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Case Report

New *PLAG1*-fusion transcripts in the spectrum of pediatric fibrotic, lipofibrotic, and mature lipomatous tumors

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Abstract: The histomorphology of liboblastoma is highly variable and comprises different patterns that are found admixed or in pure form within a tumor. The most important features - mature lipomatous, fibrotic, lipofibrous, and myxoid - overlap with the histomorphology of several other pediatric tumor entities. Regarding the morphologic overlaps, molecular diagnostics with identification of fusion transcripts involving *PLAG1* or *HMGA2* is essential to identify lipoblastomas. This paper describes the diagnostic procedure in general and two new fusion transcripts of lipoblastoma, *MEG3-PLAG1* and *COL1A1-PLAG1*. In conclusion, the algorithm to diagnose lipoblastomas among this group of pediatric fibrotic, lipofibrous and mature lipomatous tumors essentially includes histomorphology, immunohistochemistry, and molecular diagnostics.

Keywords: Lipoblastoma, molecularpathology, pediatric tumor, *PLAG1*-fusion

Introduction

The group of lipofibrous lesions includes fibrous hamartoma of infancy, fibrolipoma, lipofibromatosis, juvenile (infantile) fibromatosis (CF), hemosiderotic fibrolipomatous tumor (HFLT), lipoblastoma-like tumor (LLT), and lipoblastoma. All these entities are rare, but with the exception of HFLT, they peak in infancy and therefore are addressed as 'benign pediatric lipofibrotic soft tissue tumors'. They have a strong histomorphologic overlap with regard to main cellular and structural components, including adipocytes, spindled fibroblastic elements, and collagenous/fibrous matrix. However, there are distinct histomorphologic and especially molecular features to separate these entities from each other (**Table 1**).

The HFTL is characterized by more characteristic histomorphologic and molecular findings and seen in adults, but also in infants. A 'paucihemosiderotic' variant of the disease was recently described [1]. The CF, including the morphological subtypes diffuse/mesenchymal, desmoid, and fibroblastic, is a lesion with predominant fibroblastic differentiation. The differences in the extension of fibrocollagenous material and adipose tissue are probably attributable to the duration of the disease and the degree of maturation [2]. The LLT peaks in adults and frequently affects the vulva. The tumor has strong microscopic similarities to lipoblastoma, but the molecular background is different.

Lipoblastoma/lipoblastomatosis also belongs to the group of benign lipofibrous lesions. The localized subtype is called lipoblastoma, whereas the term lipoblastomatosis describes the diffuse, infiltrative subtype, which is more associated with tumor recurrence. Lipoblastoma is most commonly seen in children and infants, with about 80% of cases occuring before the age of 3 years and a slight male predilection. Frequently, the tumor is found on the lower

Table 1. Characteristics of myxoid liposarcoma and entities in the spectrum of pediatric fibrotic, lipofibrotic, and mature lipomatous tumors (alphabetical order)

entity	main location	histomorphology	molecular biology
fibrolipoma	heterogeneous	mature adipose tissue and fibrotic septa without cellular immaturity	no characteristic pattern
fibrous hamartoma of infancy (FH)	heterogeneous	primitive nodular fibromyxoid component, no lipoblasts/pseudolipoblasts	*EGFR* mutations
hemosiderotic fibrolipomatous tumor (HFLT)	dorsum of foot, ankle, hand region	hemosiderin deposits and hemosiderin-laden spindle cells within the fibroadipose tissue	recurrent balanced or unbalanced translocation t(1;10)(p22-p31;q24-q25) affecting *TGFBR3* and *OGA* - amplification of *VGLL3* locus on chromosome 3p12.1
juvenile (infantile) conventional fibromatosis (CF)	fascia and skeletal muscle of head and neck	solid sheet-like growth, extensive architectural effacement of fat	heterogeneous
lipoblastoma	extremities, trunk	fat lobules and fibrotic septa with or without myxoid change and cellular immaturity	rearrangement of *PLAG1* or *HMGA2*
lipoblastoma-like tumor	vulva	mature adipocytes interspersed with spindle cells	no rearrangement of *PLAG1* or *DDIT3* or *HMGA2*; lack of alterations in *MDM2*, *NCOA2* or *FOXO1*
lipofibromatosis	hand, feet; subcutis or deep soft tissues	disorganized adipose tissue dominates; no wire-like fibrocollagenous connective tissue or lipoblasts	heterogeneous (activation of the PI3K/AKT/mTOR pathway and others)
myxoid liposarcoma	deep soft tissue of extremities	primitive non-lipogenic round cells and small signet ring lipoblasts in a myxoid background, plexiform ('chicken wire') vasculature	rearrangement of *DDIT3*

extremities and trunk; rare locations include the retroperitoneum [3]. The histomorphology of lipoblastomas is highly diverse and includes a spectrum from fibrotic to mature lipomatous [4]. The tumor shows only a relatively uncharacteristic immunophenotype with variable expression of desmin by stromal cells and S-100 protein synthesis in lipogenic elements.

There is some evidence that transcriptional upregulation by the rearrangement/fusion of *PLAG1*, encoding a zinc finger transcription factor, with another gene (e.g. *COL3A1*, *CHCHD7*) is involved in the molecular pathogenesis of lipoblastoma [5, 6]. Other than *PLAG1*, the gene *HMGA2*, encoding a protein essential in the enhanceosome, can be upregulated by rearrangement/fusion with *FGD6*, *EP400,* or other genes [7]. These molecular alterations are frequently found in lipoblastomas and are of great diagnostic interest in view of the highly variable histomorphology of these tumors.

Important, myxoid liposarcoma is the most relevant malignant differential diagnosis of lipoblastoma and related neoplasias in the spectrum of pediatric lipofibrotic and mature lipomatous tumors. Adipocytic cells in different stages of maturation and a fine plexiform capillary network are found in myxoid liposarcoma as well as lipoblastoma, but a lobular pattern of growth, signet ring lipoblasts, as well as the molecular background with presence of *DDIT3* gene fusions, give evidence for liposarcoma [8].

We aim to explore and describe morphologic and molecular criteria in the diagnostic algorithm of lipomatous neoplastic lesions with a focus on lipoblastoma and report hereby two novel fusion transcripts of *PLAG1*.

Fibrotic lipoblastoma

Case 1

A tumor of unknown origin located near the right axilla was removed from a 1-year-old female infant. There were no congenital abnormalities or any evidence of a tumor at birth. Familial history was unremarkable with regard to neoplasms.

Grossly, the tumor appeared as a 6.5 × 4.3 × 2.4 cm mass (37 g) with well-defined borders and small amounts of skeletal muscle focally adherent to the capsule. After inspection, the tumor was cut into thin sections, each approximately 0.2 cm thick. The cut surfaces were almost entirely fibrotic, with sparse, slightly inhomogeneous glassy mucoid areas up to 0.6 cm (**Figure 1**).

Microscopically, the completely embedded tumor, showed an almost entirely fibrotic, hypo-

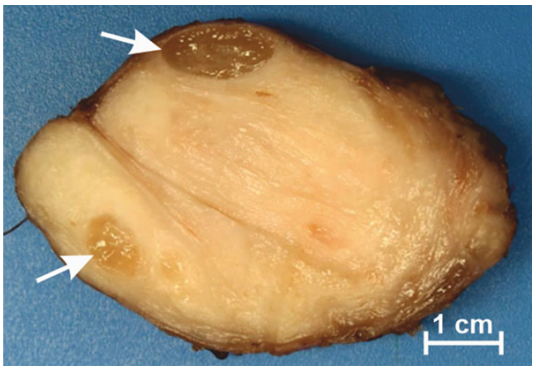

Figure 1. Gross photograph of fibrotic lipoblastoma with few mucoid islands (arrows).

cellular tissue. The small inhomogeneous areas within the fibrotic tissue consisted of myxoid material with fibrotic septa, few thin-walled vessels, some inconspicuous stromal cells, and sparse lipogenic cells in different stages of maturation. The histologic findings were highly suspicious for lipoblastoma (**Figure 2A**). The immunostaining revealed the characteristic pattern with S-100-positive adipocytes (**Figure 2B**) and desmin-positive stromal cells (**Figure 2C**). Due to the rather characteristic histomorphology of the mucoid areas with lipogenic cells adjacent to fibrotic tissues (**Figure 2D**), from the morphologic point of view, the spectrum of possible differential diagnoses was highly limited.

In order to further validate the diagnosis, a molecular analysis of formalin-fixed and paraffin-embedded tumor tissues was performed using targeted RNA sequencing strategies (QIAseq™ targeted RNAscan custom Panel; Qiagen) running on a MiSeq (Illumina). A characteristic *PLAG1* fusion (involving the translocation partner *MEG3*) was found, confirming the diagnosis of a fibrotic lipoblastoma. To our knowledge, this fusion transcript of *PLAG1* has not been reported so far.

Mature lipomatous lipoblastoma

Case 2

A resection specimen of a 10 × 6 × 3 cm tumor (80 g) of the lumbar region of a 2-year-old boy was submitted for histologic workup. The anamnestic data about congenital abnormalities or familial neoplastic diseases were unremarkable.

Grossly, the tumor comprised of lipomatous tissue enclosed by a thin fibrous capsule. In fine lamellas of the tumor, few fibrotic septa and areas were visible within the lipomatous tissue, while mucoid change, hemorrhage, or necrosis were absent.

Microscopically, mature adipocytes with large lipid vacuoles and marginalized nuclei dominated. Frequently, the adipocytes were separated by fibrotic septa and fibroblastic fascicles with an increase in stromal cells (**Figure 3A**). These cells lacked atypia. Within the lipofibrous tissue only a few foci with a minor increase in myxoid substances were identified. Myxoid change was not accompanied by occurence of lipoblasts (**Figure 3B**). The features of adipocytes intermingled with fibrotic septa were suspicious for lipoblastoma.

Using targeted RNA sequencing as described above, a fusion transcript *COL1A1-PLAG1* was identified, which is different from the *PLAG1* fusions in lipoblastomas published so far.

Discussion

Lipoblastomas are rare benign soft tissue tumors histogenetically derived from embryonal white fat and preferentially occur in infants. Microscopically, lipoblastomas show a lobular architecture including mature adipocytes arranged in sheets and intermingled with monovacuolated and multivacuolated lipoblasts, spindled mesenchymal cells, and less differentiated cells of embryonal white fat. Frequently, the adipocytic cell types are admixed with myxoid areas comprised of primitive mesenchymal cells, fibrous septa, and thin-walled vessels. The proportion of these matrix components/structures as well as these different cell types are highly variable within a tumor and from case to case. Unfortunately, the highly variable histomorphology of lipoblastomas is accompanied by a relatively nonspecific immunophenotype (variable expression of desmin). Lipoblastomas have been subdivided into a lipoma-like (mature lipomatous), a classic, and a myxoid subtype [9].

It is suggested that lipoblastomas are able to mature, which is probably associated with a substitution of mature lipomatous adipocytic and mesenchymal cells by connective tissues determining fibrolipomatous or fibrotic fea-

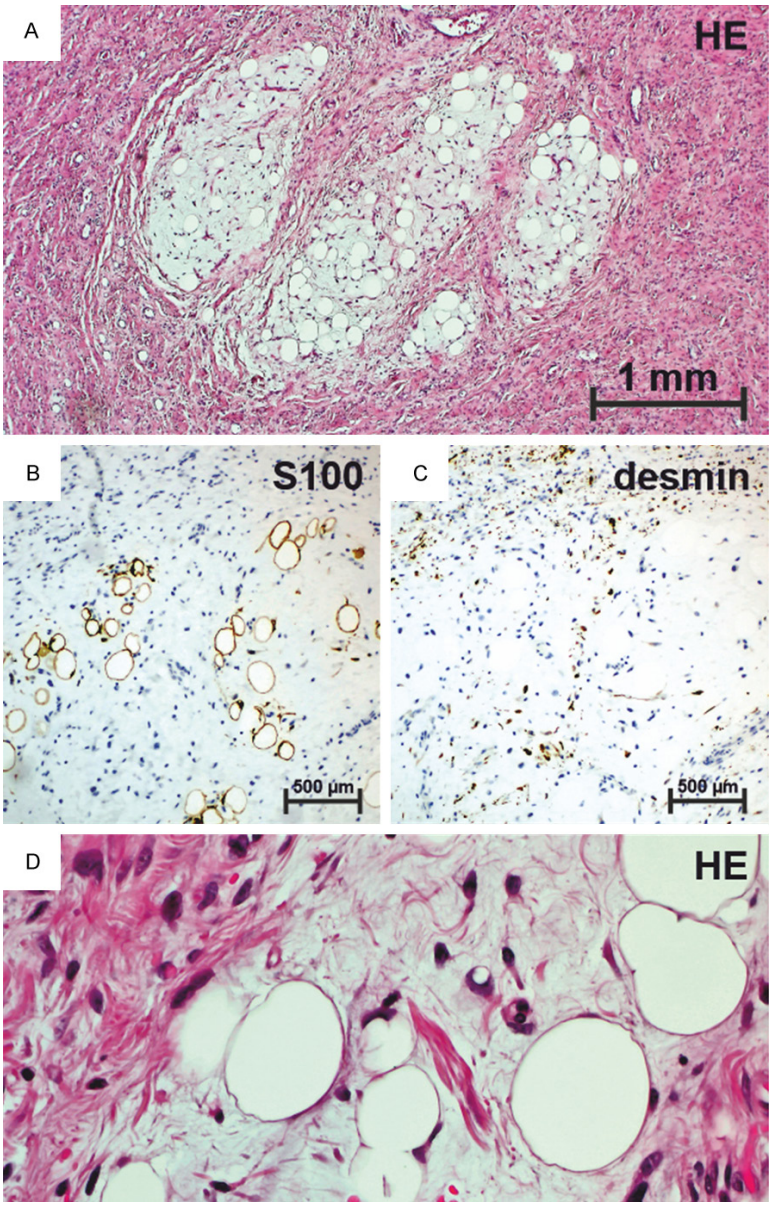

Figure 2. Histomorphology and immunohistochemistry of mucoid islands in fibrotic lipoblastoma (fusion transcript *MEG3-PLAG1*). (A) The lipogenic tissue is dominated by a fibrotic prolifertaion with small vessels and non-atypical stromal cells. The lipogenic cells are embedded in a myxoid matrix. Immunostaining of S-100 marks the lipogenic cells (B), whereas the stromal cells are positive for desmin (C). (D) The characteristic feature of well-formed lipogenic cells in a myxoid matrix adjacent to fibrotic tissue with stromal cells.

tures, that show similarities and overlap with the histomorphology of other pediatric fibrotic or lipofibrous neoplasms [4].

Regarding the histologic overlaps between the different entities, including the lipoblastoma-like tumor [10], molecular diagnostics is essential to definitively confirm the diagnosis of lipoblastomas. In a large number of molecular studies, lipoblastomas were associated with occurence of fusion transcripts, preferentially *PLAG1* with fusion partners as *COL1A2*, *COL3A1*, *HAS2*, *DDX6*, *KLF10*, *KANSL1L* or *HMGA2* with *FGD6* or *EP400* as fusion partners [5, 7]. Based on these observations it is suggested that the molecular finding of fusion transcripts involving *PLAG1* or *HMGA2* is essential in the differential diagnosis of pediatric fibrotic, lipofibrous, and mature lipomatous tumors. Unfortunately, a strong genotype (i.e. fusion-transcript) to phenotype correlation in lipoblastomas has not been identified.

Recently, the number of fusion transcripts identified in lipoblastomas has increased considerably. In particular, *SRSF3*, *HNRNPC*, *PCMTD1*, *YWHAZ*, *CTDSP2*, and *PPP2R2A* were identified as possible fusion partners of *PLAG1* [5]. We report two additional, hitherto undescribed fusion transcripts in lipoblastomas, *COL1A1-PLAG1* and *MEG3-PLAG1*, both identified with targeted RNA sequencing. Regarding the high and increasing number of known fusion transcripts as well as for a future possible correlation of genotype to phenotype in lipoblastomas, the targeted RNA-sequencing technology is probably more applicable in routine molecular diagnosis of lipoblastomas than fluorescence in situ hybridization (FISH).

In conclusion, the algorithm to diagnose lipoblastomas among the group of pediatric fibrotic, lipofibrous, and mature lipomatous tumors essentially includes two parts. First, a comprehensive histologic workup assisted by immuno-

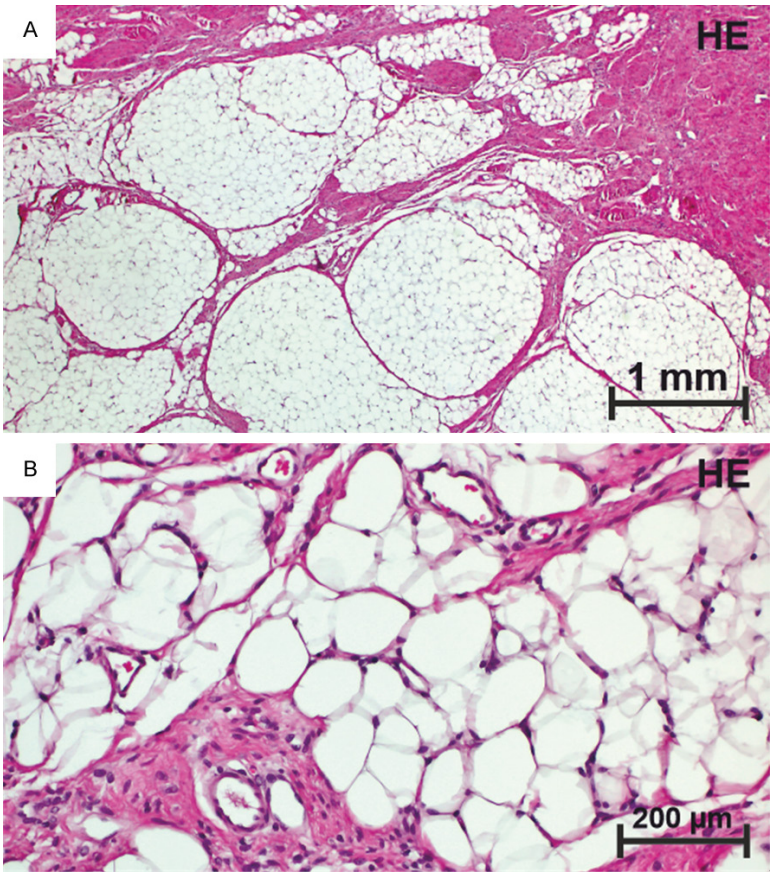

Figure 3. Histomorphology of lipogenic cells and fibrotic septa in mature lipoblastoma (fusion transcript *COL1A1-PLAG1*). A. The fibrotic tissue is dominated by a lipomature proliferation. Nodules of lipogenic cells are separated by delicate septa with some areas of fibrotic confluence. B. The lipogenic cells are without any atypia and surrounded by a few fibrotic septa. The myxoid matrix is poorly developed and mostly absent.

histochemistry in order to identify immature lipomatous as well as myxoid differentiation in a fibrotic background is warranted. Second, molecular diagnosis with targeted RNA sequencing to identify fusion transcripts affecting *PLAG1* or rarely *HMGA2* is employed for confirmation.

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In both cases an informed consent to publish the data was signed.

Disclosure of conflict of interest

None.

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