Published in final edited form as:

Int J Gynecol Pathol. 2018 September ; 37(5): 421–430. doi:10.1097/PGP.0000000000000447.

Fumarate Hydratase Mutations and Alterations in Leiomyoma with Bizarre Nuclei

Qing Zhang, M.D., Ph.D.^{1,2,*}, Kate Poropatich, M.D.^{1,*}, Julianne Ubago, M.D.¹, Jia Xie, Ph.D.¹, Xiuhua Xu, Ph.D.¹, Norma Frizzell, Ph.D.⁴, Julie Kim, Ph.D.³, Beihua Kong, M.D.², and Jian-Jun Wei, M.D.^{1,3}

¹Department of Pathology, Northwestern University, Feinberg School of Medicine

²Department of Obstetrics and Gynecology, Qilu Hospital, Shandong University, Jinan, Shandong, 250012, P.R. China

³Department of Gynecology and Obstetrics, Northwestern University, Feinberg School of Medicine

⁴University of South Carolina

Abstract

Leiomyoma with bizarre nuclei (LM-BN), is a variant of uterine smooth muscle tumor with atypical histological features. Although some LM-BN share several significant genetic alterations with leiomyosarcoma (LMS), including p16 and p53, the underlying tumorigenesis of LM-BN remains largely unknown. As we previously reported, LM-BN can be divided into two subtypes, type I and type II, based on different nuclear features. Type I LM-BN have similar histologic features as uterine smooth muscle tumors with fumarate hydratase (FH) alterations. In this study, we examined FH expression and FH mutations in 77 LM-BN (40 type I cases and 37 type II cases). FH expression was examined by immunohistochemistry (IHC) using S-(2-succino)-cysteine antibodies (2SC, a protein modification associated with FH inactivation and subsequent fumarate accumulation) and FH antibodies (fumarate hydratase gene products). Seventy-two LM-BN tumors underwent Sanger sequencing to detect *FH* mutations. We found that 51% (39/77) of LM-BN showed FH alterations detected by immunohistochemistry with both 2SC and FH. Mutational analysis showed that 21% (15/72) of LM-BN harbored *FH* gene mutations. Further analysis revealed that 85% (34/40) of those with FH alterations were type I LM-BN while 19% (7/37) were type II LM-BN. Our findings suggest that over half of histologically-diagnosed LM-BN may be related to FH alterations or FH mutations and the majority of these have the characteristic histologic features of type I LM-BN.

Corresponding Authors: Dr. J-J Wei, Department of Pathology, Northwestern University, Feinberg School of Medicine, 251 East Huron Street, Room 7-334, Chicago, IL 60611; Telephone: 312-926-1815, Fax: 312-926-3127, jianjun-wei@northwestern.edu.

*These authors contributed equally.

Conflicts of Interest: None

Keywords

Leiomyoma with bizarre nuclei; fumarate hydratase; gene mutation; immunohistochemistry; uterine smooth muscle tumor

INTRODUCTION

Uterine smooth muscle tumors consist of a diverse group of tumors based on histologic features and clinical behavior. Currently the 2014 WHO classifies them as benign (including usual-type leiomyoma, leiomyoma with bizarre nuclei, cellular leiomyoma, mitotically-active leiomyoma, and other rare variants), intermediate grade (atypical smooth muscle tumors) and malignant (leiomyosarcoma, LMS). Leiomyoma with bizarre nuclei (LM-BN) was changed from atypical leiomyoma due to its overall benign clinical course¹⁻⁴. Thus, we use the terminology LM-BN in this report based on the WHO recommendation. The biologic nature of LM-BN and its histologic relationship to benign (usual-type leiomyoma, ULM), intermediate (atypical uterine smooth tumors) and malignant (LMS) uterine smooth muscle tumors remain unclear. Recently, several independent studies have investigated the molecular relationship among these variants and the results have shown that many cell cycle regulatory proteins including p16, p21, p27 and p53 are dysregulated in both LM-BN and LMS^{2, 4-6}. Given the completely different clinical outcomes of these two entities, further investigation into LM-BN may assist in better understanding this tumor.

Fumarate hydratase (FH) mutations are most notably seen in hereditary leiomyomatosis and renal cell carcinoma syndrome (HLRCC), also known as Reed syndrome, and they can also be seen with uterine leiomyomatosis⁷. Somatic *FH* mutations have occasionally been identified in some patients treated for uterine leiomyomas⁸ with a higher incidence seen in younger patients⁹. Further histologic analysis has revealed that some ULM with FH mutations have different nuclear and histologic features and can be referred to as smooth tumors with features of fumarate hydratase alteration (SMT-FH)^{9, 10}. Historically, many sporadic SMT-FH were diagnosed as LM-BN due to the significant nuclear atypia^{11, 12}. LM-BN also present with a wide range of histologic features^{1, 3, 4} and in our set of tumors originally diagnosed as LM-BN, nearly half showed nuclear features similar to SMT-FH^{9, 10} and we therefore defined them as type I LM-BN¹³. This suggests that many uterine smooth muscle tumors with atypical histology diagnosed as LM-BN may be related to FH alterations/mutations.

In this study, we analyzed FH alterations/mutations in 77 cases of LM-BN. FH mutations were first examined by immunohistochemistry using S-(2-succino)-cysteine antibodies (2SC), a substrate of FH that accumulates following inactivation of FH, and then by FH antibody to identify the fumarate hydratase gene products. The *FH* gene was then sequenced for each tumor. Both IHC and molecular sequencing identified distinct patterns of FH alterations in the two LM-BN subtypes.

MATERIALS AND METHODS

Case Selection

This study was approved by the Northwestern University Institutional Review Board (IRB). A total of 77 cases of LM-BN were retrieved from the pathology databases at Northwestern Memorial Hospital (Northwestern University, Chicago, IL, USA) and Qilu Hospital (Shandong University, Shandong, China). This included 49 cases from Northwestern Memorial Hospital and 28 cases from Qilu Hospital. The slides from each case were reviewed by two pathologists to confirm the original diagnosis using the Bell criteria¹⁴ and clinical information from each patient's chart was also recorded. Detailed case information is summarized in Table 1.

Histologic features of type I and II LM-BN

By reviewing all tumor slides, LM-BN were divided into two subtypes purely based on distinct nuclear features. Type I LM-BN were characterized by large round or oval nuclei, distinct smooth nuclear membranes, prominent nucleoli, and open chromatin (Figure 1). Type II LM-BN were characterized by elongated or spindle nuclei, irregular nuclear membranes, pinpoint or no nucleoli, and dark smudgy chromatin (Figure 1). For those cases with mixed type I and II nuclear features, we defined cases as either type I or II when over 70% of the tumor cells appeared to show nuclear features favoring one or another. The detailed description of type I and II characterization has been outlined in a previous study¹³.

Immunohistochemical analysis

Antigen retrieval was performed by first microwaving in a citrate buffer at a pH 6.0 for 15 minutes then blocking for endogenous peroxidase. The specimen was then incubated with anti-2SC polyclonal antibody (1:5000) and washed in phosphate buffered saline (PBS). Myometrium was used as a negative control. The 2SC antibody¹⁵ was provided by Dr. Norma Frizzell and used as previously described¹⁶. FH from Santa Cruz Biotechnology was used at a dilution of 1:200 (J-13 fumarate hydratase, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The IHC stains were analyzed blindly by two pathologists and the percent staining and staining intensity (0=negative, 1+=weak, 2+=moderate, and 3+=strong) were recorded for each case. Strong and diffuse immunoreactivity for 2SC in the cytoplasm was considered positive. FH immunostaining was considered negative when there was no detectable signals in the tumor cells and FH staining was positive when the tumor cells showed strong cytoplasmic staining. Endothelial cells showed positive staining and were used as an internal control.

Semiquantitative scores for each tumor and matched myometrial controls were calculated using the staining intensity and percentage. We found that both 2SC and FH IHC had specific staining patterns and were easily identified by their positive or negative stain. 2SC positivity was defined as diffusely strong immunoreactivity for 2SC while weak or focal immunoreactivity for 2SC was considered negative. FH negativity was defined as complete absence of immunoreactivity for FH in tumor cells.

Molecular analysis

Mutational analysis for *FH* was performed on 72 of the LM-BN cases. Pure tumor tissue were sectioned at 10 μ m and genomic DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) tissue using the QIAamp DNA FFPE Tissue Kit (QIAGEN, 56404). For *FH* mutational analysis, 50ng DNA was amplified by PCR with HotStarTaq Master Mix (QIAGEN, 203446) at all ten *FH* exons. PCR products were then purified using the Gel Extraction and PCR Clean-Up Kit (Clontech) according to the user's manual. DNA sequencing of the purified DNA products was performed using the ABI 3730 High-Throughput DNA Sequencer. The mutations and variations were analyzed using DNASTAR Lasergene 9 software. Detailed information regarding primers can be found in Supplementary Table 1.

Statistical analysis

SPSS Version 19.0 was used for statistical analysis. The mean differences between clinical parameters and histologic features were analyzed using a student T-test. Positivity rates for each gene were performed using Pearson's chi-squared test and Fisher exact test. The association between 2SC and *FH* IHC expression was evaluated using the Pearson coefficient. *P* values less than 0.05 were considered statistically significant.

RESULTS

General case information

We reviewed and confirmed the diagnosis for 77 LM-BN cases by Bell's criteria¹⁴. The patient age ranged from 21 to 65 years with a median age of 40 years and the mean tumor size was 7.7 cm (Table 1). LM-BN were further divided into two categories, type I and type II, based on nuclear features¹³. Type I LM-BN showed round-to-oval nuclei, distinct and smooth nuclear membranes, prominent nucleoli with perinucleolar halos, and open chromatin (Figure 1). Type II LM-BN showed large elongated or spindle nuclei, irregular nuclear membranes, pinpoint or absent nucleoli, and dark smudgy chromatin (Figure 1). In general, type I LM-BN patients were younger (mean age of 38 years) than that of type II (mean age of 43 years, $p < 0.01$) and the mean overall tumor size was larger in type I than in type II (8.7 cm vs. 6.7 cm, $p < 0.05$) (Table 1). Seventy-two of the 77 patients had more than one uterine nodule. The additional nodules were either additional LM-BN or usual-type leiomyomas (ULM). The general information is summarized in Table 1.

Immunohistochemical analysis of 2SC

S-(2-succino)-cysteine (2SC) is a downstream biomarker for biallelic *FH* inactivation and strong IHC reactivity has been shown to be a sensitive marker in detecting defective *FH* enzyme function in tumor cells (Figure 2). In the 77 LM-BN cases, diffuse positivity for 2SC was found in 53% (41/77) of cases while all 60 myometrial controls were completely negative (Figure 2B). Overall, patients with 2SC immunoreactivity were younger and had a larger tumor burden than the 2SC negative cases ($p < 0.05$). Additionally, histologic features including prominent nucleoli, perinucleolar halos, hyaline globules, and staghorn vessels had high concordance with 2SC IHC positivity (Table 2).

Next, immunoreactivity for 2SC was compared between type I and type II LM-BN. Of the 40 type I LM-BN cases, 85% (34/40) were 2SC positive (Figure 2), while 19% (7/37) of type II were positive for 2SC ($p<0.01$) (Table 3). These findings suggest that the majority of type I LM-BN were related to FH alterations and only a small fraction of type I LM-BN (7/40) were completely negative for 2SC by IHC (Figure 3). As shown in Figure 3, tumors with characteristic nuclear features of type I LM-BN that were completely negative for 2SC were re-reviewed. To confirm that there were no misdiagnoses of uterine PEComa, immunohistochemical stains for Melanin A and HMB45 were performed and were negative for all cases. Of the 37 type II LM-BN, 81% (30/37) were negative for 2SC (Figure 4) and only 19% were positive for 2SC by immunohistochemistry (Figure 5). While the nuclear features of type I LM-BN strongly correlated with 2SC immunopositivity, this alone was not always an accurate predictor of 2SC positivity (Table 3). The positive rates of 2SC in LM-BN between the two different institutions were also similar (55% vs 51%, respectively).

Immunohistochemical analysis of FH

2SC immunoreactivity detects the byproduct of accumulated fumarate in tumors with defective FH. While it appears to be sensitive, its specificity for FH gene mutations in uterine smooth muscle tumors is still unclear^{8, 10, 17}. Thus, to test this concept further to see whether 2SC-positive tumors are indeed caused by FH genetic alterations, all cases were also stained with FH antibodies. FH IHC should be negative in tumors with loss of FH and is quite specific¹⁷. As illustrated in Figure 2, complete absence of the immunostain in tumor cells for FH was identified and compared to myometrial tissue and internal endothelial cells. Among the 41 cases of 2SC-positive LM-BN, 95% (39/41) were negative for FH by IHC (Figure 2). A Pearson rate correlating the 2SC and FH immunohistochemical staining was -0.939 (Table 4). Only two cases were positive for both markers and FH mutational analysis revealed that neither of those cases had FH mutations (see below). All 36 2SC-negative cases were also FH-positive (Figure 4). Overall, IHC for 2SC and FH were complimentary to each other in almost all cases and can be reliably used to detect FH alterations in LM-BN (Figure 2 and 4, Table 3).

Molecular analysis

The immunohistochemical analysis of 2SC and FH appears to be both sensitive and specific in identifying FH alterations in LM-BN cells. To test whether the alterations of FH expression by IHC were due to *FH* gene mutations, *FH* gene mutation analysis was conducted in 72 LM-BN cases using Sanger sequencing, specifically looking at the ten exons of the *FH* gene. Among the 72 LM-BN cases, 21% (15/72) harbored *FH* mutations. In our current study, 36.6% (15/41) of 2SC-positive LM-BN harbored *FH* gene mutations, mainly comprised of missense mutations (Figure 5). *FH* mutations were found in 38.5% (15/39) cases that were negative for FH by IHC. The *FH* mutant tumors were exclusively identified in tumors with positive 2SC and negative FH by IHC (Table 3, *FH*Figure 6). Yet, there was still a large number of 2SC-positive and FH-negative cases by IHC with no detectable mutations by sequencing analysis.

DISCUSSION

FH alterations are seen in HLRCC (Reed Syndrome) and *FH* germline mutations are present in 85% of these cases¹⁸. In contrast, the incidence of *FH* mutations in sporadic leiomyoma is very low. Previous studies have shown only 1% of unselected leiomyomas will have FH-deficiency and most are somatically-acquired *FH* mutations (Table 4)^{8, 9, 19, 20}. In uterine leiomyomas from patients younger than 40 years of age, *FH* mutations have been reported to be only slightly higher, accounting for 2.57% of these cases⁹. Additionally, *FH* mutations in LMS have occasionally been reported^{21, 22}. In those leiomyomas with *FH* gene mutations, some have characteristic histologic features, defined as SMT-FH^{10, 11} (Table 4). These tumors are typically cellular with eosinophilic macronucleoli, perinucleolar halos, and eosinophilic cytoplasmic globules. They also frequently show branching staghorn vessels. Miettinen et al. examined FH IHC staining in different types of uterine smooth muscle tumors and found 37% of LM-BN were negative for FH by IHC (Table 4). S-(2-succino)-cysteine (2SC) is another protein that may be used as a sensitive and specific marker for fumarate in the setting of defective FH and can be used as a surrogate marker for FH by immunohistochemistry¹⁰. Absent immunohistochemical staining for FH has also been shown to have high specificity when evaluating FH alterations^{8, 10, 23}. Using the two IHC stains in combination, along with *FH* mutational analysis, appears to be an extremely reliable way to confirm FH alterations and mutations in LM-BN.

In our study, the 77 cases with an LM-BN diagnosis were divided into two subtypes based on cytologic features¹³, with type I LM-BN showing features similar to SMT-FH (Figure 1)¹³. In total, there were 40 cases showing type I features and 37 with type II features. We found that 85% of type I LM-BN were strongly and diffusely immunoreactive for 2SC (Table 3, Table 3, *FH*Figure 2) and all but two of those cases also showed loss of immunoreactivity for FH. These findings suggest that most, if not all, type I LM-BN are related to FH alterations. We found that roughly 20% of all LM-BN had mutations and 37% (15/41) of those with 2SC positivity by IHC harbored *FH* mutations (Table 3). The underlying molecular mechanisms for FH alterations in the remaining cases needs to be further investigated. Our findings suggest that for those sporadic tumors originally diagnosed as LM-BN, careful evaluation of their nuclear features may be helpful in the potential differential diagnosis of an FH-related tumor. All type I LM-BN tumors and tumors with SMT-FH features should be tested for FH alterations by 2SC/FH IHC or by mutational analysis. Recently Joseph et al⁹ proposed an algorithm for screening SMT-FH in young patients and we believe their approach should also be applied to all LM-BN cases. Since nearly 20% of the type II LM-BN in our study presented with FH alterations by both 2SC and FH IHC and none had recognizable features of SMT-FH, immunohistochemistry may be the only method for detection of FH alterations in this tumor subset.

As summarized in Table 4, the rate of *FH* mutations in HLRCC is quite high¹⁸ while the rate in sporadic LM-BN is largely unknown. The results from Miettinen et al²⁴ concur with our current study. We have shown that FH alterations can be detected by immunohistochemistry for 2SC and that *FH* mutations appear to be disproportionately higher in LM-BN than in other types of sporadic uterine smooth muscle tumors including usual-type leiomyomas, although additional *FH* gene mutation analysis is still needed. Our current study reveals that

over 20% (15/72) of LM-BN harbor *FH* mutations and over one-third with FH IHC alterations also have corresponding *FH* gene mutations (Figure 6). The subset that shows FH alterations by IHC without an associated genetic cause requires further study. In a recent study, Liegl-Atzwanger et al. reported that *FH* genomic deletions can be found in over 30 % LM-BN ²⁵. Preliminary data from our cohort also showed that in 4 cases of LM-BN with extracted DNA failed to amplify in any of the 10 *FH* exons, suggestive of *FH* genomic deletions.

2SC is a sensitive and specific immunohistochemical marker for detection of FH deficiency, whereas negative staining for FH by IHC is more specific. We found a high concordance rate between these two markers in FH-altered tumors, with 39 of 41 2SC-positive cases also showing loss of FH by IHC (Table 4). For those two cases with immunoreactivity for both 2SC and FH, we postulate that there are likely other molecular defects resulting in succinate accumulation in this small subset of LM-BN (~5%). Yet FH by IHC alone is sensitive enough to detect over 95% of FH alterations and it can be used alone for routine screening.

The correlation of 2SC and FH by immunohistochemistry and their concordance with FH gene mutations in uterine smooth muscle tumors is of great clinical interest. The most common type of mutations are missense mutations (57%), followed by frameshift and nonsense mutations (27%), with the remainder consisting of a diverse array of deletions, insertions and duplications ²⁶. In our current study, 36.6% (15/41) of 2SC positive LM-BN harbored *FH* gene mutations and they were mainly missense mutations (Figure 6). Since the *FH* gene is located in chromosome 1q and this region has been found to be deleted frequently in uterine smooth muscle tumors ^{27–29}, analysis of additional chromosomal alterations deserves further investigation.

This study examined FH alterations and mutations by immunohistochemistry and gene mutation analysis in 77 LM-BN. More than 50% of cases originally diagnosed as LM-BN were related to FH alterations and over 20% of them harbored FH gene mutations. FH alterations and mutations were more frequently identified in type I LM-BN, suggesting a different mode of tumorigenesis in this histologic tumor subtype and which may have important clinical implications in this subset of patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank the Pathology Core Facility at Northwestern Lurie Comprehensive Cancer Center for their technical support. Part of this work was supported by P01HD057877 (NIH, NICHD).

References

1. Ly A, Mills AM, McKenney JK, et al. Atypical leiomyomas of the uterus: a clinicopathologic study of 51 cases. The American journal of surgical pathology. 2013; 37:643–649. [PubMed: 23552381]

2. Mills AM, Ly A, Balzer BL, et al. Cell cycle regulatory markers in uterine atypical leiomyoma and leiomyosarcoma: immunohistochemical study of 68 cases with clinical follow-up. *The American journal of surgical pathology*. 2013; 37:634–642. [PubMed: 23552380]
3. Croce S, Young RH, Oliva E. Uterine leiomyomas with bizarre nuclei: a clinicopathologic study of 59 cases. *The American journal of surgical pathology*. 2014; 38:1330–1339. [PubMed: 25140893]
4. Zhang Q, Ubago J, Li L, et al. Molecular analyses of 6 different types of uterine smooth muscle tumors: Emphasis in atypical leiomyoma. *Cancer*. 2014; 120:3165–3177. [PubMed: 24986214]
5. Atkins KA, Arronte N, Darus CJ, et al. The Use of p16 in enhancing the histologic classification of uterine smooth muscle tumors. *Am J Surg Pathol*. 2008; 32:98–102. [PubMed: 18162776]
6. Bodner-Adler B, Bodner K, Czerwenka K, et al. Expression of p16 protein in patients with uterine smooth muscle tumors: an immunohistochemical analysis. *Gynecol Oncol*. 2005; 96:62–66. [PubMed: 15589581]
7. Tomlinson IP, Alam NA, Rowan AJ, et al. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nat Genet*. 2002; 30:406–410. [PubMed: 11865300]
8. Harrison WJ, Andrici J, Maclean F, et al. Fumarate Hydratase-deficient Uterine Leiomyomas Occur in Both the Syndromic and Sporadic Settings. *Am J Surg Pathol*. 2016; 40:599–607. [PubMed: 26574848]
9. Joseph NM, Solomon DA, Frizzell N, et al. Morphology and Immunohistochemistry for 2SC and FH Aid in Detection of Fumarate Hydratase Gene Aberrations in Uterine Leiomyomas From Young Patients. *The American journal of surgical pathology*. 2015; 39:1529–1539. [PubMed: 26457356]
10. Reyes C, Karamurzin Y, Frizzell N, et al. Uterine smooth muscle tumors with features suggesting fumarate hydratase aberration: detailed morphologic analysis and correlation with S-(2-succino)-cysteine immunohistochemistry. *Mod Pathol*. 2014; 27:1020–1027. [PubMed: 24309325]
11. Wheeler KC, Warr DJ, Warsetsy SI, et al. Novel fumarate hydratase mutation in a family with atypical uterine leiomyomas and hereditary leiomyomatosis and renal cell cancer. *Fertil Steril*. 2016; 105:144–148. [PubMed: 26493120]
12. Wei JJ. Atypical Leiomyoma With Features Suggesting of Fumarate Hydratase Mutation. *Int J Gynecol Pathol*. 2016; 35:531–536. [PubMed: 26919578]
13. Ubago JM, Zhang Q, Kim JJ, et al. Two Subtypes of Atypical Leiomyoma: Clinical, Histologic, and Molecular Analysis. *Am J Surg Pathol*. 2016; 40:923–933. [PubMed: 27015034]
14. Bell SW, Kempson RL, Hendrickson MR. Problematic uterine smooth muscle neoplasms. A clinicopathologic study of 213 cases. *Am J Surg Pathol*. 1994; 18:535–558. [PubMed: 8179071]
15. Nagai R, Brock JW, Blatnik M, et al. Succination of protein thiols during adipocyte maturation: a biomarker of mitochondrial stress. *J Biol Chem*. 2007; 282:34219–34228. [PubMed: 17726021]
16. Bardella C, El-Bahrawy M, Frizzell N, et al. Aberrant succination of proteins in fumarate hydratase-deficient mice and HLRCC patients is a robust biomarker of mutation status. *J Pathol*. 2011; 225:4–11. [PubMed: 21630274]
17. Buelow B, Cohen J, Nagymanyoki Z, et al. Immunohistochemistry for 2-Succinocysteine (2SC) and Fumarate Hydratase (FH) in Cutaneous Leiomyomas May Aid in Identification of Patients With HLRCC (Hereditary Leiomyomatosis and Renal Cell Carcinoma Syndrome). *The American journal of surgical pathology*. 2016; 40:982–988. [PubMed: 26945337]
18. Kiuru M, Launonen V. Hereditary leiomyomatosis and renal cell cancer (HLRCC). *Curr Mol Med*. 2004; 4:869–875. [PubMed: 15579034]
19. Barker KT, Spendlove HE, Banu NS, et al. No evidence for epigenetic inactivation of fumarate hydratase in leiomyomas and leiomyosarcomas. *Cancer Lett*. 2006; 235:136–140. [PubMed: 15949892]
20. Barker KT, Bevan S, Wang R, et al. Low frequency of somatic mutations in the FH/multiple cutaneous leiomyomatosis gene in sporadic leiomyosarcomas and uterine leiomyomas. *Br J Cancer*. 2002; 87:446–448. [PubMed: 12177782]
21. Ylisaukko-oja SK, Kiuru M, Lehtonen HJ, et al. Analysis of fumarate hydratase mutations in a population-based series of early onset uterine leiomyosarcoma patients. *Int J Cancer*. 2006; 119:283–287. [PubMed: 16477632]

22. Kiuru M, Lehtonen R, Arola J, et al. Few FH mutations in sporadic counterparts of tumor types observed in hereditary leiomyomatosis and renal cell cancer families. *Cancer Res.* 2002; 62:4554–4557. [PubMed: 12183404]
23. Chen YB, Brannon AR, Toubaji A, et al. Hereditary leiomyomatosis and renal cell carcinoma syndrome-associated renal cancer: recognition of the syndrome by pathologic features and the utility of detecting aberrant succination by immunohistochemistry. *Am J Surg Pathol.* 2014; 38:627–637. [PubMed: 24441663]
24. Miettinen M, Felisiak-Golabek A, Wasag B, et al. Fumarase-deficient Uterine Leiomyomas: An Immunohistochemical, Molecular Genetic, and Clinicopathologic Study of 86 Cases. *The American journal of surgical pathology.* 2016; 40:1661–1669. [PubMed: 27454940]
25. Liegl-Atzwanger B, Heitzer E, Flicker K, et al. Exploring chromosomal abnormalities and genetic changes in uterine smooth muscle tumors. *Mod Pathol.* 2016; 29:1262–1277. [PubMed: 27363490]
26. Bayley JP, Launonen V, Tomlinson IP. The FH mutation database: an online database of fumarate hydratase mutations involved in the MCUL (HLRCC) tumor syndrome and congenital fumarase deficiency. *BMC Med Genet.* 2008; 9:20. [PubMed: 18366737]
27. Christacos NC, Quade BJ, Dal Cin P, et al. Uterine leiomyomata with deletions of 1p represent a distinct cytogenetic subgroup associated with unusual histologic features. *Genes, chromosomes & cancer.* 2006; 45:304–312. [PubMed: 16320247]
28. Hodge JC, Pearce KE, Clayton AC, et al. Uterine cellular leiomyomata with chromosome 1p deletions represent a distinct entity. *American journal of obstetrics and gynecology.* 2014; 210:572–577. e571–577. [PubMed: 24412114]
29. Mehine M, Kaasinen E, Heinonen HR, et al. Integrated data analysis reveals uterine leiomyoma subtypes with distinct driver pathways and biomarkers. *Proceedings of the National Academy of Sciences of the United States of America.* 2016

Figure 1.

Photomicrographs illustrating hematoxylin and eosin (H&E) stained slides in three randomly selected cases of type I (left) and type II (right) LM-BN. Type I LM-BN have large round to oval nuclei, prominent nucleoli and perinucleoli halos. They also have open chromatin and eosinophilic hyaline globules. Type II LM-BN have elongated and spindle nuclei with dark, hyperchromatic, and smudgy chromatin. (Magnification 40X).

Figure 2.

Side-by-side comparison of immunoreactivity for 2SC and FH by IHC in two randomly selected type I LM-BN (A) and control myometrium (B). Strong and diffuse immunoreactivity for 2SC can be appreciated in both the nucleus and cytoplasm. Loss of immunoreactivity for FH is evident in all tumor cells while positive staining for FH in endothelial cells is present as an internal positive control. H&E sections (40X) are presented on the left.

Figure 3.

Histologic and immunohistochemical analysis of 2SC in two randomly selected type I LM-BN. H&E stained slides (60X) reveal the large tumor cells with round to oval nuclei, prominent nucleoli, perinucleoli halos, open chromatin, and distinct nuclear membranes. Occasional eosinophilic hyaline globules are seen. Stains for Melanin A and HMB45 were performed (not shown) and were negative, excluding the differential diagnosis of PEComa. The tumor cells showed weak or negative immunostaining for 2SC.

Figure 4.

Side-by-side comparison of immunoreactivity for 2SC and FH in two randomly selected type II LM-BN (A) and control myometrium (B). Strong and diffuse immunoreactivity for FH can be appreciated in the cytoplasm of both the tumor cells and the endothelial cells. Immunostaining for 2SC was negative in both cases. H&E sections (40X) are presented on the left.

Figure 5.
Photomicrographs illustrating examples of diffuse immunoreactivity for 2SC in two cases with type II LM-BN nuclear features.

Figure 6.

Sequencing analysis of FH mutations in uterine smooth muscle tumors. A) Sequencing plots with red arrows designating the mutation sites, B) Mutation distribution of fumarate hydratase (red dots) in each of the ten FH exons.

Table 1

General information for LM-BN cases and subtypes

Clinical parameters	Measurement	Total LM-BN	Type I	Type II	p value
No. Cases		77	40	37	
Age (years)	Med (Range)	40 (21–65)	38 (21–57)	43 (28–65)	0.003
Hysterectomy	Percent (n)	54.5 (42/77)	42.5 (17/40)	67.6 (25/37)	0.027
No. Tumor slides	Med (Range)	7 (2–20)	7 (3–19)	6 (2–20)	0.063
Tumor size (cm)	Mean±sem	7.7±0.5	8.7±0.7	6.7±0.6	0.728

Table 2

Correlation between 2SC status and histologic features

	Immunoreactivity for 2SC		p value
	Positive	Negative	
No. cases	53.2% (41/77)	46.8% (36/77)	
Age (years)	38	43	0.045
Size (cm)	8.6	6.7	0.024
Hyaline globules	96.8%	30.7%	<0.001
Prominent nucleoli	80.6%	17.9%	<0.001
Perinucleolar halo	83.3%	16.7%	<0.001
Staghorn vessels	91.7%	33.3%	<0.001

Table 3

FH IHC expression and mutational analysis in LM-BN

	No. cases	2SC +ve % (n)	FH -ve % (n)	FH mutation % (n)	2SC +ve FH -ve *	2SC +ve mutation *
Total LM-BN	77	53.2 (41/77)	50.6 (39/77)	20.8 (15/72)	-0.939	0.428
Type I	40	85.0 (34/40)	82.5 (33/40)	26.3 (10/38)	-0.791	0.224
Type II	37	18.9 (7/37)	16.2 (6/37)	14.7 (5/34)	-1.000	0.808
2SC Positive	41		95.1 (39/41)	36.6% (15/41)		
2SC Negative	36		0 (0/36)	0 (0/33)		

* : Pearson correlation

Table 4

FH alterations and mutations in sporadic uterine leiomyomas, hereditary uterine leiomyoma and atypical leiomyoma

	2SC +ve % (n)	FH –ve % (n)	FH mutation % (n)	References
ULM	N/A	1.3 (31/2449)	1.3 (5/413)	Five studies ^{8, 9, 19, 20}
HLRCC			85 (89/105)	Kiuru et al ¹⁸
SMT-FH	100% (9/9)	N/A	66.7 (2/3)	Reyes; Wheeler et al ^{10, 11}
ALM	N/A	37.3 (68/182)	N/A	Miettinen et al ²⁴
LM-BN	53.2 (41/77)	50.6 (39/77)	20.8 (15/72)	Current study

ULM: usual leiomyoma; SMT-FH: smooth muscle tumor with features of fumarate hydratase alterations; ALM: atypical leiomyoma; LM-BN: atypical leiomyoma/leiomyoma with bizarre nuclei