Virchows Archiv (2022) 480:281–291
https://doi.org/10.1007/s00428-021-03217-z

ORIGINAL ARTICLE

# Uterine cellular leiomyomas are characterized by common HMGA2 aberrations, followed by chromosome 1p deletion and *MED12* mutation: morphological, molecular, and immunohistochemical study of 52 cases

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Received: 30 July 2021 / Revised: 14 September 2021 / Accepted: 30 September 2021 / Published online: 9 October 2021


## Abstract

Cellular leiomyoma (CL) represents an uncommon variant of uterine leiomyoma with limited data concerning its immunohistochemical and molecular profile. We performed a comprehensive analysis of 52 CL cases all of which were analyzed immunohistochemically. Molecular analysis was possible in 32 cases with sufficient DNA, and 38 cases with sufficient RNA. The immunohistochemical results showed a high expression of smooth muscle markers (calponin (100%), desmin (100%), smooth muscle actin (98.1%), caldesmon (96.1%), transgelin (96.1%), smooth muscle myosin heavy chain (86.5%), and smoothelin (61.5%)). Concerning markers of endometrial stromal differentiation, the expression of CD10 was observed in 65.4% cases (42.2% with *H*-score > 50), and IFITM1 in 36.5% cases (1.9% with *H*-score > 50). 36.5% showed HMGA2 overexpression at the IHC level, associated with increased mRNA expression in 14/14 cases. The rearrangement of the *HMGA2* gene was detected in 13.2%. Chromosome 1p deletion was found in 19.3%, while 9.4% of tumors showed a pathogenic mutation in the *MED12* gene. In conclusion, CL is immunohistochemically characterized by a high expression of "smooth muscle" markers commonly associated with a co-expression of "endometrial stromal" markers, where IFITM1 shows superior performance compared to CD10 regarding its specificity for differentiation from endometrial stromal tumors. The sensitivity of smoothelin in CL seems rather low, but no data is available to assess its specificity. On a molecular level, the most common mutually exclusive aberration in CL affects HMGA2, followed by chromosome 1p deletions and *MED12* mutations.

**Keywords** Cellular leiomyoma · MED12 · HMGA2 · Chromosome 1p · NGS · ddPCR

## Introduction

Cellular leiomyoma (CL) is uncommon and represents less than 5% of all uterine leiomyomas [1]. A correct diagnosis is crucial, as the differential diagnosis includes not only benign lesions such as endometrial stromal nodule (ESN), but also malignant tumors, especially low grade endometrial stromal sarcoma (LG-ESS) and leiomyosarcoma (LMS) [2–5]. The morphologic diagnostic criteria are well established, but there are cases which cannot be diagnosed with certainty based on the morphology alone and ancillary methods are needed. Currently, the molecular classification of tumors is gaining significance and the knowledge of recurrent molecular aberrations is increasingly used in the differential diagnostics of several tumors. The spectrum of aberrations occurring in usual leiomyomas, which are

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mostly characterized by the *MED12* mutation and less commonly by HMGA2 overexpression, is well known [6–9]. The third molecular subtype of leiomyoma is characterized by fumarate hydratase (FH) deficiency, which is typical for FH-deficient leiomyoma and a subset of leiomyoma with bizarre nuclei (LBN), but is absent or very rare in usual leiomyoma (UL) [10–12]. However, the knowledge of molecular findings occurring in CL is limited. In our study, we focused on molecular aberrations occurring in CL with respect to the three molecular leiomyoma subtypes, their mutual exclusivity, the occurrence of cases with chromosome 1p deletion, and the assessment of other molecular changes with a possible recurrent pattern. Moreover, we performed a complex immunohistochemical analysis using a broad panel of antibodies potentially useful for differential diagnosis between CL and endometrial stromal tumors (EST), including some less commonly used antibodies (transgelin, IFITM1) and antibodies whose expression has not yet been analyzed in CL (smoothelin). We also analyzed the expression of other antibodies with possible diagnostic meaning whose expression has not yet been analyzed in CL (BCOR, NTRK, ALK, HMB45) or has been analyzed in only a limited number of cases (CD117, CD44). Finally, our IHC findings were compared with the available literary data.

## Materials and methods

The archive files of our department were searched for cases diagnosed as CL. All cases were reviewed independently by two pathologists (PD and KN) and only the 52 consensual cases meeting the strict diagnostic criteria of CL were included in the study. The main diagnostic criterium for differentiation between usual and cellular leiomyoma represented a substantially increased cellularity compared to the surrounding myometrium (if present). If the myometrium was absent (myomectomy or morcellated specimens), only cases in which the subjective impression of hypercellularity was supported by other features typical of CL, such as thick-walled vessels and the presence of clefts, were included in the study. Microscopic features assessed in each tumor selected for the study included: nuclear atypia, mitotic figures (per 10 HPF equal to 2.4 $mm^2$), margins (sharp; irregular—undulating uneven margins with possible intersecting fascicles of myometrial smooth muscle; infiltrative—tongue-like infiltrative appearance with a dissection of myometrial smooth muscle), hyalinization (absent; present; rare), hyaline plaques—plaque-like areas usually occurring in endometrial stromal tumors (absent; present; rare), neural-like areas (absent; present; rare), satellite nodules (absent; present), cleft-like spaces (absent; present; rare); and large vessels with a thick muscular wall (absent; present; rare).

### Immunohistochemical analysis

The immunohistochemical (IHC) analysis was performed using 4 µm thick sections of formalin-fixed and paraffin-embedded (FFPE) tissue using tissue microarrays (TMAs). The eligible areas of tumor were identified and two tissue cores (each 2.0 mm in diameter) were drilled from the donor block using the tissue microarray instrument TMA Master (3DHISTECH Ltd., Budapest, Hungary). The only exception was the expression of smoothelin, which was assessed on whole tissue sections due to its limited and commonly weak expression. The expression of the following antibodies was examined in each tumor: transgelin, smoothelin, smooth muscle actin (SMA), IFITM1, fumarate hydratase (FH), CD10, desmin, caldesmon, BCOR, CD44, calponin, smooth muscle myosin heavy chain (SMMHC), pan-TRK, ALK, CD117, and HMB45. The clones, manufacturers, dilution, and staining instruments for all antibodies are summarized in Table 1. The immunohistochemical results were assessed according to the overall percentage of positive cells (0–100%) and then also semi-quantitatively, using the H-score. This method is based on the assessment of the percentage of positive cells based on the level of staining intensity (1 + for weak intensity, 2 + for moderate, and 3 + for strong intensity). The final H-score for each case is then calculated by adding the multiplication of the different staining intensities according to the following formula: 1x (% of cells 1 +) + 2x (% of cells 2 +) + 3x (% of cells 3 +), resulting in an H-score value of 0 – 300. For the comparison of our results with literature data, cases were classified based only on the extent (not intensity) of expression as negative (0%), positive (any positivity), and 3 + positive (≥ 50%). The literary data concerning the extent of HMGA2 expression necessary to be classified as an "overexpression" is not well defined. In our study, we used the same criteria as Bertsch et. al., and moderate to strong expression in ≥ 50% of tumor cells was classified as "overexpression" [13].

### Molecular analysis

Capture NGS analysis of DNA and RNA was performed for all qualitatively sufficient cases: 32/52 (61.5%) DNA and 38/52 (73.1%) RNA.

Genomic DNA and RNA were isolated by the DNA/RNA FFPE isolation kit (ZymoResearch) according to the manufacturer's protocol. DNA quality control was performed by qPCR assay, as described previously [14]. The isolated total RNA samples were characterized by Fragment Analyser capillary electrophoresis system (AATI) using Standard RNA kit (AATI), resulting in an RNA

**Table 1** List of immunohistochemical antibodies

| Antibody | Clone | Dilution | Producer | Platform | Detection |
|---|---|---|---|---|---|
| Transgelin | 2A10C2 | 1:300 | Cell Marque, Rocklin, CA, USA | Ventana BenchMark ULTRA (Roche, Basel, Switzerland) | OptiView |
| Smoothelin | R4A | 1:50 | Zeta Corporation, Sierra Madre, CA, USA | Ventana BenchMark ULTRA (Roche, Basel, Switzerland) | OptiView |
| SMA | 1A4 | 1:800 | Dako, Glostrup, Denmark | PT-link (Agilent, Santa Clara, CA, USA) | EnVision FLEX (Agilent) |
| IFITM1 | polyclonal | 1:300 | Abcam, Cambridge, United Kingdom | Ventana BenchMark ULTRA (Roche, Basel, Switzerland) | OptiView |
| FH | polyclonal | 1:500 | Abcam, Cambridge, United Kingdom | PT-link (Agilent, Santa Clara, CA, USA) | EnVision FLEX (Agilent) |
| CD10 | 56C6 | 1:50 | Novocastra, Leica Biosystems, Wetzlar, Germany | Ventana BenchMark ULTRA (Roche, Basel, Switzerland) | OptiView |
| Desmin | D33 | 1:200 | Dako, Glostrup, Denmark | Ventana BenchMark ULTRA (Roche, Basel, Switzerland) | OptiView |
| Caldesmon | h-CD | 1:50 | Novocastra, Leica Biosystems, Wetzlar, Germany | PT-link (Agilent, Santa Clara, CA, USA) | EnVision FLEX (Agilent) |
| BCOR | C-10 | 1:50 | Santa Cruz Biotechnology, Dallas, TX, USA | Ventana BenchMark ULTRA (Roche, Basel, Switzerland) | OptiView |
| CD44 | DF1485 | 1:100 | Dako, Glostrup, Denmark | PT-link (Agilent, Santa Clara, CA, USA) | EnVision FLEX (Agilent) |
| Calponin | CALP | 1:400 | Dako, Glostrup, Denmark | PT-link (Agilent, Santa Clara, CA, USA) | EnVision FLEX (Agilent) |
| SMMHC | SMMS1 | 1:50 | Dako, Glostrup, Denmark | PT-link (Agilent, Santa Clara, CA, USA) | EnVision FLEX (Agilent) |
| Pan-TRK | EPR17341 | 1:250 | Abcam, Cambridge, United Kingdom | Ventana BenchMark ULTRA (Roche, Basel, Switzerland) | OptiView |
| ALK | D5F3 | 1:100 | Cell Signalling, Danvers, MA, USA | Ventana BenchMark ULTRA (Roche, Basel, Switzerland) | OptiView |
| CD117 | polyclonal | 1:400 | Dako, Glostrup, Denmark | PT-link (Agilent, Santa Clara, CA, USA) | EnVision FLEX (Agilent) |
| HMB45 | HMB45 | 1:100 | Dako, Glostrup, Denmark | PT-link (Agilent, Santa Clara, CA, USA) | EnVision FLEX (Agilent) |
| HMGA2 | D1A7 | 1:400 | Cell Signalling, Danvers, MA, USA | PT-link (Agilent, Santa Clara, CA, USA) | EnVision FLEX (Agilent) |

*SMA* smooth muscle actin, *FH* fumarate hydratase; *SMMHC* smooth muscle myosin heavy chain

Quality Number (RQN; median 3.3; range 1.1–10). Amplification capability was assessed by qPCR assay as a part of the FusionPlex protocol (ArcherDX).

Targeted NGS DNA analysis, biostatistical evaluation, and the interpretation of data was performed as described previously with minor changes [14]. The samples were processed using KAPA HyperPlus Kit (Roche) according to the KAPA HyperCapture protocol (Roche) and custom hybridization probes (944 kbp of target sequence, including 765 kbp of coding regions of 300 genes; Supplementary Table 1; KAPA HyperChoice; Roche). The RNA samples were processed by amplicon RNA NGS using Archer FusionPlex Sarcoma Expanded Kit (ArcherDX) according to the manufacturer's protocol. Five samples with undetermined HMGA2 fusion or detected low read count fusion were reanalyzed by targeted capture RNA-Seq approach using KAPA RNA HyperPrep Kit (Roche) with a panel of hybridization probes (373 kbp of target sequence; 147 genes; Supplementary Table 2, KAPA HyperChoice; Roche). The prepared libraries were sequenced by the NextSeq instrument (Illumina) using the NextSeq500/550 High Output Kit v2.5 (300 Cycles) according to the manufacturer's protocol.

Copy number variation (CNV) was evaluated using an algorithm in the NextGENe CNV Tool (Softgenetics). The variants (including 1q, *MED12*, and *FH* deletions) were evaluated in all cases. Precise CNV data analysis of the entire sequenced region was limited in several low-quality samples (complete CNV analyses were possible in 21 cases).

Fusions were evaluated from amplicon RNA NGS data using Archer Analysis software v5.1.7 (ArcherDX). Low-reads fusions or undetermined HMGA2 fusions were further inspected based on the capture RNA-Seq data in the

CLC Genomics Workbench and compared to the Archer FusionPlex results. Low-read fusions were considered only when detected by both the amplicon and capture RNA NGS approach.

Detailed pipelines of all NGS data analysis together with module settings are available upon request.

### Droplet digital PCR (ddPCR) expression analysis of HMGA2 mRNA

Complementary DNA (cDNA) was synthesized from 2 µg of total RNA as described previously [15]. The expression analysis was performed using the QX200 ddPCR system (Bio-Rad), quantification kits for probes (Bio-Rad), and custom FAM/HEX quencher probes. The amplicon in the *POLR2A* gene (located in the exon 27 and 28) was used as a reference and the amplicon in *HMGA2* (located in the exon 1 and 2) was used as a target (Supplementary Table 3). The reactions were prepared in multiplex PCR reactions using the ddPCR Supermix for Probes (No dUTP; Bio-Rad), 10 µl of cDNA template (approx. 500 ng of total RNA; where available) and 5 pmol of each of the two primers and two probes (250 nM final concentration) in a 20 µl reaction volume. Droplets were generated in the QX200 AutoDG instrument (Bio-Rad) and amplified according to the manufacturer's protocol. The resulting data was acquired by the QX200 Droplet Reader instrument (Bio-Rad) and analyzed by QuantaSoft (Bio-Rad).

Samples with > 10 reference POLR2A templates per 1 µl of cDNA were further evaluated (the ddPCR expression analysis of HMGA2 mRNA was possible in 40/52 cases.). Given that the median of expression of POLR2A in a healthy uterus is 144.4 TPM (Transcripts per million) and expression of HMGA2 is 0.02 TPM (according to GTEx Portal Database), we considered a high HMGA2 expression to be ≥ 0.25 of POLR2A [16].

## Results

Fifty-two cases were included in the study; 50 originated from our routine in-house files and 2 cases were sourced from our consultation files. Both consultation cases were sent for a second opinion with a differential diagnosis of CL and EST. From the in-house cases, only 2/50 (4%) were diagnostically challenging and the differential diagnosis of CL and EST was mentioned in the original biopsy report. In 15/50 cases (30%), immunohistochemistry was used during the routine pathological assessment to confirm the leiomyocellular differentiation of the lesion. The basic clinico-pathological data for all patients in the study (age, type of specimen, size, or weight of the tumor) are summarized in Table 2. Microscopic findings together with IHC and molecular findings are descriptively summarized in Fig. 1. The tumors commonly showed irregular margins (19/29), but in most cases, these changes were only focal. Infiltrative margins resembling the type of growth occurring in LG-ESS were found rarely and focally (2/29 cases). Representative microscopic findings are shown in Fig. 2.

**Table 2** Basic clinico-pathological data

| Clinical and gross features (number of cases) | | |
|---|---|---|
| Age (52); years | Range | 22–74 |
| | Mean | 43.2 |
| | Median | 42.5 |
| Surgical procedure (51) | HE | 25 |
| | Myomectomy | 3 |
| | Morcellation | 23 |
| Diameter (23); millimeters; HE specimens | Range | 15–75 |
| | Mean | 39.6 |
| | Median | 40 |
| Weight (19); grams; myomectomy and morcellation specimens | Range | 23–279 |
| | Mean | 104.8 |
| | Median | 76 |

*HE* hysterectomy

### Immunohistochemical findings

Immunohistochemical results are summarized in Fig. 1 and Table 3. Briefly, from the "smooth muscle markers," positivity of calponin and desmin was seen in 52/52 cases (100%), SMA in 51/52 cases (98.1%), caldesmon and transgelin in 50/52 cases (96.1%), SMMHC in 45/52 cases (86.5%), and smoothelin in 32/52 cases (61.5%) (Fig. 3). BCOR, CD117, and S100 protein were negative in all cases. The assessment of smoothelin was complicated by the commonly weak staining intensity, as a strong intensity of staining was present in only 10/52 cases. The markers of "endometrial stromal differentiation" showed the expression of CD10 in 34/52 cases (65.4%) and IFITM1 in 19/52 cases (36.5%) (Fig. 3). The *H*-score ≥ 50 for CD10 was recorded in 23/52 cases (44.2%) and for IFITM1 in 1/52 cases (1.9%). When only the extent of staining is taken into consideration, the expression of CD10 was present in > 50% of tumor cells in 5/52 cases (9.6%) and expression of IFITM1 in 1/52 cases (1.9%).

CD44 expression was detected in 8/52 cases (15.4%) and HMB45 in 2/52 cases (3.8%). From antibodies not listed in Table 3, the expression of HMGA2 was observed in 23/52 cases (44.2%), but only in 19/52 of cases (36.5%) was the expression classified as overexpression (≥ 50% tumor cells) (Fig. 3). All 52/52 cases (100%) showed a retained expression of FH. NTRK expression was seen in 5 cases (3 nuclear,

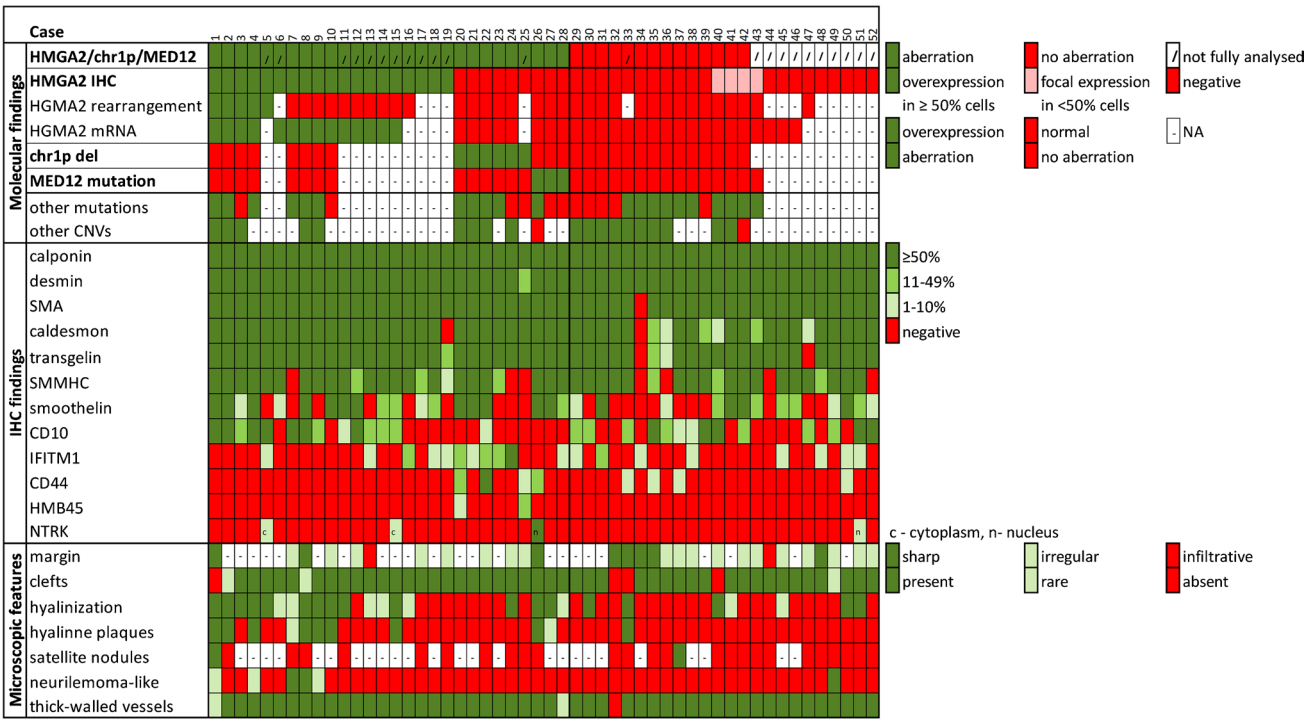


**Fig. 1** Summary of the molecular, immunohistochemical, and microscopic features

2 cytoplasmic), but no case with *NTRK* rearrangement was found. ALK was negative in all cases.

We compared our results with the available literary data concerning the expression of selected markers in CL and low grade endometrial stromal sarcoma, mostly based on our previous review focusing on the immunohistochemical findings in mesenchymal tumors of the uterus [3, 17–28]. The results are summarized in Table 3. Data for some antibodies were not available in the literature.

## Molecular genetic findings

The NGS outputs (32 DNA and 38 RNA) are summarized in Fig. 1. In total, 3/32 cases (9.4%) harbored mutations in the *MED12* gene and 6/31 cases (19.3%) had a heterozygous deletion of chromosome 1p. The RNA-seq revealed rearrangement of the *HMGA2* gene in 5/38 (13.2%) cases (all showing IHC overexpression of HMGA2). Fusion partners were identified on mRNA level in three cases: (i) HMGA2 (exon 3)—C9orf92 (exon 4); (ii) HMGA2 (exon 5)—PBX1 (exon 3); and (iii) RAD51B (exon 8)—HMGA2 (exon 2). In the remaining 2 cases, no fusion partner gene was detected. In these cases, the rearrangements were within the non-coding areas of chromosome 5 (chr5 34,437,681–34,437,906) and 6 (chr6 169,376,151–169,376,269), both connected to HMGA2 exon 4 on the RNA level. A high expression of HMGA2 mRNA was detected by ddPCR in 14/40 (35%) cases, in all of which it was associated with HMGA2 overexpression on the IHC level. In the 29/52 cases which were suitable for complete immunohistochemical and DNA/RNA NGS analyses of all the markers (*MED12* mutation, HMGA2 overexpression, *HMGA2* rearrangement, FH expression and mutation, and chromosome 1p deletion), all the aberrations were mutually exclusive.

In addition to the "driver" aberrations (mutually exclusive *MED12* mutation, deletion of chr1p, or HMGA2 overexpression) other mutations and/or copy number alterations were detected in a wide spectrum of genes and were mostly co-occurring (Supplementary Table 4 and 5). However, none of those aberrations had a recurrent pattern. Pathogenic mutations in the *FH* gene were not detected. Only two cases showed a variant of uncertain significance (class 3) and in one case a heterozygous deletion of the *FH* locus (all cases with retained FH expression on IHC level).

## Discussion

CL are defined as leiomyocellular tumors having a "significantly" greater cellularity compared to the adjacent myometrium, the microscopic features of which have already been well described in the literature [1, 4, 29–31]. The differential diagnosis of CL includes especially leiomyosarcoma (LMS) and EST. CL compared to LMS lacks significant nuclear atypia, substantial mitotic activity, and tumor-type necrosis. Based on this, the differential diagnosis between

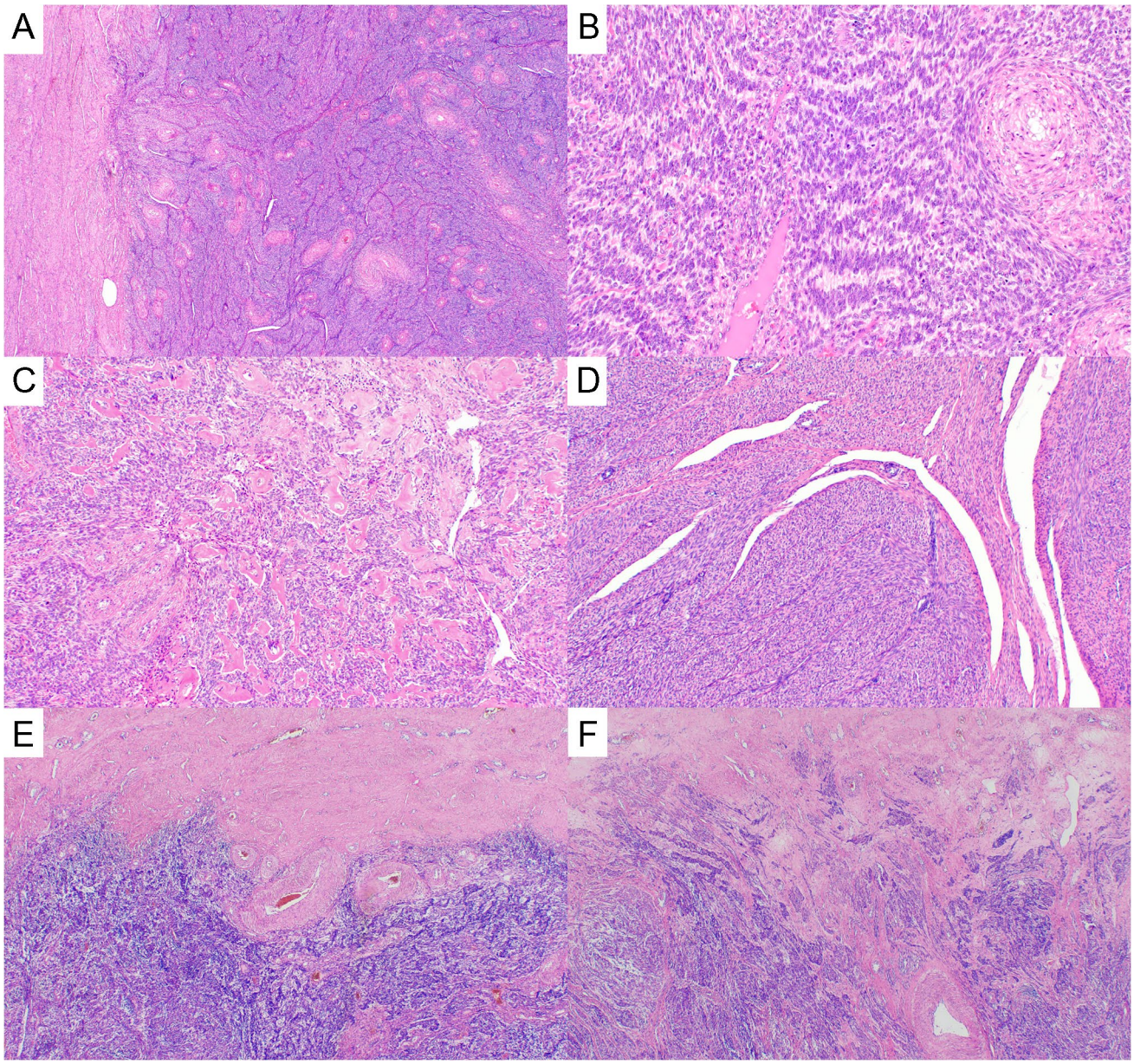


**Fig. 2** Cellular leiomyomas. **A** Lesion with substantially increased cellularity, numerous thick-walled vessels, and sporadic "clefts" (case no. 36, HE, ×40). **B** Neurilemoma-like areas with palisading of the nuclei (case no. 7, HE, ×200). **C** Multiple hyaline plaques (case no. 2, HE, ×200). **D** Areas with vessels forming the so-called "clefts" (case no. 28, HE, ×100). Some lesions showed irregular (**E**) and/or infiltrative margins (**F**) (HE, ×40)

CL and LMS is usually straightforward. However, the differential diagnosis between CL and EST is more complicated, especially in cases of the so-called highly cellular leiomyomas [1, 4, 5]. EST consists mostly of oval cells, and the fascicles of spindle cells are usually absent. Similarly to CL, the tumor cells have regular nuclei and a high nuclear-cytoplasmic ratio. However, the mitotic activity may be high, even in benign ESN. The characteristic feature of EST is the presence of small arterioles surrounded by whirls of tumor cells. Large thick-walled vessels and clefts are usually absent. The small arterioles typical for EST may be present also in CL, but tumor cells whorls surrounding these arterioles are absent. Increased perivascular cellularity due to the proliferation of pericytes may be confused with tumor cells whirling. Since ESN represents a benign entity, the most important differential diagnosis is between CL and LG-ESS. In this context, we should be aware that despite the relation to the surrounding myometrium being a defining feature for the discrimination between ESN and LG-ESS, this is not necessarily helpful in the distinction between CL and

**Table 3** Comparison of immunohistochemical findings in our CL cases with literary data (CL, LG-ESS)

| | CL (our cases) | 1 + positive/all (%) | 2 + positive/all (%) | 3 + positive/all (%) | | CL (literary data) | | LG-ESS (literary data) | |
|---|---|---|---|---|---|---|---|---|---|
| | Positive/all (%) | HS < 50 | HS 50–149 | HS ≥ 150 | > 50%; positive/all (%) | Positive/all (%) | > 50%; positive/all (%) | Positive/all (%) | > 50%; positive/all (%) |
| Calponin | 52/52 (100) | 0/52 (0) | 2/52 (3.8) | 50/52 (96.1) | 51/52 (98.1) | 9/9 (100) | 9/9 (100) | 17/41 (41.5) | 1/41 (2.4) |
| Caldesmon | 50/52 (96.1) | 4/52 (7.7) | 7/52 (13.5) | 39/52 (75) | 43/52 (82.7) | 74/79 (93.7) | 17/34 (50) | 20/158 (12.7) | 4/90 (4.4) |
| Desmin | 52/52 (100) | 0/52 (0) | 10/52 (19.2) | 42/52 (80.8) | 50/52 (96.1) | 77/78 (98.7) | 61/68 (89.7) | 100/241 (41.5) | 15/180 (8.3) |
| SMA | 51/52 (98.1) | 0/52 (0) | 0/52 (0) | 51/52 (98.1) | 51/52 (98.1) | 57/58 (98.3) | 57/58 (98.3) | 108/215 (50.2) | 33/143 (23.1) |
| SMMHC | 45/52 (86.5) | 7/52 (13.5) | 34/52 (65.4) | 4/52 (7.7) | 34/52 (65.4) | NA | NA | 3/10 (30) | 0/10 (0) |
| Smoothelin | 32/52 (61.5) | 16/52 (30.8) | 13/52 (25) | 3/52 (5.8) | 16/52 (30.8) | NA | NA | NA | NA |
| Transgelin | 50/52 (96.1) | 4/52 (7.7) | 9/52 (17.3) | 37/52 (71.1) | 46/52 (88.5) | NA | NA | 0/7 (0) | 0/7 (0) |
| CD10 | 34/52 (65.4) | 11/52 (21.1) | 11/52 (21.1) | 12/52 (23.1) | 5/52 (9.6) | 25/67 (37.3) | 1/58 (1.7) | 351/405 (86.7) | 157/285 (55.1) |
| IFITM1 | 19/52 (36.5) | 14/52 (26.9) | 1/52 (1.9) | 0/52 (0) | 1/52 (1.9) | 7/16 (43.8) | 0/16 (0) | 26/28 (92.9) | 16/28 (57.1) |
| BCOR | 0/52 (0) | 0 | 0 | 0 | 0 | NA | NA | 4/67 (6) | 0/67 (0) |
| CD44 | 8/52 (15.4) | 7/52 (13.5) | 1/52 (1.9) | 0/52 (0) | 1/52 (1.9) | 25/25 (100) | NA | 3/29 (10.3) | 0/20 (0) |
| HMB45 | 2/52 (3.8) | 1/52 (1.9) | 1/52 (1.9) | 0./52 (0) | 0/52 (0) | NA | NA | 6/102 (5.9) | 5/101 (4.9) |
| CD117 | 0/52 (0) | 0 | 0 | 0 | 0 | 0/9 (0) | 0/9 (0) | 14/104 (13.5) | 2/75 (2.7) |

*CL* cellular leiomyoma, *LG-ESS* low grade endometrial stromal sarcoma, *HS H*-score, *NA* not available, *SMA* smooth muscle actin; *SMMHC* smooth muscle myosin heavy chain

LG-ESS [32, 33]. The reason being that CL may commonly manifest irregular tumor borders with undulating margins and can even show infiltrative features. We should also be aware of the possibility of satellite small CL in the vicinity of the main tumor, which can give the impression of infiltrative invasion, mimicking the tongue-like myometrial infiltration of LG-ESS. In summary, because in some CL cases morphology alone is not sufficient to achieve diagnosis, especially in cases of the so-called highly CL, ancillary methods are needed [1]. These methods include immunohistochemical analysis and molecular testing. However, these diagnostically challenging cases are rare (in our study, they represented only 4% of the in-house cases) and in most cases of CL the diagnosis is straightforward, without the need for using ancillary methods.

The most commonly used antibodies in this context are CD10, h-caldesmon, desmin, smooth muscle actin, and recently IFITM1 [3, 22, 23, 34]. Previously, CD10 expression was regarded as a relatively specific marker of endometrial stromal differentiation, but current knowledge is that CD10 expression is relatively common in leiomyocellular tumors. In LG-ESS, CD10 expression is present in about 87% of cases, but can also occur in CL and has been described in 37.3% of CL (according to 6 studies analyzing the total of 67 CL cases) [17, 18]. The extent and intensity of CD10 expression may be an important feature because in LG-ESS significant positivity (which is quantified differently in individual studies) occurs in c. 55% of cases, while in CL the expression is usually only focal and significant expression has been seen in 1.7% of cases (according to the limited literature data). However, in our study, the expression of CD10 was present in 65.4% of CL and significant in 23.1% of all cases (in 9.6% of cases present in ≥ 50% of tumor cells). With respect to other markers—according to literature data desmin is expressed in about 40% of LG-ESS and 98% of CL, muscle-specific actin in 20% of LG-ESS and 100% of CL, SMA in 50% of LG-ESS and 98% of CL, and caldesmon in 13% of LG-ESS and 94% of CL. Literary data suggests that the IFITM1 antibody has a significantly higher specificity than CD10 while maintaining high sensitivity (93%) for LG-ESS. However, according to the only study focusing on IFITM1 expression in CL, the expression was present in 7/16 cases (43.7%), but never strong. The results of this study were confirmed by our work, in which the expression of IFITM1 was found in 19/52 cases (36.5%), but in only one case was the expression present in more than 50% of tumor cells. In summary, immunohistochemistry is an essential part of the differential diagnosis between CL and EST, but a panel of antibodies should be used, and we should be aware of the limitations due to the overlapping immunohistochemical profile in some cases. Our data suggests that out of the "endometrial stromal markers" IFITM1

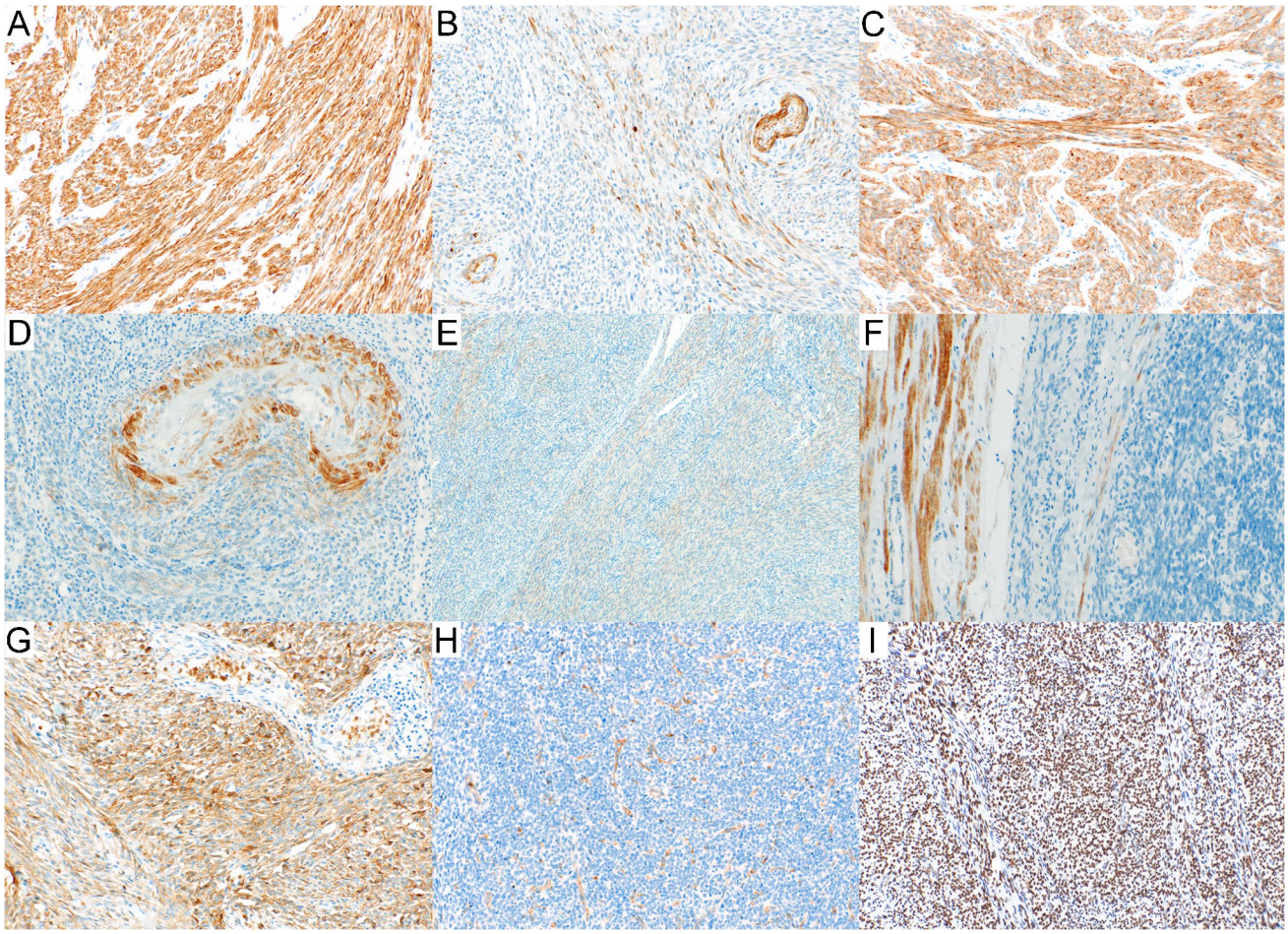


**Fig. 3** Immunohistochemical findings in cellular leiomyomas. **A** Tumor cells showing diffuse and strong positivity for transgelin (case no. 26, × 200). **B** Another example with focal and weak positivity for transgelin (case no. 35, × 200). **C** Diffuse and strong expression of smoothelin (case no. 1, × 200). **D** Focal weak positivity of smoothelin (note the strong expression in smooth muscle cells of the vessel wall) (case no. 3, × 200). **E** Weak expression of smoothelin in some tumor cells (case no. 18, × 100). **F** Negative staining of smoothelin in tumor cells (note the positivity in the adjacent myometrium) (case no. 7, × 200). **G** Diffuse and strong expression of CD10 (case no. 35, × 200). **H** Negativity of IFITM1 (note the positivity in endothelial cells) (case no. 40, × 200). **I** Diffuse and strong expression of HMGA2 (case no. 14, × 200)

clearly outperforms CD10, especially if we consider not only the presence of positivity but also the intensity and extent of the staining.

In some CL, the correct diagnosis cannot be achieved with certainty and molecular testing can be helpful. However, knowledge of molecular aberrations occurring in CL is, contrary to UL, limited. UL have been shown to share recurrent molecular aberrations and can be classified into three molecular subtypes including tumors with *MED12* mutation, HMGA2 overexpression, and FH deficiency [6, 8]. The *MED12* mutations occur in approximately 40–75% of UL cases and according to literary data their frequency differs based on ethnicity/nationality [6]. On the contrary, in CL the mutation of *MED12* is present in approximately 5–16% of cases [35–37]. Only one study reported a higher percentage of 33% of CL showing *MED12* mutations, but as only 6 cases of CL were analyzed in this study, it is not statistically significant [38]. In our study, *MED12* mutations were present in 9.4% of cases. HGMA2 overexpression occurs in 10–25% of UL cases [13, 36, 37]. Some of these are associated with *HMGA2* intergenic rearrangement, but in most studies, the overexpression and rearrangements of HMGA2 were not analyzed simultaneously and the percentage of cases with overexpression showing *HMGA2* rearrangement is not entirely clear [9, 38–42]. The knowledge concerning HMGA2 aberrations in CL is very limited. In one study, overexpression of HMGA2 was seen in 32% of CL (8/25 cases) [36]. The "highly" CL in this study showed overexpression of HMGA2 in 10.8% (4/37 cases). In another study of 6 CL cases, no case with HMGA2 overexpression was found [37]. In another recent study, the authors performed RNA sequencing of 11 CL cases and identified three

cases with fusions, including *HMGA2-TRAF3IP2*, *HMGA2-NAA11*, and *TPCN2-YAP1* [27]. In our work, HMGA2 overexpression (≥ 50% of tumor cells) was present in 18/52 cases (34.6%). The *HMGA2* rearrangement was found in 5/38 cases (13.2%), all of which showed strong IHC expression of HMGA2 in 100% of tumor cells. Other mechanisms of HGMA2 overexpression in leiomyomas without *HMGA2* intergenic rearrangement have also been suggested. Rearrangements in flanking areas of the *HMGA2* gene, which are usually not detected by standard methods, could potentially lead to HMGA2 overexpression, as well as *HMGA2* promoter DNA hypomethylation [40, 43]. Another recurrent aberration detected in uterine leiomyomas is FH deficiency [44]. Inactivation of FH occurs mostly in leiomyoma variants such as LBN and FH-deficient leiomyoma [10, 11, 45]. In UL, FH deficiency is very rare, occurring in 0–2.5% of cases [36, 37, 46]. In CL the data is limited. In one study, FH-deficiency occurred in 1/25 of CL cases (4%) [36]. In our study, no pathogenic mutations or homozygous deletions were found, and all cases showed retained expression of FH. Finally, another recurrent aberration detected in CL is the loss of the short arm (p) of chromosome 1, which can be found in up to 25% of cases (in our work it was 19.3%) [47, 48]. According to some authors, these tumors seem to represent a distinct entity with potentially more aggressive behavior [48].

According to the literature, the aberrations occurring in the leiomyoma molecular subtypes seem to be mutually exclusive [9, 13]. Our results are in concordance with this data on both the molecular and immunohistochemical levels. We have confirmed that the deletion of chromosome 1p is mutually exclusive with the other molecular subtypes as well. However, in one study the overexpression of HMGA2 on mRNA level has also been described in a leiomyoma with *MED12* mutations [49]. In our work, the mRNA expression was high only in cases with overexpression detected by IHC. The molecular findings in CL may be useful in practice in the differential diagnosis with EST. Knowledge of molecular aberrations occurring in endometrial stromal tumors is rapidly evolving and a substantial part of these tumors is characterized by known aberrations, mostly chromosomal rearrangements of genes involving *JAZF1*, *EPC1*, *CXorf67*, and *BCOR* (such as JAZF1-JJAZ1, EPC1-PHF1, PHF1-MEAF6, *MBTD1-CXorf67*, and *ZC3H7B-BCOR* [50–61].

In conclusion, the results of our study showed that on a molecular level CL represents a heterogeneous group of lesions. The most common abnormality occurring in CL seems to be affecting HMGA2, as a high percentage of cases showed an overexpression on the IHC level (36.5%). Where molecular analysis was possible, 50% of these cases were associated with *HMGA2* rearrangements. The second most common aberration was chromosome 1p deletion, present in 19.3% of cases. A minority of CL cases may have *MED12* mutations (9.4%), which are far less common in CL than in UL. The FH-deficient subtype of leiomyoma seems to be very rare in CL, and in our study, we have not detected any such case. All molecular aberrations including chromosome 1p deletions were mutually exclusive, which suggests that CL leiomyoma with chromosome 1p deletions represents a distinct molecular subtype. Even though we have detected 8 other gene mutations and deletions and/or duplications of several genes, we have not identified any other molecular aberration with a recurrent pattern and there remains a substantial number of CL cases which do not fit any of the defined molecular subtypes. From a practical point of view, the molecular aberrations occurring in CL may be used as an ancillary marker in the differential diagnosis of equivocal cases with overlapping features with EST, which are characterized by different recurrent molecular aberrations. Regarding immunohistochemical analysis, we should be aware that in a high percentage of cases CL can express markers traditionally regarded as more specific for endometrial stromal differentiation, such as CD10 and IFITM1. In our study, we also analyzed the expression of smoothelin, which is regarded as highly specific for differentiated smooth muscle cells but its expression has not yet been studied in smooth muscle uterine tumors. The results showed the expression of smoothelin in 61.5% of cases, but the intensity of staining was commonly weak, which seems to be a limiting factor for its practical use. Nevertheless, the literary data concerning its expression in EST is missing and further research is needed to assess its practical use in the differential diagnosis of equivocal cases.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00428-021-03217-z.

**Acknowledgements** This work was supported by the Ministry of Health, Czech Republic (MH CZ DRO-VFN 64165 and AZV NU22-03-00122), by Charles University (Project Progress Q28/LF1 and UNCE204065), and by the European Regional Development Fund (CZ .02.1.01/0.0/0.0/18_046/0015959; BBMRI_CZ LM2018125).

The authors wish to thank Zachary Harold Kane Kendall, B.A. (Institute for History of Medicine and Foreign Languages, First Faculty of Medicine, Charles University) for the English proofreading.

**Author contribution** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Pavel Dundr, Mária Gregová, Jan Hojný, Eva Krkavcová, Romana Michálková, Kristýna Němejcová, Michaela Bártů, Nikola Hájková, Jan Laco, Michal Mára, Adéla Richtárová, Tomáš Zima and Ivana Stružinská. The first draft of the manuscript was written by Pavel Dundr and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Data availability** All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).

## Declarations

**Ethical approval** The study has been approved by the Ethics Committee of General University Hospital in Prague in compliance with the Helsinki Declaration (ethical approval number EK RVO 210/19 S-IV). The Ethics Committee waived the requirement for informed consent, as according to the Czech Law (Act. no. 373/11, and its amendment Act no. 202/17) it is not necessary to obtain informed consent in fully anonymized studies.

**Conflict of interest** The authors have no conflicts of interest to declare that are relevant to the content of this article.

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