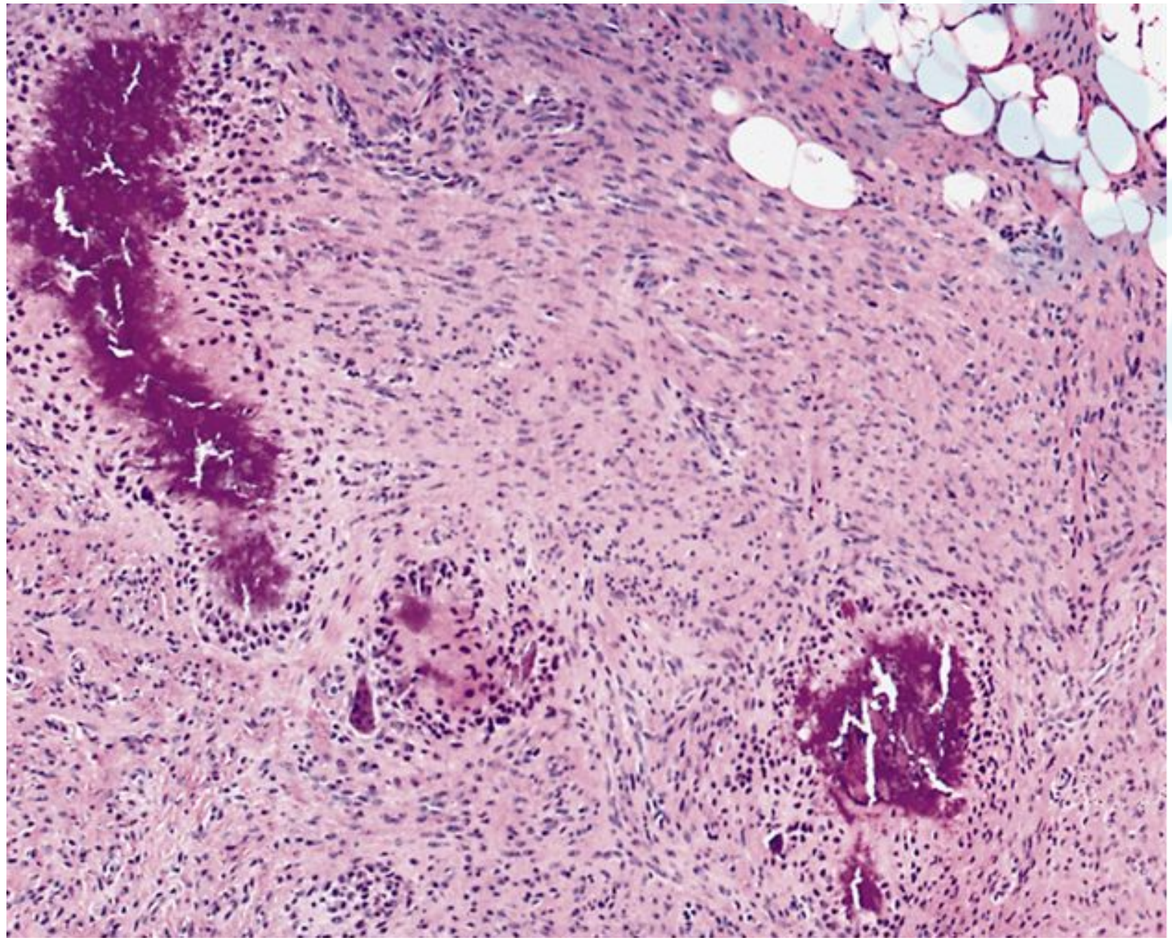
–Case #3 also recurred 5 months later, as a subcutaneous tumor with irregular borders, composed of cellular spindle cell fascicles without a hypocellular center

–No metastatic disease developed in these 2 patients with available follow-up

–No follow-up information was available for case #2.

DISCUSSION

1. Differential diagnosis——（1）calcifying aponeurotic fibroma



Positive： SMA

Sometimes: EMA S100

Negative： CD34, Desmin , β-catenin

DISCUSSION

1. **Differential diagnosis——（2）lipofibromatosis**

   – All 3 cases showed limited adipose tissue infiltration compared with the typical long dissecting fascicles of fibroblastic cells seen in lipofibromatosis.

DISCUSSION

1. Differential diagnosis——（2）lipofibromatosis

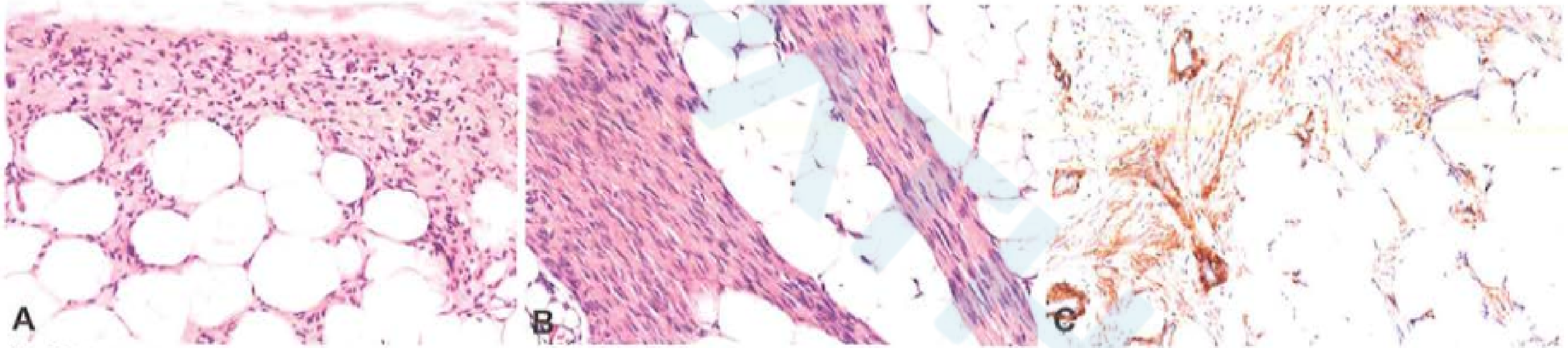


Fig. 3.053 Lipofibromatosis. **A** The spindle-cell component is bland and may have a rather primitive fibroblastic appearance. **B** Fascicular growth of the fibroblastic element. **C** Focally positive immunostaining for SMA, consistent with fibroblastic/myofibroblastic differentiation.

DISCUSSION

1. **Differential diagnosis——（3）lipofibromatosis-like neural tumors with NTRK1 fusion**

   – the index case was negative for both S100 and CD34 immunostains

   – Additional immunostains performed showed negative SOX10 in 2 cases and NTRK1 in case #3.

Recurrent NTRK1 Gene Fusions Define A Novel Subset Of Locally Aggressive Lipofibromatosis-Like Neural Tumors

Am J Surg Pathol. 2016 October ; 40(10): 1407–1416.

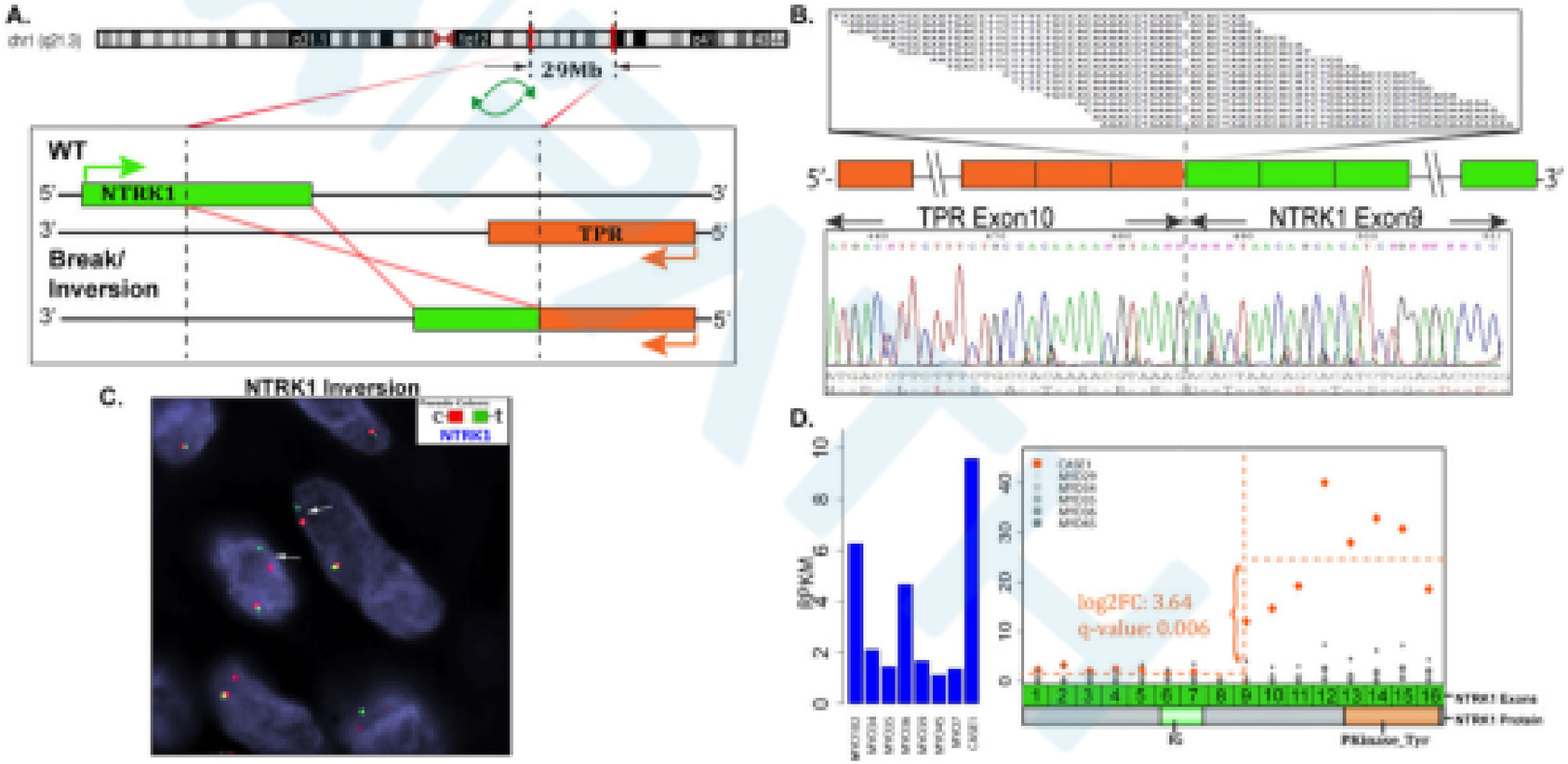


Figure 1. *TPR-NTRK1* gene fusion in LPF-like NT (case 1)

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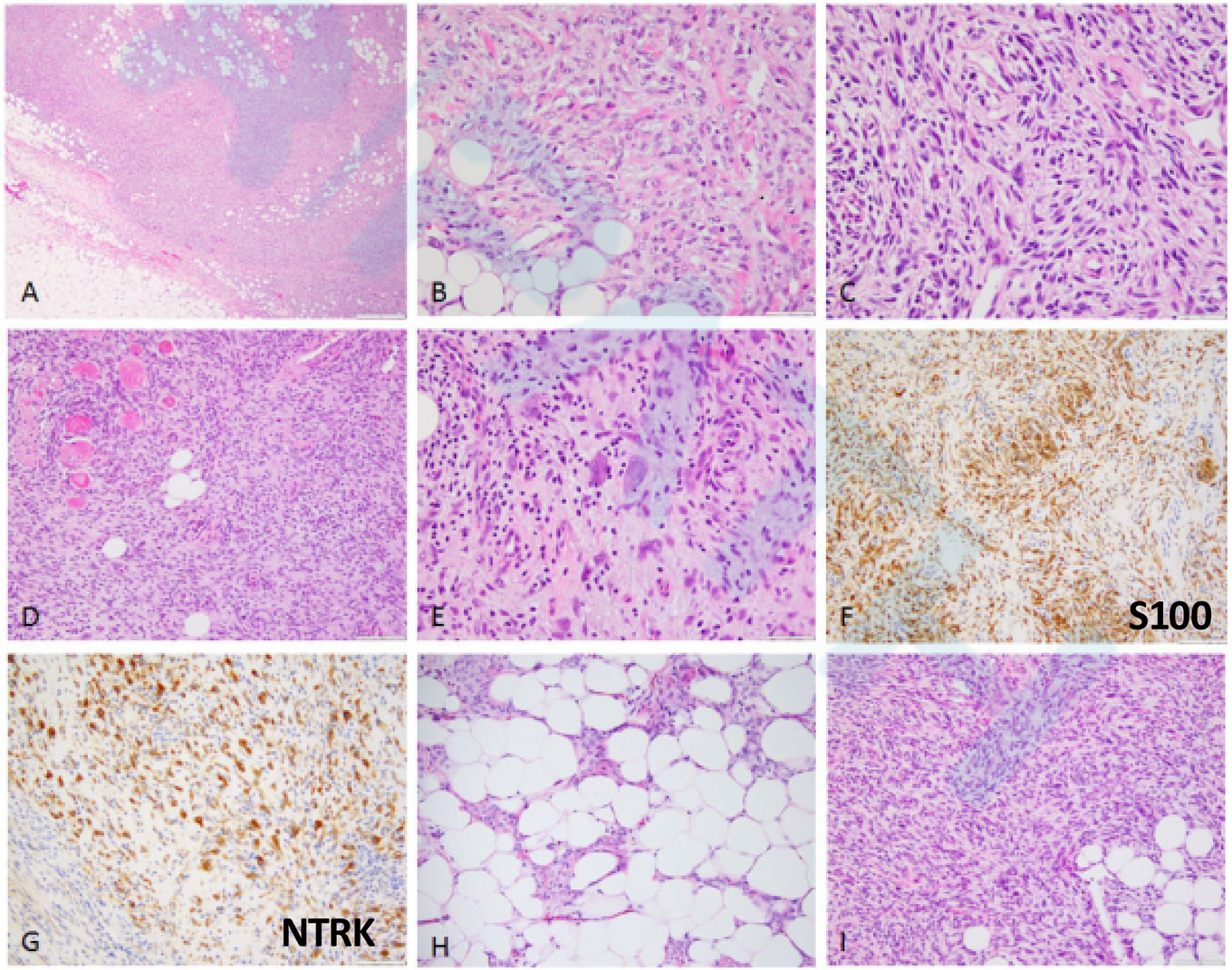
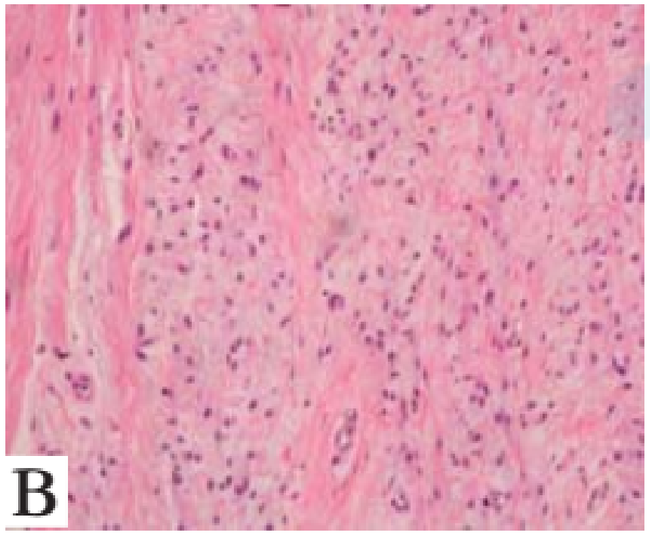


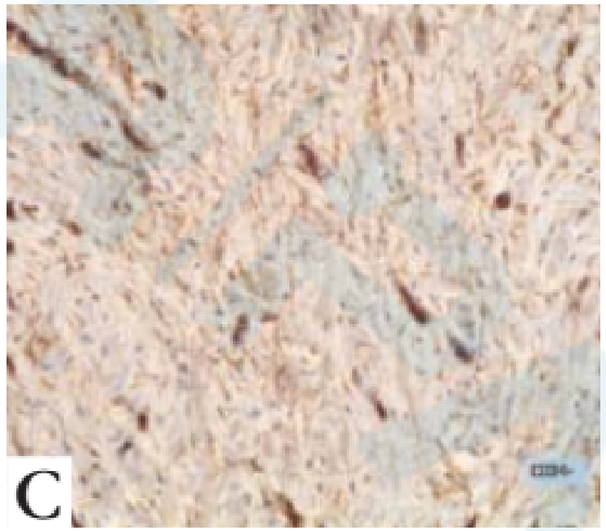
DISCUSSION

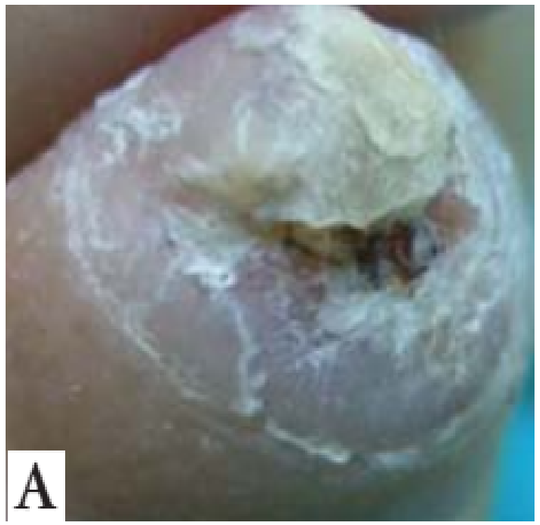
1. **Differential diagnosis——（4）superficial acral fibromyxoma**

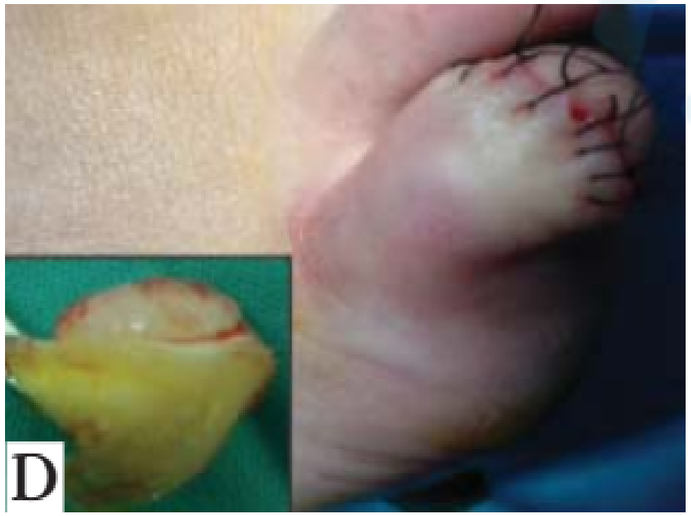
   – the lesions in our study cohort showed a distinct fascicular growth pattern and lacked myxoid stroma

Superficial Acral Fibromyxoma involving the nail's apparatus. Case report and literature review









An Bras Dermatol. 2014;89(1):147-9.

A: Loss of the distal end of the 5th toe of the right foot and partial onycholysis;

B: Fusiform cell proliferation in a myxoid stroma (HE,x100);

C: Fusiform cells show cytoplasmic positivity to CD34 (PAP, x200);

D: Image after excision. The macroscopic part of the tumor along with nail apparatus can be seen

DISCUSSION

1. **Differential diagnosis——（5）infantile digital fibroma**

   – our index case do not present cytoplasmic inclusions

Congenital infantile digital fibromatosis: a case report and review of the literature

Rare Tumors 2009; volume 1:e47

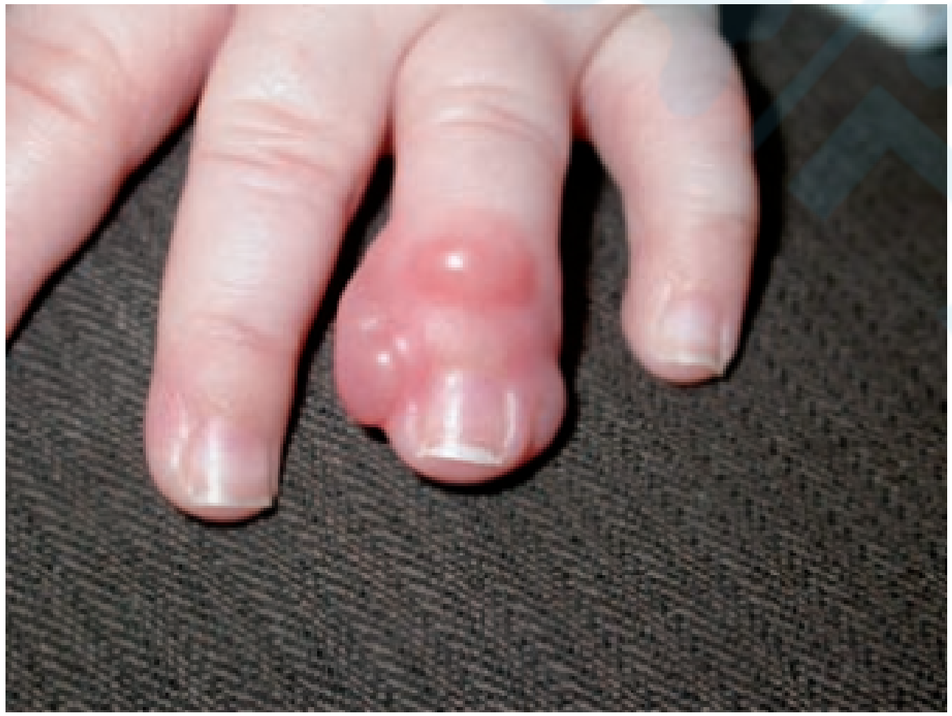
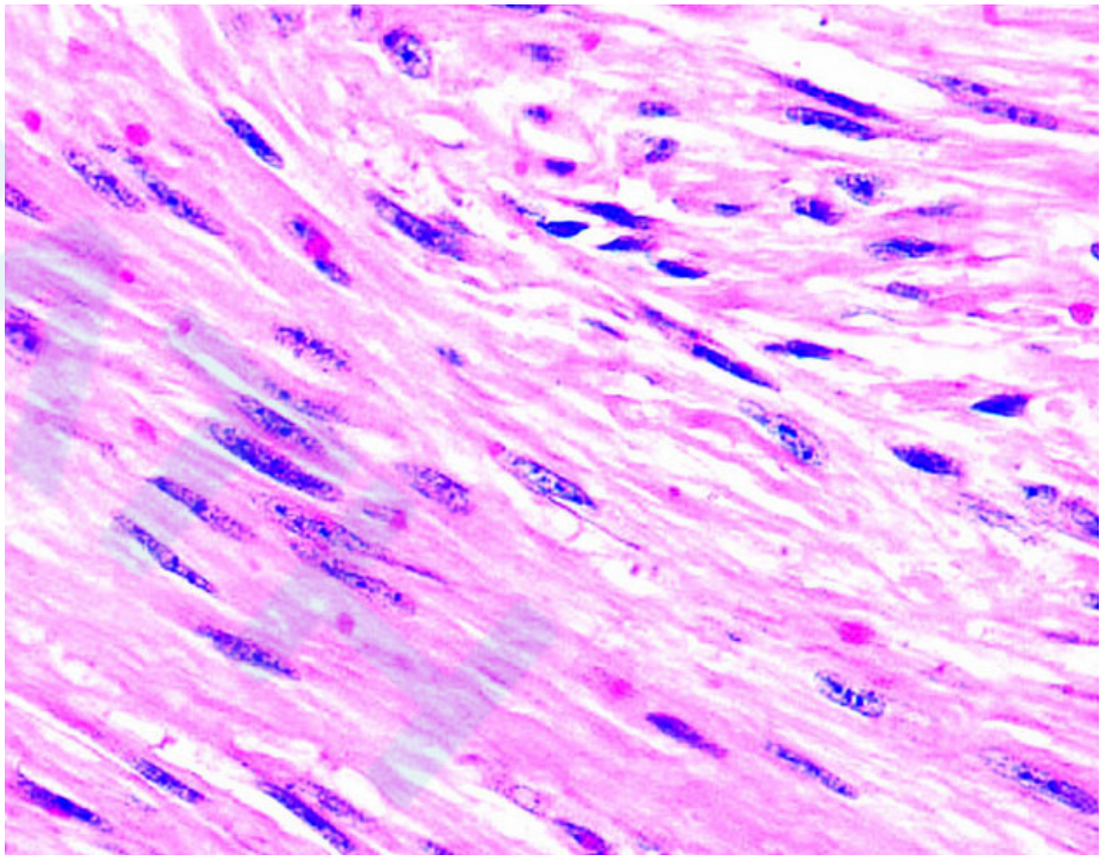


Figure 1. Infantile digital fibromatosis affecting the fourth digit.



DISCUSSION

1. **Differential diagnosis——myofibroblastic line**

   – The lack of staining for muscle markers further excluded a myofibroblastic line of differentiation

DISCUSSION

1. Differential diagnosis

– The 2 adult cases displayed **a peculiar zonation pattern**, with an acellular center and cellular peripheral areas, distinctive from most other tumors considered in the differential diagnosis

DISCUSSION

2. **The consistent diffuse and strong ERG immunostaining in all 3 cases**
   - ERG ——— vascular and cartilaginous differentiation
   - often positive in Ewing sarcomas with the variant EWSR1-ERG gene fusion
   - the RNAseq data from the index case showed corresponding high levels of ERG mRNA levels; however, this upregulation was not due to gene amplifications or gene rearrangements, as FISH was negative in 1 case tested.
   - Thus, the mechanism of aberrant ERG expression in this subset of EWSR1-SMAD3 fusion positive fibroblastic tumors remains elusive.

DISCUSSION

3. SMAD3 gene

- a novel EWSR1 fusion partner in translocation-associated neoplasia
- an important signal transducer in the TGF-β/SMAD signaling pathway, which is involved in extracellular matrix synthesis by fibroblasts.
- Dysfunction of SMAD family is associated with scleroderma, renal fibrosis, and radiation-induced fibrosis.
- Therefore，SMAD3 gene abnormalities through chromosomal translocation is also in keeping with the presumed fibroblastic differentiation

SUMMARY

- We report recurrent EWSR1-SMAD3 gene fusions in a distinctive group of acral superficial tumors with presumed fibroblastic lineage and local recurrence potential.

- Despite an otherwise nonspecific immunoprofile, diffuse ERG expression.

- At transcriptional level, the tumors also show overexpression of FN1 (fibronectin)

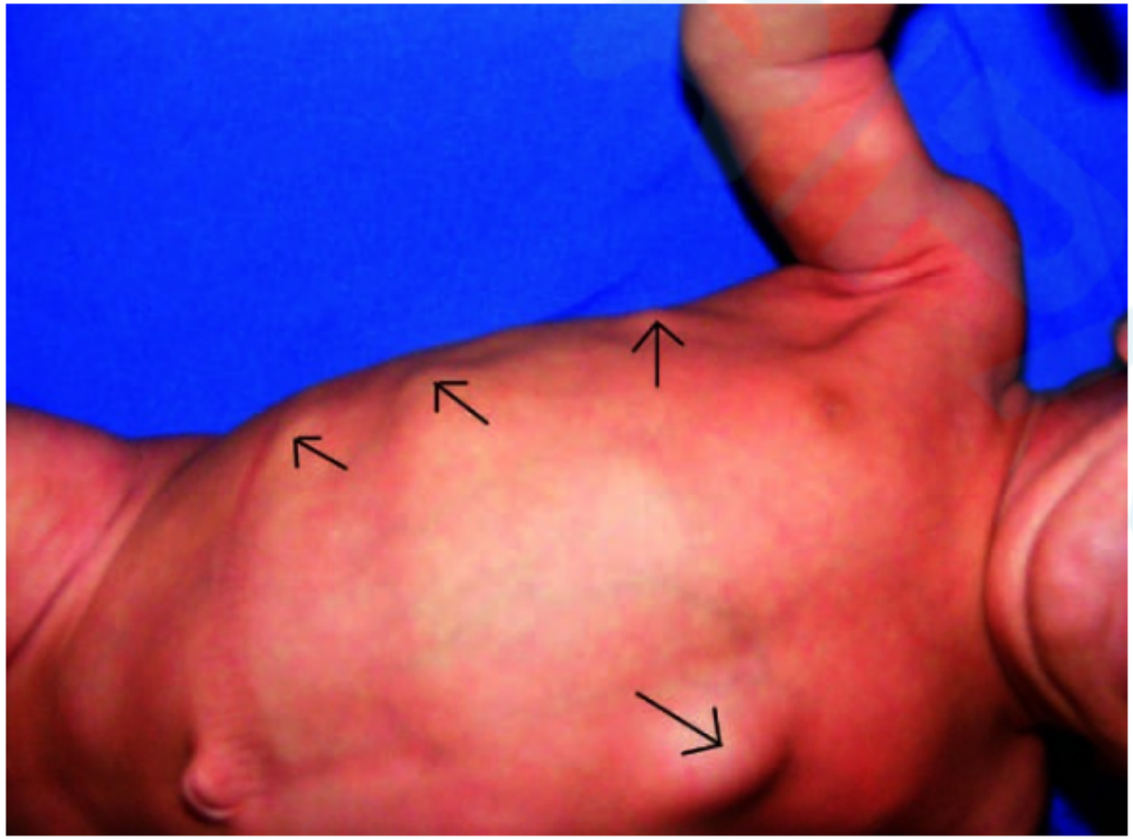
SUMMARY

- Dysfunction of SMAD family, previously associated with extracellular matrix abnormalities, such as scleroderma and renal fibrosis, are now implicated for the first time in translocation-associated mesenchymal neoplasia.

- Their similar morphology, anatomic location, and novel EWSR1-SMAD3 gene fusion strongly suggest that these tumors represent a novel and distinct entity.

THANK YOU

Infantile Digital Fibromatosis (Inclusion Body Fibromatosis) Observed in a Baby Without Finger Involvement



IDF can be observed in babies without finger involvement.
SMA was positive; desmin, S100, and CD34 were negative;
No staining with PAS was observed.

Indian J Dermatol. 2013 Mar-Apr; 58(2): 160.

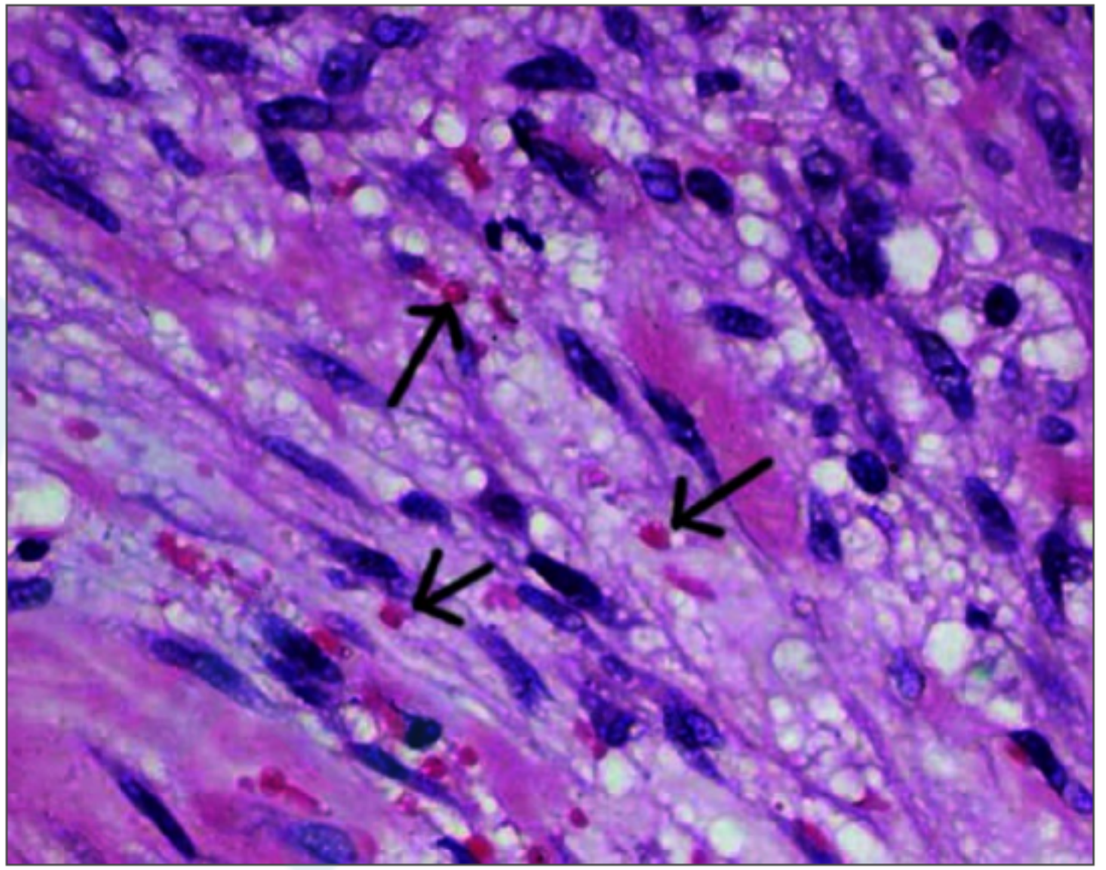


Figure 3

The plump spindle cells, uniform nuclei and scattered intracytoplasmic eosinophilic round inclusions (H and E, × 400)

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(D) cellular LPF-like NT infiltrating fat and skeletal muscle, which at higher power
(E) showed scattered pleomorphic nuclei and was strongly and diffusely positive for (F) S100

positive for **S100, SMA and CD34**； negative for desmin, GFAP, HMB45, Melan A and SOX10

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(G)Diffuse NTRK1 immunostaining (Case 6, LMNA-NTRK1);
(H) Spindle cells intimately associated with adipose tissue (LPF-like, case 14);
(I) densely cellular lesion with streaming fascicles infiltrating fat (Case 9, NTRK1 rearrangement positive).

DISCUSSION

3. SMAD3 gene

- SMAD3 mRNA expression level is not significantly upregulated in the index case
  - the truncation of the SMAD3 protein due to the translocation event might lead to abnormal localization and functional alterations of its downstream transcriptional targets
- In our index case, the mRNA expression levels of several SMAD3 target collagen genes, including COL1A1, COL1A2, COL3A1, and COL6A3, were also up-regulated