HELDA
HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

UNIVERSITY OF HELSINKI

https://helda.helsinki.fi

Lung metastases and subsequent malignant transformation of a fumarate hydratase-deficient uterine leiomyoma

Ahvenainen, Terhi

2022-06

Ahvenainen , T , Khamaiseh , S , Alkodsi , A , Mehine , M , Nevala , R , Äyräväinen , A , Bützow , R & Vahteristo , P 2022 , ' Lung metastases and subsequent malignant transformation of a fumarate hydratase-deficient uterine leiomyoma ' , Experimental and Molecular Pathology , vol. 126 , 104760 . https://doi.org/10.1016/j.yexmp.2022.104760

http://hdl.handle.net/10138/345045
https://doi.org/10.1016/j.yexmp.2022.104760

cc_by
publishedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

Experimental and Molecular Pathology 126 (2022) 104760

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Short report

Lung metastases and subsequent malignant transformation of a fumarate hydratase -deficient uterine leiomyoma

Terhi Ahvenainen [a,b,c,1], Sara Khamaiseh [a,b,c,1], Amjad Alkodsi [a], Miika Mehine [a,b], Riikka Nevala [d], Anna Äyräväinen [a,b,e], Ralf Bützow [a,f], Pia Vahteristo [a,b,c,*]

[a] Applied Tumor Genomics Research Program, University of Helsinki, Helsinki, Finland
[b] Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland
[c] iCAN Digital Precision Cancer Medicine Flagship, Helsinki, Finland
[d] Comprehensive Cancer Center, Helsinki University Hospital (HUH), Helsinki, Finland, and Medical Faculty, University of Helsinki, Helsinki, Finland
[e] Department of Obstetrics and Gynecology, Helsinki University Hospital, Helsinki, Finland
[f] Department of Pathology, University of Helsinki, Helsinki, Finland, Laboratory of Helsinki University Hospital (HUSLAB), Helsinki University Hospital, Finland

ARTICLE INFO

Keywords:
Uterine leiomyoma
Pulmonary leiomyosarcoma
Fumarate hydratase
Benign metastasizing leiomyoma
Malignant transformation
Metastasis

ABSTRACT

Uterine leiomyomas, or fibroids, are very common smooth muscle tumors. Their potential to metastasize or transform into leiomyosarcomas is extremely low. Here, we report a patient who underwent hysterectomy due to a large leiomyoma and who was diagnosed with pulmonary tumors seven and nine years later. Histopathological re-evaluation confirmed the cellular leiomyoma diagnosis for the uterine tumor, whereas the pulmonary tumors met the diagnostic criteria of a leiomyosarcoma. Whole-exome sequencing revealed very similar mutational profiles in all three tumors, including a somatic homozygous deletion in a rare, but well-established leiomyoma driver gene *FH*. Tumor evolution analysis confirmed the clonal origin of all three tumors. In addition to mutations shared by all three tumors, pulmonary tumors harbored additional alterations affecting e.g. the cancer-associated genes *NRG1* and *MYOCD*. The second pulmonary leiomyosarcoma harbored additional changes, including a mutation in *FGFR1*. In global gene expression profiling, the uterine tumor showed similar expression patterns as other FH-deficient leiomyomas. Taken together, this comprehensive molecular data supports the occasional metastatic capability and malignant transformation of uterine leiomyomas. Further studies are required to confirm whether FH-deficient tumors and/or tumors with cellular histopathology have higher malignant potential than other uterine leiomyomas.

1. Introduction

Uterine leiomyomas, or fibroids, are the most common gynecological tumors with a lifetime prevalence of ~70% (Bulun et al., 2015). They are typically found in women of reproductive age and tumors tend to shrink or disappear after menopause. Leiomyomas are often asymptomatic, but can cause symptoms such as heavy and prolonged menstrual bleeding, abdominal pain, and reproductive dysfunction. Most tumors are driven by one of the three well-established driver alterations: mediator complex subunit 12 (*MED12*) mutations, high-mobility group AT-hook 2 (*HMGA2*) overexpression, or fumarate hydratase (*FH*) inactivation (Mehine et al., 2014). FH-deficient tumors are rare accounting for 1–2% of all uterine leiomyomas, but they form a clinically relevant subgroup as germline *FH* mutations predispose to hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome (Mehine et al., 2014; Tomlinson et al., 2002). FH-deficient tumors typically display specific morphological features including staghorn vasculature, eosinophilic inclusions, bizarre nuclei, and perinucleolar haloes (Ip et al., 2020). In addition to molecular changes, uterine leiomyomas can be classified based on histopathology. The vast majority are diagnosed as conventional tumors, whereas 10% display variant histopathology. These variants include e.g. cellular and mitotically active leiomyomas, and leiomyomas with bizarre nuclei (Ip et al., 2020).

Leiomyomas are benign tumors whose malignant potential is

* Corresponding author at: Applied Tumor Genomics Research Program, Department of Medical and Clinical Genetics, Biomedicum Helsinki, University of Helsinki, P.O. Box 63, Haartmaninkatu 8, FIN-00014, Finland.
E-mail address: pia.vahteristo@helsinki.fi (P. Vahteristo).
[1] Equal contribution.

https://doi.org/10.1016/j.yexmp.2022.104760
Received 10 February 2022; Received in revised form 21 March 2022; Accepted 28 March 2022
Available online 31 March 2022
0014-4800/© 2022 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

extremely low. Rarely, however, leiomyomas have metastasized to extrauterine sites in a condition referred to as benign metastasizing leiomyoma (BML) (Barnaś et al., 2017). Pathogenesis of BML is poorly understood. The most common theory is hematogenous spreading of leiomyoma cells following uterine surgery. Histopathological appearance of primary leiomyoma and metastatic tumor are usually similar, which has been considered as evidence of metastasis. We recently reported a patient who underwent hysterectomy due to a large uterine leiomyoma and who was diagnosed with pulmonary leiomyosarcomas seven and nine years later (Ahvenainen et al., 2018). All three tumors displayed loss of FH and ATRX protein expression and the pulmonary tumors showed elongated telomeres. Here, we performed whole-exome sequencing, evolution analysis, and expression profiling of all three tumors to determine their mutational landscape and clonal relationship.

2. Materials and methods

2.1. Patient description

Gynecologic ultrasound revealed a large, atypical-looking uterine leiomyoma and a hysterectomy was performed when the patient was 63 years old. The tumor was diagnosed as a leiomyoma. Seven years later, a chest radiograph revealed a tumor of over seven centimeters in the right medial pulmonary lobe. The tumor was removed, and its core met the diagnostic criteria of a leiomyosarcoma. The tumor did not invade the pleural tissue and a whole-body CT scan showed no suspicion of malignancy in other sites of the body. The surgical treatment was considered sufficient, and a regular follow-up was initiated. Two years after the pulmonary surgery, a CT scan revealed novel metastatic lesions in the mediastinum. Despite treatment with multiple chemotherapeutic agents (doxorubicin, gemcitabine-docetaxel, pazopanib), the mediastinal metastases continued to grow. The patient received radiation therapy. Two years after the second pulmonary diagnosis, a palliative surgery was performed and two large metastatic lesions in the mediastinum were removed. A pathologist confirmed the leiomyosarcoma diagnosis. After the operation, active follow-up continued. During the last five years, no recurrence has been observed.

2.2. Tissue specimens and ethics approval

Archival formalin-fixed paraffin-embedded (FFPE) tissue samples from the uterine leiomyoma, two pulmonary tumors from different operations, and normal ovarian tissue were collected at the Department of Pathology, Helsinki University Hospital, Helsinki, Finland. Tissue samples were collected with permission from the National Supervisory Authority for Welfare and Health. Research has been approved by the appropriate ethics review board of the Hospital District of Helsinki and Uusimaa, Helsinki, Finland.

2.3. Exome sequencing, evolution analysis, and expression profiling

Exome capture was executed with SeqCap MedExome EZ Library SR (Roche, Madison, WI). Paired-end sequencing was performed with an Illumina NextSeq 500 system (Illumina, San Diego, CA). Somatic copy number alterations and small nucleotide variants and indels were determined with an in-house pipeline including tools from Genome Analysis ToolKit (GATK) (McKenna et al., 2010). A corresponding normal tissue sample (ovarian), an in-house panel of normal tissue exomes, and Genome Aggregation Database (gnomAD) were used to remove germline variation. For evolution analysis, single-nucleotide variants and allele-specific copy number variants were combined and total allele-specific copy numbers were computed from tumor cell content and ploidy. The clonal structure was inferred with PyClone (v0.13.0) (Roth et al., 2014). ClonEvol was used to infer the clonal ordering and to reconstruct the phylogenetic tree from the detected clones (Dang et al., 2017). In 3′RNA sequencing, dual-indexed mRNA libraries were prepared using QuantSeq 3′RNA-Seq Library Prep Kit FWD (Lexogen Gmbh, Vienna, Austria). The libraries were multiplexed and sequenced using the NovaSeq 6000 System (Illumina). FASTQ preprocessing was performed using the Integrated Data Analysis Pipeline version 2.3.1 FWD UMI (Lexogen Gmbh) implemented on the Bluebee® Genomics analysis platform. Reads were analyzed together with a previously published dataset of 44 leiomyomas (13 leiomyomas with a *MED12* mutation, 15 with HMGA2 overexpression, and 16 with FH-deficiency) and 5 myometrium samples (Mehine et al., 2020). Expression patterns were defined by principal component analysis. See Supplemental material for detailed description of the methods.

3. Results

3.1. Histopathological re-evaluation confirms benign histopathology of the primary tumor

A pathologist specialized in gynecological tumors (RB) re-evaluated the tissue slides and confirmed the benign histopathology of the primary tumor. More specifically, the tumor was diagnosed as a highly cellular uterine leiomyoma with generally low-grade and focally moderate atypia. The tumor had <5 mitotic figures per 10 high power field (HPF). No coagulative (tumor) necrosis was detected. The tumor also displayed features associated with FH-deficiency, including staghorn vessels, nuclear atypia, and eosinophilic nucleoli (Fig. 1A**, Supplemental table 1).** The first pulmonary tumor was a highly cellular leiomyosarcoma with generally moderate and focally strong atypia (Fig. 1B and C**).** It showed a mitotic index of over 10/10 HPF. No coagulative (tumor) necrosis was observed. The tumor displayed similar FH-deficiency-associated features as the primary tumor **(**Fig. 1B and C**, Supplemental table 1)**. The second pulmonary tumor showed similar morphology as the first leiomyosarcoma, with slightly more pronounced FH-deficiency-associated features. **(**Fig. 1D**, Supplemental table 1)**.

3.2. Exome sequencing detects similar somatic copy number profiles in all three tumors and additional amplifications and deletions in the leiomyosarcomas

All three tumors displayed very similar somatic copy number profiles (Fig. 2A). Euclidean hierarchical clustering showed that the pulmonary tumors where more similar to each other than to the uterine tumor. Several identical somatic amplifications and deletions were detected in all three tumors, including deletions in 1p36 (**Supplemental table 2)**. Most of the shared amplified and deleted regions were in chromosomes one and eight (Fig. 2A). Analysis focusing on cancer genes revealed several shared somatic alterations, including an amplification of Janus kinase 1 (*JAK1*) (1p31.3) and a homozygous deletion of *FH* (1q43) (Fig. 2A). Pulmonary tumors harbored additional amplifications and deletions, including deletions in 19q13 (**Supplemental table 3)**. A highly amplified region in the pulmonary tumors encompassed neuregulin 1 (*NRG1*) (8p12) (Fig. 2A). See Supplemental tables 2 and 3 for complete lists of shared copy number alterations. Next, the pulmonary tumors were examined for alterations previously reported in leiomyosarcomas. A gain in 17p12 encompassing mitogen-activated protein kinase kinase 4 (*MAP2K4*) and myocardin (*MYOCD*) was detected in both pulmonary tumors, with the first tumor displaying moderate and the second tumor high-level amplification (Fig. 2A). A loss in 19q13.43 was also detected in both pulmonary tumors, with the second tumor showing a homozygous deletion.

3.3. Tumors share several identical single-nucleotide variants and microindels

Altogether 224 somatic single-nucleotide variants and microindels were detected in three tumor specimens (Fig. 2B**, Supplemental table 4)**. All tumors shared 38 somatic variants, of which 11 were non-

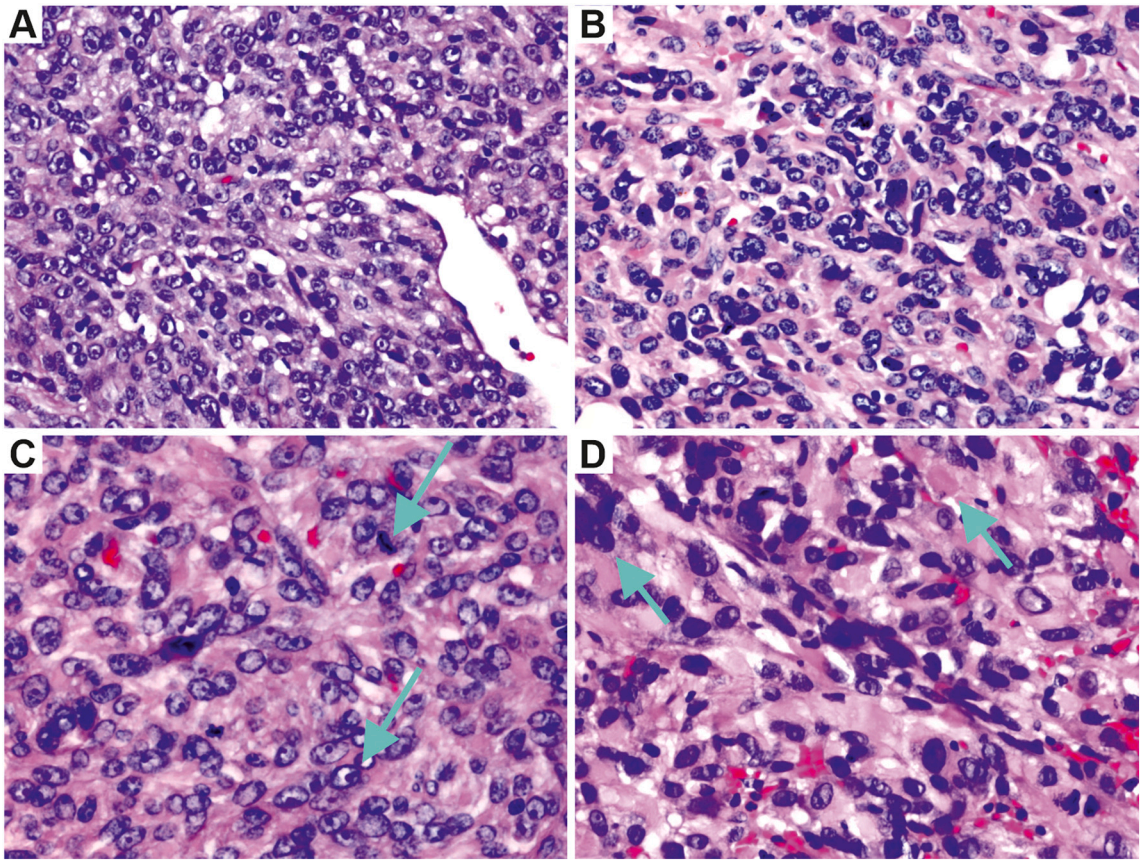

Fig. 1. Hematoxylin and eosin-stained tissue sections of the uterine leiomyoma and two pulmonary leiomyosarcomas. (A) Uterine leiomyoma displays highly cellular histopathology, mild to moderate nuclear atypia, central eosinophilic nucleoli, and staghorn vessels. (B, C) First pulmonary leiomyosarcoma in the right medial pulmonary lobe, removed seven years after hysterectomy. The tumor also displays FH-deficiency-associated features, including eosinophilic nucleoli (lower arrow). The nuclear atypia is pronounced (moderate, focally strong) and there is increased number of mitotic figures (upper arrow). (D) Second pulmonary leiomyosarcoma in mediastinum, diagnosed two years after the first pulmonary leiomyosarcoma. The tumor displays more prominent malignant features and characteristics associated with FH-deficiency (eosinophilic inclusions, upper arrow; bizarre nuclei, lower arrow). All images at 40× magnification.

synonymous alterations or changes affecting a splice site. One alteration was a splice site mutation (c.2111-1_2111delGGinsAA) in a tumor suppressor EPH receptor A7 (*EPHA7*). Pulmonary leiomyosarcomas shared additional 88 somatic variants, of which 40 were non-synonymous exonic or splice site alterations. When scrutinizing cancer genes for mutations predicted damaging by *in silico* analysis, two missense mutations in APOBEC1 complementation factor (*A1CF*; c.298 T > A, p.(Phe100Ile) and c.355 T > C, p.(Tyr119His)) and one in filamin A (*FLNA*; c.7348 T > C, p.(Phe2450Leu)) were detected in both pulmonary tumors. In addition, the second leiomyosarcoma harbored a mutation in fibroblast growth factor receptor 1 (*FGFR1*; c.1247 T > C, p.(Phe416Ser)).

3.4. Evolution analysis confirms the clonal origin of the tumors

Five cell clusters were extracted from the tumors (**Supplemental fig. 1**). An ancestral clone (C1) that was present in all tumors included 33 genetic alterations. In addition to the ancestral clone, evolution analysis identified a uterine leiomyoma -specific subclone (C3) that included 41 alterations and three subclones that were present exclusively in the pulmonary tumors (subclone C2 with 91 alterations present in both pulmonary tumors, subclone C5 with 36 alterations in the first pulmonary tumor, and subclone C4 with 29 alterations in the second pulmonary tumor). Taken together, evolution analysis indicates a clonal origin for all three tumors and identifies tumor-specific subclones that have developed later during tumor evolution (Fig. 2C**, Supplemental fig. 1)**.

3.5. 3'RNA sequencing reveals similar gene expression patterns

Principal component analysis revealed that the uterine leiomyoma clustered among other FH-deficient leiomyomas while the pulmonary tumors formed a separate nearby cluster **(**Fig. 2D**).** *FH* was downregulated in the uterine and pulmonary tumors when compared to myometrium samples and leiomyomas with other driver mutations **(**Fig. 2E**).**

4. Discussion

Benign metastasizing leiomyoma is a controversial entity; it refers to a histopathologically benign condition, but the behavior resembles that of a malignant tumor. The average age when BML-associated uterine leiomyoma is operated on is 39 and the metastasis is diagnosed nine years later (Barnaś et al., 2017). Only occasionally has the condition been reported in postmenopausal women (Barnaś et al., 2017). Here, the patient was 63 years old when hysterectomy was performed, and the metastases were diagnosed seven and nine years later. While the patient was relatively old for a leiomyoma surgery, the delay in the metastatic disease is typical for BML. Usually, BML is found in patients with a history of uterine surgery, raising the possibility of an iatrogenic spread of leiomyomatous cells (Barnaś et al., 2017). Also our patient had undergone hysterectomy, which may have facilitated metastasis. The exact mechanism of metastasis is unknown, but vasculature system has been suggested to be involved.

The clonality of BML tumors has been confirmed through identical

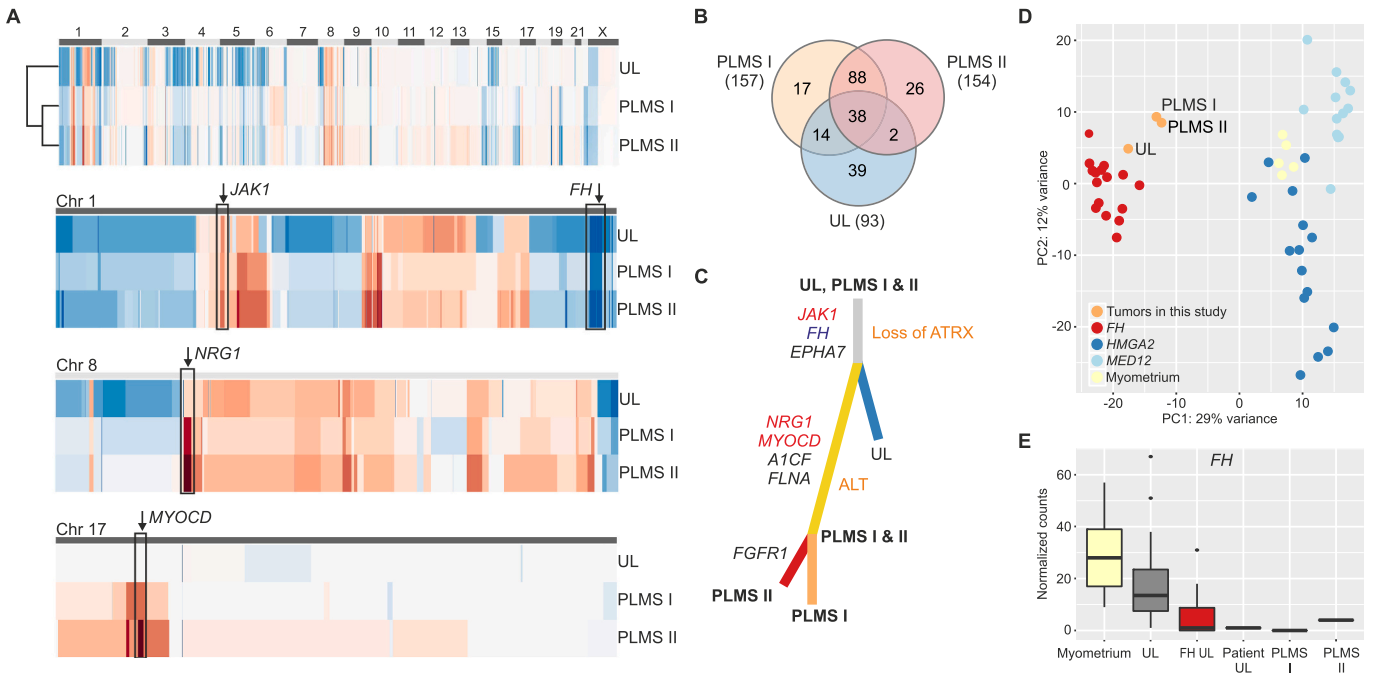

Fig. 2. Whole-exome sequencing, evolution analysis, and differential gene expression profiling of uterine leiomyoma and pulmonary leiomyosarcomas. (A) Somatic copy number analysis shows similar copy number profiles in the uterine leiomyoma (UL) and two pulmonary leiomyosarcomas (PLMS) in whole-genome view (upper panel). Chromosome one harbors the largest number of shared amplifications and deletions, including an amplification of *JAK1* and a homozygous deletion of *FH*. Alterations that are more evident in PLMSs include amplifications in chromosome eight affecting *NRG1* and in chromosome 17 encompassing *MYOCD*. (B) A Venn diagram illustrating the number of somatic microindels and single-nucleotide variants detected in the UL and PLMS. (C) A phylogenetic tree with a trunk and four branches represents the five cell clusters that were extracted from the tumors, and order of the events that likely occurred during tumor evolution. All tumors share an ancestral clone (gray), supporting their clonal origin. Cancer genes highlighted in the discussion are shown. Genes with amplifications in red, deletions in blue, and single-nucleotide alterations and microindels in black. Previously (Ahvenainen et al., 2018) identified loss of ATRX expression and alternative lengthening of telomeres (ALT) are presented in orange. (D) Principal component analysis shows similar gene expression profiles in the UL and other FH-deficient leiomyomas. The tumors under study were analyzed together with a previously published set of 44 leiomyoma and five myometrium samples (Mehine et al., 2020). (E) *FH* expression is downregulated in all three tumors. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

molecular findings in the primary and metastatic tumors (Barnaś et al., 2017). Previously, we have shown that all three tumors of this patient displayed FH and ATRX loss (Ahvenainen et al., 2018). Here, numerous additional shared alterations were identified in high-throughput sequencing analysis. Clonality was confirmed by evolution analysis, which identified an ancestral clone shared by all tumors as well as uterine and pulmonary tumor-specific subclones. A phylogenetic tree further visualized the branched tumor evolution. While these findings by us and others provide molecular evidence for clonality, the exact alterations contributing to metastatic spread have remained elusive. Previously, a few recurrent alterations have been discovered, including 6p21 rearrangements and losses in 1p, 3q, 19q, and 22q (Raposo et al., 2018; Wu et al., 2017; Nucci et al., 2007; Bowen et al., 2012). In our patient, most clonal copy number alterations were in chromosomes one and eight. All tumors harbored identical amplified regions of 1p31.3, which contains a well-established oncogene *JAK1* (Flex et al., 2008). We also discovered deletions in 1p36 in all tumors, and similar alterations have been previously reported in one BML patient's metastases (Bowen et al., 2012). All tumors shared a somatic homozygous deletion in 1q43 including *FH*. Tumors also shared a splice-site mutation in the *EPHA7* tumor suppressor gene. Ephrin (Eph) family receptor tyrosine kinases and their ligands are known to promote tumor invasion and metastasis (Li et al., 2016). Interestingly, biallelic loss of *EPHA7* has also been identified in an FH-deficient renal adenocarcinoma (Smith et al., 2016). Overall, shared single-nucleotide variants in cancer genes were rare.

One proposed explanation for BML has been that the uterine tumor is not a leiomyoma but a low-grade leiomyosarcoma. Here, even after careful re-examination of the primary tumor, histopathological criteria of malignancy (Ip et al., 2020) were not met. The tumor was not, however, a conventional leiomyoma, but it displayed increased cellularity and features associated with FH-deficiency. Previously, leiomyomas with increased cellularity have been shown to cluster between conventional leiomyomas and leiomyosarcomas in expression profiling (Christacos et al., 2006). Still, leiomyomas with increased cellularity are considered benign at the clinical setting and are treated similarly as other leiomyomas. Leiomyosarcomas, on the other hand, are rare but highly aggressive cancers with early metastases and poor survival (Ip et al., 2020). Their genomes are extremely complex and they are almost universally driven by alterations in the well-known cancer genes *TP53*, *ATRX, PTEN*, and *RB1* (Choi et al., 2021). Here, the primary tumor showed more complexity in its genome compared to typical leiomyomas, which have a near-complete absence of amplifications (Mehine et al., 2014). However, the genome was still relatively stable, nearly all typical sarcoma-associated alterations were absent, histopathological diagnosis remained leiomyoma after thorough re-evaluation, and the first metastatic tumor developed long (seven years) after hysterectomy. While all these observations support the leiomyoma diagnosis, the possibility of an undiagnosed sarcomatous component cannot be unambiguously excluded.

Metastases associated with BML typically display benign histopathology (Barnaś et al., 2017). In addition to our patient, there are only five other individuals whose uterine leiomyoma-derived metastases have undergone malignant transformation (Ogawa et al., 2011; Song et al., 2017; Esteban et al., 1999; Ventura et al., 2021; Fischer et al., 2022). Molecular data is available in only one study, and that data supports the clonality between the uterine tumor and malignant pulmonary metastasis (Fischer et al., 2022). Here, the diagnostic criteria for leiomyosarcoma were met in both pulmonary tumors. Particularly, the nuclear atypia was pronounced and the mitotic index was increased. When compared to the uterine tumor, pulmonary tumors had acquired

additional molecular alterations. Many of the changes were in chromosome one, which displayed multiple amplifications. The pulmonary tumors also shared an amplification in 8p12. This region includes an oncogene *NRG1*, in which recurrent rearrangements have been reported in lung cancers (Suda and Mitsudomi, 2020) . Also, alterations previously reported in leiomyosarcomas, including a deletion in 19q13.43 and amplification in 17p12, were present in the pulmonary tumors (Choi et al., 2021). The 17p12 region includes *MYOCD*, which has been shown to induce smooth muscle differentiation and promote cell migration (Pérot et al., 2009). We also detected deletions in 19q13, which have been previously reported in several BML tumors (Raposo et al., 2018; Nucci et al., 2007). Both pulmonary tumors harbored mutations in cancer genes *A1CF* and *FLNA,* while a mutation in *FGFR1* was only seen in the second leiomyosarcoma. Even though the pulmonary tumors harbored some mutations in cancer genes, also these tumors lacked the most typical sarcoma-associated alterations. This may explain the less aggressive clinical course in this patient.

We have previously shown the loss of FH protein expression in the uterine and pulmonary tumors of this patient (Ahvenainen et al., 2018) and here we showed *FH* downregulation also in the RNA-level. In addition, high 2SC level has been identified in the primary tumor validating FH-deficiency (Mäkinen et al., 2017). At the DNA-level, we now detected a somatic homozygous deletion in the *FH* locus in all three tumors, which explains the diminished expression and confirms the tumors as sporadic FH-deficient lesions. The normal ovarian tissue sample did not show any alterations in *FH* and thus the patient does not have HLRCC. Interestingly, Gilhooley et al. have reported pulmonary leiomyomas in an individual with HLRCC and thus with a germline *FH* mutation (Gilhooley et al., 2018). FH-deficiency is a well-established driver alteration accounting for 1–2% of leiomyomas (Mehine et al., 2014). It has been associated with epithelial-to-mesenchymal transition, which enables tumor cell dissemination and metastasis (Guerra et al., 2017). FH-deficient uterine leiomyosarcomas have also been discovered, albeit rarely (Mäkinen et al., 2017). Further studies are required to confirm whether FH-deficient leiomyomas are more prone to local recurrence, intraperitoneal or distant metastases, and/or transforming into malignancy compared to other leiomyoma subtypes.

To conclude, we present molecular evidence for lung metastasis and subsequent malignant transformation of a sporadic FH-deficient uterine leiomyoma. The uterine tumor displayed both an unconventional histopathology and a rare molecular subtype. Further studies are warranted to determine whether these characteristics contribute to metastatic capability and malignant potential.

Declaration of Competing Interests

The authors declare no competing interests.

Author contributions

T.A. and P.V.: conceptualization and study design. T.A., S.K, and R. B.: investigation and visualization. T.A., S.K., A.A., and M.M.: formal analysis of the whole-exome sequencing data. S.K.: formal analysis of the 3'RNA sequencing data. R.B., R.N., and A.Ä.: resources. P.V.: supervision and funding acquisition. T.A., S.K., and P.V.: writing the original draft. All authors reviewed and approved the manuscript.

Funding

This study was supported by Sigrid Jusélius Foundation, Academy of Finland (307773), Cancer Foundation Finland, the iCAN Digital Precision Cancer Medicine Flagship, Biomedicum Helsinki Foundation, and Orion Research Foundation. The funding sources were not involved in the study design or the writing or the decision to submit the article for publication.

Acknowledgements

We express our gratitude for the patient's contribution. We thank Lotta Katainen for excellent technical assistance. Exome sequencing was performed at Biomedicum Functional Genomics Unit (FuGU) and 3RNA sequencing at the Sequencing Unit of the Institute for Molecular Medicine Finland (FIMM) Technology Centre, University of Helsinki, Finland.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yexmp.2022.104760.

References

Ahvenainen, T.V., Mäkinen, N.M., von Nandelstadh, P., Vahteristo, M.E.A., Pasanen, A. M., Bützow, R.C., et al., 2018. Loss of ATRX/DAXX expression and alternative lengthening of telomeres in uterine leiomyomas. Cancer 124, 4650–4656.
Barnaś, E., Książek, M., Raś, R., Skręt, A., Skręt-Magierło, J., Dmoch-Gajzlerska, E., 2017. Benign metastasizing leiomyoma: a review of current literature in respect to the time and type of previous gynecological surgery. PLoS One 12, e0175875.
Bowen, J.M., Cates, J.M., Kash, S., Itani, D., Gonzalez, A., Huang, D., et al., 2012. Genomic imbalances in benign metastasizing leiomyoma: characterization by conventional karyotypic, fluorescence in situ hybridization, and whole genome SNP array analysis. Cancer Gene Ther. 205, 249–254.
Bulun, S.E., Moravek, M.B., Yin, P., Ono, M., JST, Coon, Dyson, M.T., et al., 2015. Uterine leiomyoma stem cells: linking progesterone to growth. Semin. Reprod. Med. 33, 357–365.
Choi, J., Manzano, A., Dong, W., Bellone, S., Bonazzoli, E., Zammataro, L., et al., 2021. Integrated mutational landscape analysis of uterine leiomyosarcomas. Proc. Natl. Acad. Sci. U. S. A. 118.
Christacos, N.C., Quade, B.J., Dal Cin, P., Morton, C.C., 2006. Uterine leiomyomata with deletions of Ip represent a distinct cytogenetic subgroup associated with unusual histologic features. Genes Chromosom. Cancer 45, 304–312.
Dang, H.X., White, B.S., Foltz, S.M., Miller, C.A., Luo, J., Fields, R.C., et al., 2017. ClonEvol: clonal ordering and visualization in cancer sequencing. Ann. Oncol. 28, 3076–3082.
Esteban, J.M., Schaerf, Allen W.M., RH., 1999. Benign metastasizing leiomyoma of the uterus: histologic and immunohistochemical characterization of primary and metastatic lesions. Arch. Pathol. Lab. Med. 123, 960–962.
Fischer, J.V., Mejia-Bautista, M., Vadasz, B., Yin, P., Bulun, S., Tanner, E.J., et al., 2022, published online ahead of print. Uterine Leiomyosarcoma associated with leiomyoma with bizarre nuclei: histology and genomic analysis of 2 cases. Int. J. Gynecol. Pathol.
Flex, E., Petrangeli, V., Stella, L., Chiaretti, S., Hornakova, T., Knoops, L., et al., 2008. Somatically acquired JAK1 mutations in adult acute lymphoblastic leukemia. J. Exp. Med. 205, 751–758.
Gilhooley, E., Fahy, C., Hanrahan, E., Keane, M., Swan, N., Lally, A., 2018. Multiple cutaneous and uterine leiomyomata with features of benign metastasing leiomyomatosis: a novel mutation of the fumarate hydratase gene. Clin. Exp. Dermatol. 43, 334–335.
Guerra, F., Guaragnella, N., Arbini, A.A., Bucci, C., Giannattasio, S., Moro, L., 2017. Mitochondrial dysfunction: a novel potential driver of epithelial-to-mesenchymal transition in cancer. Front. Oncol. 7, 295.
Ip, P.P.C., Bennett, J.A., Croce, S., et al., 2020. Tumours of the uterine Corpus. In: Kim, K.-R., Lax, S.F., Lazar, A.J., Longacre, T.A., Malpica, A., Matias-Guiu, X., Nucci, M.R., Oliva, E. (Eds.), WHO Classification of Tumors: Female Genital Tumours, 5th ed. IARC Publications, Lyon, France, pp. 272–276.
Li, R., Sun, Y., Jiang, A., Wu, Y., Li, C., Jin, M., et al., 2016. Knockdown of ephrin receptor A7 suppresses the proliferation and metastasis of A549 human lung cancer cells. Mol. Med. Rep. 13, 3190–3196.
Mäkinen, N., Kämpjärvi, K., Frizzell, N., Bützow, R., Vahteristo, P., 2017. Characterization of MED12, HMGA2, and FH alterations reveals molecular variability in uterine smooth muscle tumors. Mol. Cancer 16, 101.
McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., et al., 2010. The genome analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20, 1297–1303.
Mehine, M., Mäkinen, N., Heinonen, H.R., Aaltonen, L.A., Vahteristo, P., 2014. Genomics of uterine leiomyomas: insights from high-throughput sequencing. Fertil. Steril. 102, 621–629.
Mehine, M., Khamaiseh, S., Ahvenainen, T., Heikkinen, T., Äyräväinen, A., Pakarinen, P., et al., 2020. 3'RNA sequencing accurately classifies formalin-fixed paraffin-embedded uterine leiomyomas. Cancers (Basel) 12.
Nucci, M.R., Drapkin, R., Dal Cin, P., Fletcher, C.D., Fletcher, J.A., 2007. Distinctive cytogenetic profile in benign metastasizing leiomyoma: pathogenetic implications. Am. J. Surg. Pathol. 31, 737–743.
Ogawa, M., Hara, M., Ozawa, Y., Moriyama, S., Yano, M., Shimizu, S., et al., 2011. Benign metastasizing leiomyoma of the lung with malignant transformation mimicking mediastinal tumor. Clin. Imaging 35, 401–404.
Pérot, G., Derré, J., Coindre, J.M., Tirode, F., Lucchesi, C., Mariani, O., et al., 2009. Strong smooth muscle differentiation is dependent on myocardin gene amplification in most human retroperitoneal leiomyosarcomas. Cancer Res. 69, 2269–2278.

Raposo, M.I., Meireles, C., Cardoso, M., Ormonde, M., Ramalho, C., Pires, M., et al., 2018. Benign metastasizing leiomyoma of the uterus: rare manifestation of a frequent pathology. Case Rep. Obstet. Gynecol. 2018, 5067276.
Roth, A., Khattra, J., Yap, D., Wan, A., Laks, E., Biele, J., et al., 2014. PyClone: statistical inference of clonal population structure in cancer. Nat. Methods 11, 396–398.
Smith, S.C., Trpkov, K., Chen, Y.B., Mehra, R., Sirohi, D., Ohe, C., et al., 2016. Tubulocystic carcinoma of the kidney with poorly differentiated foci: a frequent morphologic pattern of fumarate hydratase-deficient renal cell carcinoma. Am. J. Surg. Pathol. 40, 1457–1472.
Song, K.S., Hwang, Keum D.Y., IS., 2017. Malignant transformation of pulmonary benign metastasizing leiomyoma. Kor. J. Thorac. Cardiovasc. Surg. 50, 59–63.
Suda, K., Mitsudomi, T., 2020. Emerging oncogenic fusions other than ALK, ROS1, RET, and NTRK in NSCLC and the role of fusions as resistance mechanisms to targeted therapy. Transl. Lung Cancer Res. 9, 2618–2628.
Tomlinson, I.P., Alam, N.A., Rowan, A.J., Barclay, E., Jaeger, E.E., Kelsell, D., et al., 2002. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nat. Genet. 30, 406–410.
Ventura, L., Gnetti, L., Rossi, M., Tiseo, M., Giordano, G., Corradi, M., et al., 2021. The role of miRNA-221 and miRNA-126 in patients with benign metastasizing leiomyoma of the lung: an overview with new interesting scenarios. Mol. Biol. Rep. 48, 3485–3494.
Wu, R.C., Chao, A.S., Lee, L.Y., Lin, G., Chen, S.J., Lu, Y.J., et al., 2017. Massively parallel sequencing and genome-wide copy number analysis revealed a clonal relationship in benign metastasizing leiomyoma. Oncotarget 8, 47547–47554.