*Genes, Chromosomes and Cancer*

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BRIEF REPORT OPEN ACCESS

# Fibromyxoid aSoft Tissue Tumor With PLAG1 Fusion—The First Case in an Adult Patient

M. Strnadová[1] | J. Balko[1] | P. Brož[2,3] | L. Wagenknecht[4] | L. Krsková[1,3]

[1]Department of Pathology and Molecular Medicine, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic | [2]Bioxsys Ltd., Czech Republic | [3]Department of Pediatric Hematology and Oncology, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic | [4]Department of Adult and Pediatric Orthopedic Surgery and Traumatology, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic

**Correspondence:** L. Krsková (lkrskova@email.cz)

**Received:** 15 September 2024 | **Revised:** 20 October 2024 | **Accepted:** 2 November 2024

**Funding:** This work was supported by Research Project of the Ministry of Health of Czech Republic No 00064203 and Modern Therapies Project 9782.

**Keywords:** fibromyxoid soft tissue tumor | *H3-3B* | mesenchymal neoplasm | NGS | nodular fasciitis | *PLAG1*

## ABSTRACT

With the expanding possibilities of human genome research in recent years, the number of cases of soft tissue tumors that we are able to classify into the correct subgroups and to reveal their molecular profile is increasing. Among such tumors, we can also consider neoplasms that have a specific fusion of genes, in our case namely the pleomorphic adenoma gene 1 (*PLAG1)* and its partner. *PLAG1* gene fusions were previously associated mainly with salivary gland pleomorphic adenomas, lipoblastomas, myoepithelial tumors, uterine epitheloid, myxoid leiomyosarcomas, and, recently, with PLAG1-rearranged fibromyxoid soft tissue tumors. To our knowledge, we report the first case of a soft tissue tumor with a *PLAG1* fusion gene in an adult. In our case, we detected a new *H3-3B::PLAG1* fusion in a soft tissue tumor, which originally appeared as nodular fasciitis.

## 1 | Introduction

Chromosomal rearrangements can lead to the formation of chimeric transcripts or gene fusions. Some gene fusions are specific to one diagnosis, while others are common to multiple types of diagnoses [1]. Pleomorphic adenoma gene 1 (*PLAG1*) rearrangements are among such fusions. Genes from the *PLAG1* family encode developmentally regulated zinc-finger transcription factors that recognize specific DNA-consensus sequences and control their expression [2].

Overexpression of the *PLAG1* gene is most often associated with the development of pleomorphic adenomas of the salivary gland, lipoblastomas, myoepithelial tumors [3], uterine epitheloid and myxoid leiomyosarcomas, and other mesenchymal tumors with fibromyxoid histology, known as "PLAGomas" [4–6]. Chromosome 8q11-13 rearrangement causes the replacement of the PLAG1 promoter with the active promoter of another gene and the subsequent overexpression of the PLAG1 protein. This results in the up-regulation of direct target genes leading to increased cell proliferation and transformation [7, 8].

In this case, we report a new and previously undescribed fusion gene, *H3-3B::PLAG1*, of a 60-year-old female patient with a symphysis tumor.

## 2 | Materials And Methods

### 2.1 | Histopathology and Immunohistochemistry

The tissue obtained by biopsy was fixed in 4% formalin and subsequently embedded in paraffin. Hematoxylin and eosin were used for histological staining. Sliced paraffin blocks were also subjected to immunohistochemical examination. The following antibodies were used: CD34 (QB/10), CD117,

BCL2 (124), CD99 (O13), STAT6 (YE361), ALK, S100 (EP32) and SOX10 (EP268), desmin (D33), h-caldesmon (BSB-19), and Ki-67 (MIB-1).

### 2.2 | Molecular Examination

Tumor RNA was purified from the FFPE block using the High Pure FFPET RNA Isolation Kit (Roche Diagnostics). The FusionPlex Sarcoma V2 panel (ArcherDX) was used to prepare the NGS library, and the final amplicons were subsequently sequenced on a MiSeq (Illumina) instrument. This panel includes 60 of the most common genes associated with diagnostics of sarcomas and soft tissue tumors. The Archer panel was used as a manufacturer's instruction. Archer Analysis 6.0 and Arriba software were used for the data analysis. We then designed primers for the rearrangement and confirmed the new fusion gene using Sanger sequencing.

## 3 | Case Report

A woman (60 years old) was examined at the Department of Adult and Pediatric Orthopedic Surgery and Traumatology in Motol University Hospital for painless moveable resistance in the area of the symphysis, which arose after an injury. According to magnetic resonance, it was an encapsulated hematoma or seroma in the subcutaneous tissue, which was subsequently surgically removed. This nodularly built oval formation, with a size of 40×27×25mm, was completely covered with a thin fibrous capsule on the surface.

Microscopically, the lesion corresponded to a storiform to a slightly fascicularly arranged lesion consisting of oval- to spindle-shaped cells with oval nuclei without significant atypia (Figure 1A). Small vessels with subtle extravasation of erythrocytes were visible. We detected an intermixed population of mast cells within the stroma. Mitotic activity was sparse. Giant multinucleated cells were not detected. The lesion was bordered by a thin fibrous capsule.

Subsequently, an immunohistochemical examination was performed, showing positivity for CD34 protein (Figure 1B). Positivity of the muscle-specific desmin protein was also noted in the tumor cells (Figure 1C). The proliferation index was low using Ki-67. Other investigated proteins (H-caldesmon, CD117, BCL2, CD99, STAT6, ALK, S100, and SOX10) were negative. According to the histopathological and immunohistochemical examination of the tumor, the possibility of a diagnosis of nodular fasciitis was admitted.

Nodular fasciitis is a relatively rare benign disease of fibrous tissue that, due to its histological nature and rapid growth rate, can easily be confused with malignant soft tissue tumors. It is caused by the non-neoplastic proliferation of fibroblasts and myofibroblasts in the subcutaneous tissue and deep fascia [9].

Nodular fasciitis is characterized by a *MYH9::USP6* gene fusion [10], which occurs in the majority of cases. However, molecular examination did not confirm the expected fusion. Using the NGS method, a fusion of exon 3 of the *PLAG1* gene (chr8:57080945) with the exon 1 of the *H3-3B* gene (chr17:73775738) was found (Figure 2A), as confirmed by RT-PCR and Sanger sequencing (Figure 2B). According to the literature, there has not yet been a case where the *H3-3B* gene was described as an alternative fusion partner of the *PLAG1* gene.

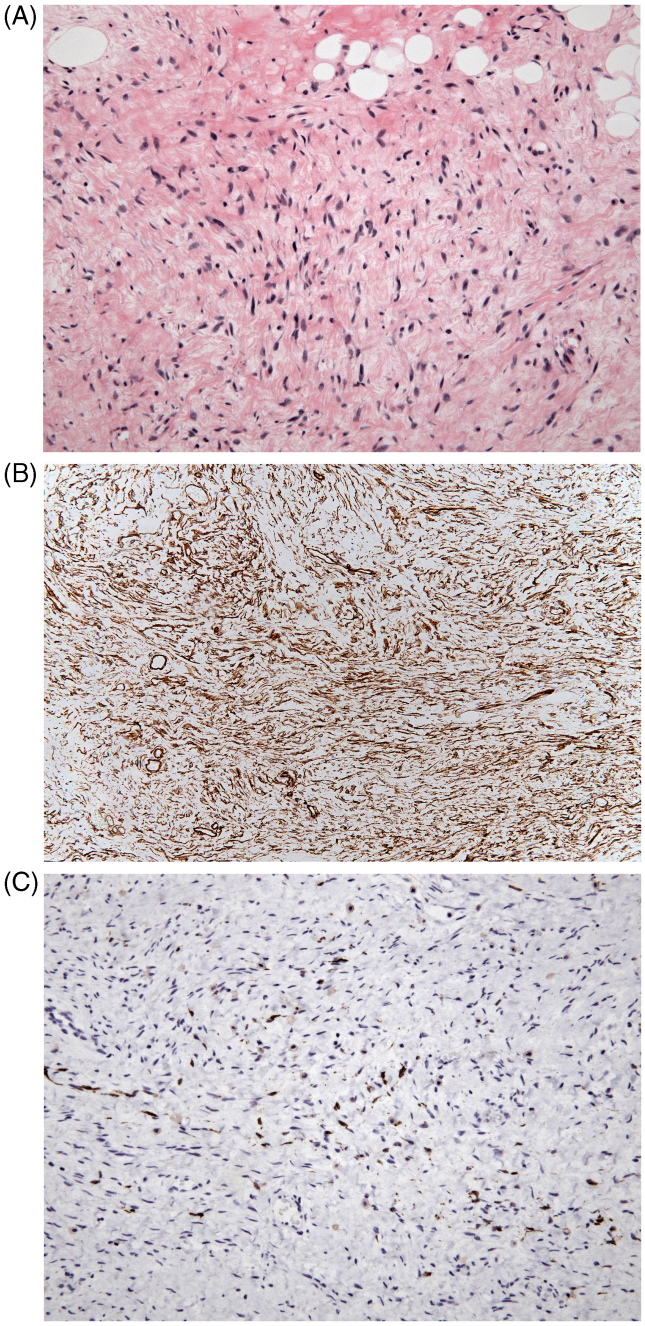


**FIGURE 1** | Light microscopy—A solid tumor composed of oval to spindle-shaped cells. The CD34 protein, h-caldesmon and desmin protein were found to be positive. (A)—Hematoxylin and eosin staining (20×); (B)—CD34 staining (20×); (C)—Desmin staining (20×).

## 4 | Discussion

To our knowledge, we report the first case of fibromyxoid soft tissue tumor with a *PLAG1* fusion gene in an adult patient. Our

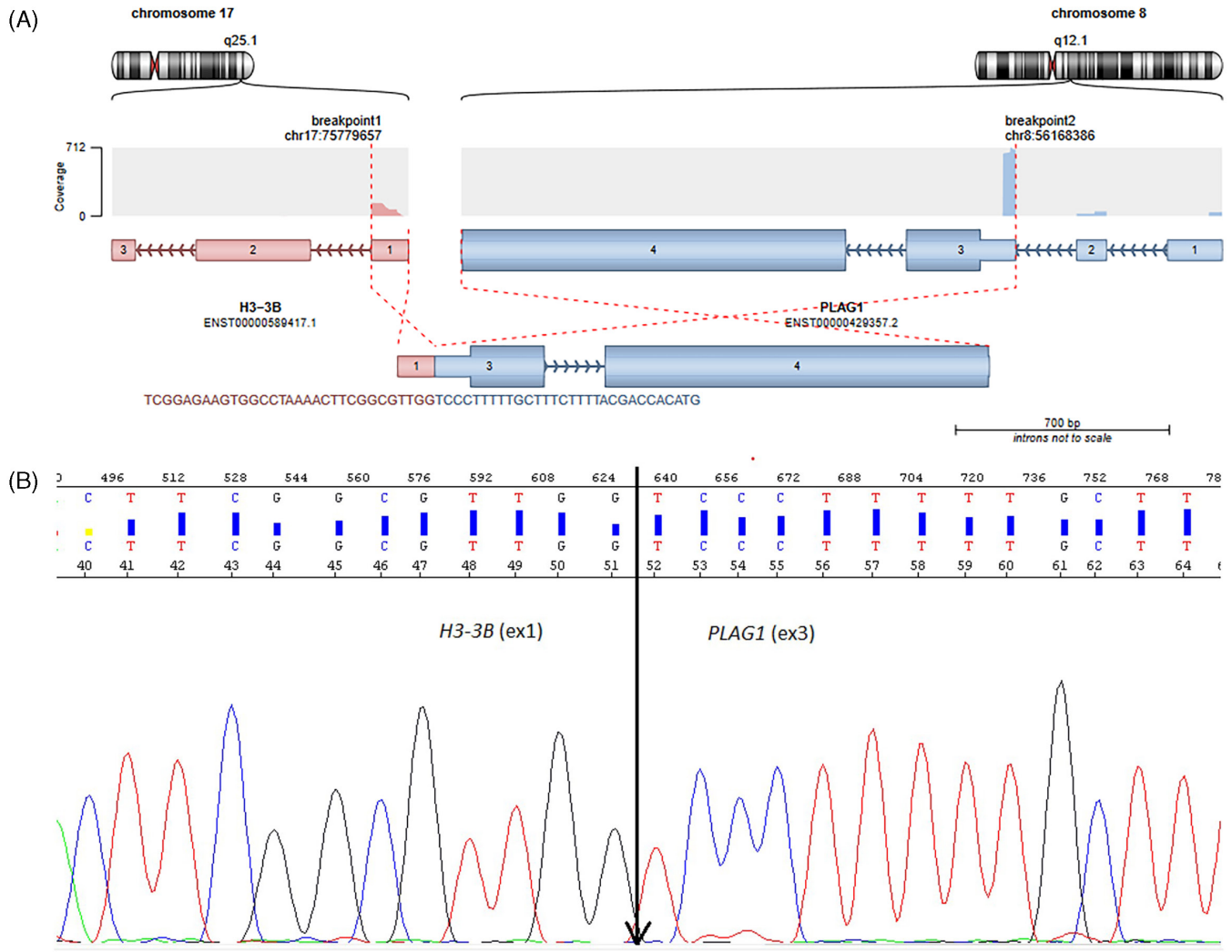


**FIGURE 2** | (A)—Schematic visualization of the fusion gene *H3-3B::PLAG1* using Arriba software (https://github.com/suhrig/arriba/); (B)—The sequence of the *H3-3B* (ex1) and *PLAG1* (ex3). The fusion caused by translocation t(8;17). The fusion is shown by the arrow.

patient has had more than 1 year of follow-up with no recurrence. This case was also unique because of detection of the previously undescribed *H3-3B::PLAG1* fusion. The evidence of fusion gene *H3-3B::PLAG1* in correlation with morphological and immunohistochemical findings support the diagnosis of a PLAG1-rearranged fibromyxoid soft tissue tumor. A PLAG1-rearranged soft tissue tumor is a newly emerging subset of soft tissue tumors in pediatric patients with a bland fibromyxoid morphological pattern, which is distinguished on the basis of a molecular profile.

*PLAG1*-rearranged tumors demonstrate a range of morphologies, both within and between entities [5]. Our case exhibited a mixed fibroblastic and myxoid morphology, but no other lines of differentiation, similar to the report of Chung et al. 2020 [6]. All together shared a distinctive immune profile, and all expressed CD34 and desmin.

Currently, over 20 fusion partners of *PLAG1* are already known across different diagnoses. *PLAG1* is an oncogene that was first observed in pleomorphic adenoma of the salivary gland [3, 11, 12]. Although this type of tumor is benign and grows slowly, recurrence and subsequent malignant transformation often occur [13]. Although there are large numbers of fusion partners of the *PLAG1* gene, the result of the rearrangement is the same. The gene fusion involves either exon 2 or 3 of the *PLAG1* gene, so the promoter of *PLAG1* is replaced by the promoter region from one of the various partner genes. The rearrangement results in the overexpression of *PLAG1*. This, in turn, produces the up-regulation of direct target genes, including growth factor-binding proteins, growth factor receptors, cell cycle-related proteins and growth factors, and mitogen-activated protein leading to increased cell proliferation and transformation [5–8, 14–17].

Chromosomal rearrangements of the *PLAG1* gene lead to urine myxoid leiomyosarcoma [18] or chondroid syringoma [19], myoepithelioma with ductal differentiation of the skin and soft tissue [3], and other neoplasms.

The fusions involving the *H3-3B* gene were detected in two cases of adenocarcinoma *H3-3B::TRIM47* [20] and *ZDHHC7::H3-3B* [21]—and in one case of astrocytoma *H3-3B::CCT5* [22]. The *H3-3B* gene is located at 17q25.1 and encodes a replication-independent histone that is a member of the histone H3 family. Histones are basic nuclear proteins that are responsible for the nucleosome structure of the chromosomal fiber in eukaryotes.

Somatic mutations in the *H3-3B* gene are associated with chondroblastoma [23].

The described case clearly documents the necessity of tumor analysis at the RNA level and the search for new molecular markers for better characterization, understanding of the biology of cancer, and establishing the correct diagnosis.


### Acknowledgments

Open access publishing facilitated by Univerzita Karlova, as part of the Wiley - CzechELib agreement.


### Ethics Statement

This project was approved by the Research Ethics Board of The Motol University Hospital.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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