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Two subtypes of atypical leiomyoma: Clinical, histologic and molecular analysis

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Abstract

Atypical leiomyoma (ALM) is a rare variant of uterine smooth muscle tumors. Several recent studies have suggested that ALM has distinct, but also heterogeneous, histologic and molecular features, yet little is known about the biology and histogenesis of ALM. Some have even postulated whether the atypical histologic features represent true atypia or simply degenerative changes. In this study, we analyzed the cytologic features of 60 ALM cases and found that ALM could be further divided into two subtypes, type I and type II, based primarily on nuclear features. Type I ALM showed round or oval nuclei, distinct and smooth nuclear membranes, prominent nucleoli with perinucleolar halos, and open coarse chromatin. Type II ALM showed elongated or spindle nuclei, irregular nuclear membranes, pinpoint or no nucleoli, and dark smudgy chromatin. There were also architectural differences between type I and type II ALM. Type I ALM often showed diffuse atypia within the tumor while the atypia in type II ALM was patchy, surrounded by usual-type leiomyoma. The two subtypes also differed when we compared the immunohistochemical and molecular patterns. Type II tumors showed significantly higher rates of immunoreactivity for p16, p53 and HMGA2 and showed *MED12* mutations more frequently than the type I counterparts. Our findings suggest that the type I and type II subtypes of ALM may arise from two different pathways. Type I tumors may be related to fumarate hydratase mutations while type II ALM appear to arise in a background of usual-type leiomyomas.

Keywords

Atypical leiomyoma; histology; immunohistochemistry; gene mutations; tumor types

INTRODUCTION

Atypical leiomyoma (ALM) is a rare variant of uterine smooth muscle tumors. It was originally defined by Bell's criteria in 1994 (1) which described a triad of histologic features

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that included moderate to severe cytologic atypia, less than ten mitoses per ten high power fields (HPF) and no coagulative tumor necrosis. According to the study by Bell et al, a small proportion of atypical leiomyoma had a low risk of malignant transformation and thus they classified it as an intermediate grade uterine smooth muscle tumor (1).

More recent studies focusing on histologic features and clinical follow-up data have shown that ALM may actually act in a benign fashion. Occasionally, ALM can recur, but it has never been reported to contribute to patient death (2–5). The new WHO Classification of Tumors of Female Reproductive Organs (2014) has renamed this variant leiomyoma with bizarre nuclei (LM-BN). However, in clinical practice, the diagnosis and management of patients with ALM/LM-BN remains a challenge. Many different names are still used both in daily practice and in the literature, including symplastic leiomyoma, leiomyoma with bizarre nuclei, and atypical leiomyoma with low risk of recurrence.

For the purposes of this study, we chose to use the term ALM since we still believe it is histologically atypical and also has been known to gain additional oncogenic alterations, but it may be thought of as analogous to the current LM-BN definition in the 2014 WHO. These different names for the same entity reflect the fact that its biologic nature and relationship in the spectrum of benign usual-type leiomyoma (ULM) to malignant leiomyosarcoma (LMS) is still poorly understood. Although ALM is a rare variant, it often presents a diagnostic dilemma for the practicing gynecologic pathologist. Of particular clinical concern is the fact that some of the histologic features of ALM mimic those of LMS and may lead to misdiagnosis. Two previous large studies found that 17 to 27% of ALM and other benign variants of uterine smooth muscle tumors were misinterpreted as LMS (4, 6). Recent studies that have focused on the molecular genetics of these tumors have also begun to raise the question whether ALM is a single disease or multiple distinct entities (4, 5).

In this study, we reviewed 60 ALM cases and defined two subtypes of ALM based on their characteristic nuclear features. We further compared and analyzed the tumor types in association with clinical follow-up data, histologic findings, immunohistochemistry and molecular alterations and discuss the possible tumor origins of each subtype of ALM.

MATERIALS AND METHODS

Case selection

We reviewed the pathology databases from Northwestern Memorial Hospital (Northwestern University, Illinois) and Qilu Hospital (Shandong University, China) from 1993 to 2015 and retrieved cases with the following diagnoses: atypical leiomyoma, atypical leiomyoma with low risk of recurrence, atypical leiomyoma with less experience, symplastic leiomyoma and leiomyoma with bizarre nuclei. A total of 60 patients met the criteria; 45 from Northwestern Memorial Hospital and 15 from Qilu Hospital. Each patient's clinical chart at Northwestern Memorial Hospital was reviewed and the original diagnosis, additional pathology results, clinical follow-up, current disease status and survival outcomes were recorded through June 2015 (Table 1).

Slide review for histologic features for type I and type II ALM

All tumor slides (2–20 slides per case) were reviewed by two pathologists and a diagnosis of ALM was confirmed using the Bell criteria (moderate to severe cytologic atypia, less than 10 mitoses/10 HPF, and no evidence of tumor necrosis). Architectural and histologic features were recorded including growth pattern (pushing/well-demarcated or infiltrating borders), distribution of nuclear atypia (focal, multifocal or diffuse), density of nuclear atypia (low <10%, intermediate 10–30%, or high >30%), tumor cellularity (low, intermediate, or high), stromal hyalinization, vessel changes (staghorn vessels), the presence or absence of eosinophilic hyaline globules and mitotic activity. In addition, the cytologic features of each tumor were also assessed which included nuclear shape (round/oval or spindled), nuclear membrane (distinct and smooth or irregular), presence of multinucleated cells (rare or frequent), “bizarre nuclei” (large irregular nuclei identified on 2X objective), presence or absence of large nucleoli/perinucleolar halos and chromatin pattern (coarse and clumped or smudgy and degenerative) (Table 2).

Immunohistochemical analysis

Six immunohistochemical (IHC) markers that are involved in tumor growth and differentiation pathways were analyzed and included ER, PR, p16, p53, Ki-67 and HMGA2 (Supplementary Table 1). The slides were first deparaffinized then followed by antigen retrieval. After blocking in 1.5% normal goat serum, slides were then incubated with primary antibodies. The staining was detected with the I-View 3, 3'-diaminobenzidine (DAB) detection system. Antibodies used in this study are listed in Supplementary Table 2. The IHC staining patterns on tissue microarray (TMA) slides were blindly reviewed separately by two pathologists and staining percent and intensity were recorded for each case. Percentage was scored as 0, 1 (1–25%), 2 (26–50%), 3 (51–75%) and 4 (76–100%). Intensity was scored as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong).

Molecular analysis

Gene mutation analysis for *P53*, *PTEN* and *MED12* was also performed (Supplementary Table 2). Genomic DNA for 59 cases of ALM was first extracted from sections of formalin-fixed and paraffin-embedded (FFPE) tissue using the QIAamp DNA FFPE Tissue Kit (QIAGEN, 56404). For *P53* (exon 4–9) and *MED12* (exon 2) mutation analysis, 50ng DNA was amplified by PCR with HotStarTaq Master Mix (QIAGEN, 203446) and PCR products were purified using the Gel Extraction and PCR Clean-Up Kit (Clontech) according to the manufacturer's instructions. DNA sequencing of the purified DNA products was then performed by an ABI 3730 High-Throughput DNA Sequencer. The mutations and variations were analyzed using DNASTAR Lasergene 9 software. For *PTEN* deletion analysis, absolute quantification PCR (A-QPCR) was performed on the Applied Biosystems StepOne-Plus Real-Time PCR System and γ -*IFN* on 12q14–15 was used as a stable diploid control gene. A copy number ratio of less than 0.5 *PTEN* to γ -*IFN* was considered positive for a *PTEN* deletion. Detailed information for the primers is listed in Supplementary Table 3.

Statistical analysis

SPSS Version 19.0 was used for the statistical analysis. Age range and tumor size were analyzed by T-test. Pearson Chi-square, Fisher exact, Kruskal-Wallis, and Mann-Whitney tests were performed to evaluate the association of pathologic and molecular findings for each subtype of ALM. The association between *P53* mutations and IHC expression was evaluated using the Kappa coefficient. *P* values less than 0.05 were considered statistically significant.

RESULTS

We retrospectively reviewed all cases with a diagnosis of atypical leiomyoma, atypical leiomyoma with low risk of recurrence, atypical leiomyoma with less experience, symplastic leiomyoma or leiomyoma with bizarre nuclei. Sixty cases met the Bell criteria. The mean patient age was 40.5 years and the mean tumor size was 7.6 cm (Table 1).

Of the 60 ALM cases, 91.7% (55/60) of patients had more than one uterine nodule. The additional nodules were either additional ALM or additional usual-type leiomyomas. Only 5 patients presented with solitary ALM. When we analyzed the histologic features, including growth pattern, distribution and density of nuclear atypia, cellularity, stromal hyalinization, vessel changes, and eosinophilic hyaline globules, we found that more than 62% (37/60) of all ALM showed a diffuse distribution of atypia. The density of nuclear atypia varied from 5.0% (3/60) with low, 62% (37/60) with intermediate and 33% (20/60) with high density. Overall, 47% (28/60) of the ALM cases were hypercellular while only 5% (3/60) of the cases were hypocellular. Staghorn vessels were noted in 63% (38/60) of ALM. Eosinophilic hyaline globules (EG) were noted in 48% (29/60) of ALM cases. The mitotic index ranged from 8% (5/60) with <1/10 HPF, 82% (49/60) with 1 to 5/10 HPF, and 10% (6/60) with >5/10 HPF. No significant correlation between these histologic features and the six examined immunohistochemical markers was found. Forty-three cases had clinical follow-up data (Table 1). There were three patients with recurrence. One patient presented with a pelvic nodule after hysterectomy and two patients had uterine recurrences in their subsequent hysterectomy specimens several years later.

Nuclear features define two subtypes of ALM

Next, we examined the cytologic features of ALM which included nuclear size, nuclear shape, chromatin pattern, nucleoli/perinucleolar halo, and nuclear membrane. Interestingly, the nuclear features appeared to distinctly separate ALM into two types of tumors (Table 2, Figures 1 and 3) which we refer to as type I and type II. Type I ALM were characterized by round or oval nuclei, distinct smooth nuclear membranes, prominent nucleoli with perinucleolar halos, and open, coarse chromatin (Figures 1 and 2). Type II ALM showed elongated or spindle nuclei, irregular nuclear membrane, pinpoint or no nucleoli, and dark smudgy chromatin (Figures 3 and 4). Based on these specific nuclear features, 95% (57/60) of our ALM cases could be clearly classified as type I or type II. Three cases had mixed nuclear features of both type I and II and we chose to include these three cases in the type II category since greater than 70% of the tumor cells appeared to show features of type II nuclei.

We then compared the clinical and histologic features between type I and type II ALM. The mean patient age for type I was 37.7 ± 1.7 years compared to 43.7 ± 1.5 years for type II, a statistically significant difference ($P=0.012$). The average tumor size was larger in type I (8.5 ± 0.8 cm) than in type II (6.6 ± 0.7 cm), although this was not statistically significant ($P=0.061$). The density of nuclear atypia was much greater in type II than in type I. Type I tumors had 3.1% of cases with low density, 84.4% of cases with intermediate density and 12.5% of cases with high density of nuclear atypia. This was in comparison to type II tumors which showed 7.1% of cases with low density, 35.7% of cases with intermediate density and 57.1% of cases with high density ($P<0.001$) (Table 3). Yet, 90.6% (29/32) of type I cases showed a diffuse pattern of distribution of nuclear atypia while 71.4% of type II cases showed only focal or multifocal atypia (21.4% focal and 50.0% multifocal) ($P<0.001$) (Table 3). The smaller round intermixed nuclei in type I tumors showed similar nuclear features as the intermixed larger atypical cells (Figure 1). In contrast, the type II tumors that showed focal/multifocal atypia were surrounded by bland cells reminiscent of usual-type leiomyoma. While multinucleation was frequently seen in both type I (75.0% (24/32)) and in type II (71.4% (20/28)) ($P=0.755$), bizarre nuclei (nuclei seen from a 2X objective) were more commonly seen in type II (64.3% (18/28) vs 18.8% in type I (6/32)) ($P<0.001$). Type I tumors were also less cellular than type II tumors, but this was not statistically significant ($P=0.163$).

Eosinophilic globules are composed of round and dense hyaline-like material present either in an intracellular or extracellular distribution (Figure 1). We evaluated the presence of eosinophilic globules using the following criteria: absent, low (2/10x field) or high (2/10x field) and found that eosinophilic globules were significantly higher in type I. Seventy-two percent (23/32) of type I cases showed high amounts of eosinophilic globules while only 21.4% (6/28) of type II cases had high amounts of eosinophilic globules ($P<0.001$). In addition, staghorn vessels were far more common in type I (81.2% (26/32)) than in type II (42.9% (11/28)) ($P<0.001$) (Table 3).

Immunohistochemical and molecular analysis in type I and II ALM

We examined six immunohistochemical biomarkers: ER, PR, p16, p53, Ki-67 and HMGA2 (Figures 5–6) and compared the staining patterns between the two subtypes of ALM. Strong positivity for ER, PR, p16, p53 and HMGA2 were defined as >50% of tumor cells with moderate to strong (2–3) staining. Both type I and type II ALM had similar rate of immunoreactivity for ER (61.3% (19/31) vs 74.1% (20/27)) and PR (100% (32/32) vs 96.0% (24/25)) (Figure 7).

P16 was found to be diffusely positive in 54.4% (31/57) of all ALM cases, but it showed significantly higher immunoreactivity in type II ALM. While 72.0% (18/25) of type II ALM showed diffuse positivity for p16, only 40.6% (13/32) of type I ALM were diffusely positive for p16 ($P=0.018$) (Figures 6 and 7). For p53 expression, 37.0% (10/27) of type II tumors were positive for p53, while only 12.5% (4/32) of type I tumors were positive ($P=0.027$) (Figures 5–7).

Past studies have shown variable Ki-67 percentages among ALM cases, ranging from 0 to 30% (3, 4). Our study showed similar variability, although most cases had low proliferation

rates. No statistically significant difference was found between type I and II ALM (Figure 7).

HMGA2 is another marker that has previously been reported to be overexpressed in 10 to 15% of ALM (7). Similar rates of HMGA2 overexpression were found in our series with five positive cases (10.4% (5/48)). All five cases were found to be type II ALM and 21.7% (5/23) of type II tumors showed HMGA2 immunoreactivity (Figure 7).

As we reported in a previously published study, gene mutation analysis of *MED12*, *P53* and *PTEN* was performed on 59 of our cases (5). *MED12* mutations were only found in 11.9% (7/59) of ALM compared to 60–70% of ULM cases. However, all seven ALM cases with *MED12* mutations were type II ALM. Overall, 25.9% of type II ALM showed *MED12* mutations which was statistically significant ($P=0.003$). There were also seven cases with *P53* mutations. Two of 32 type I ALM had *P53* mutations and five of 28 type II ALM had *P53* mutations. There was also correlation between *P53* mutations and p53 immunohistochemical staining patterns (Kappa coefficient=0.288, $P=0.019$). *PTEN* mutations were slightly more common in type I (8/25) than type II ALM (5/23), but this was not a statistically significant difference (Table 3).

DISCUSSION

ALM has characteristic gross and microscopic features that differentiate it from both the benign and malignant counterparts. In general, when the histology of a tumor is carefully evaluated for cytologic atypia, mitotic rate, and lack of tumor necrosis, a straightforward diagnosis of ALM can often be made. Yet, in difficult cases, histologic and molecular analysis may be helpful in understanding the nature of this group of tumors. Published data on selected genetic alterations in ALM seem to show that ALM shares more molecular alterations with leiomyosarcoma than leiomyoma (3, 5, 8–10). However, given the broad spectrum of molecular changes, these changes may only have limited value in the differential diagnosis of ALM (3).

One of major challenges in diagnosing ALM is the histologic heterogeneity seen in this group of tumors. In 1994, Bell et al were the first to provide an in depth analysis of clinical and histologic features of ALM. They studied a variety of uterine smooth muscle tumors and classified 56 cases within their series as “atypical leiomyoma with low risk of recurrence.” Of these cases, 89% (50/56) showed medium or high tumor cellularity. No cases had coagulative necrosis, but three cases had hyaline necrosis. Forty-five percent (25/56) of cases showed moderate density of nuclear atypia and 55% (31/56) showed high density of nuclear atypia. When the mitotic index was analyzed, 21% of cases (12/56) had 1 to 5 mitoses per 10 HPF and 61% (34/56) had 6 to 9 mitoses per 10 HPF. They determined that this new group of tumors may represent a new intermediate-grade smooth muscle tumor which they classified as “atypical leiomyoma with low risk of recurrence.”

Croce et al (4) provided a comprehensive evaluation of histologic features in their study of 59 ALM cases where they analyzed features including cellularity, density of nuclear atypia, mitotic count, presence of prominent nucleoli, karyorrhectic nuclei, pseudonuclear

inclusions, rhabdoid-like cells, vascular changes, eosinophilic hyaline globules, and ischemic necrosis. In this study, they found that 18 cases showed diffuse atypia, 26 showed multifocal atypia and 15 showed focal atypia. The mitotic counts ranged from less than 2/10 HPF (37 cases) to greater than 5/10 HPF (3 cases). Nineteen cases showed 2 to 5 mitoses per 10 HPF. Almost all showed karyorrhectic nuclei (55 cases). Twenty-one cases showed ischemic necrosis, 24 had rhabdoid features, and 25 had hyaline changes. They identified one patient with recurrence who underwent a second myomectomy procedure and one patient with a benign leiomyoma metastasis nine months after her initial hysterectomy who was otherwise alive and well. While all of the histologic features that they described are commonly seen in ALM, none of them have been previously found to be specifically valuable in subclassifying ALM cases.

Another study by Ly et al (2) reviewed the histology and clinical data for 51 ALM cases. They found that 30 cases showed diffuse atypia while 21 showed focal or patchy atypia. Thirty-seven cases had less than 1 mitosis/10 HPF while thirteen cases had 1 to 3 mitoses/10 HPF. They also had 12 cases with ischemic necrosis. Of the patients who underwent hysterectomy, there was one recurrence in the retroperitoneum at 87.5 months. This tumor measured 5.8 cm, had foci of coagulative necrosis and had 6 mitoses/10 HPF. Of the myomectomy specimens, two patients had residual ALM, one patient had recurrent ALM, and one had recurrent ULM. Ly et al further postulated that the cellular atypia identified within the tumor may either be due to degenerative changes or abnormal cell cycle proliferation leading to atypical nuclei and mitoses. These authors also postulated that there are likely two possible processes leading to the development of atypical leiomyoma; either reactive degeneration (non-neoplastic) or DNA methylation alterations causing deactivation (neoplastic).

In the current study, we examined and reviewed the clinical and histologic features of 60 ALM cases. When combined with the other four studies (1, 2, 4, 11), a total of 250 ALM cases have reported clinical and histologic features (Table 4). Overall 50% of cases showed a diffuse distribution of nuclear atypia, while 16.5% of cases had only focal atypia and 33.5% had multifocal areas with atypia. Others have postulated that diffuse cytologic atypia in ALM may be considered a potential risk factor for recurrence and malignant potential (1, 4). The clinical and histologic parameters among these studies (Table 4) show that ALM presents in middle age (mean age of 40–45 years old) with a relatively large tumor size (6.8–7.6 cm) and with a low rate of recurrence (2–7%).

In addition to the general histologic features, we found that specific nuclear features can be useful in defining two distinct subtypes of ALM (Table 2). Type I ALM cases had round or oval nuclei, distinct smooth nuclear membranes, prominent nucleoli with perinucleolar halos, and open and coarse chromatin (Figures 1 and 2). Type II ALM showed elongated or spindle nuclei, irregular nuclear membranes, pinpoint or no nucleoli, and dark smudgy chromatin (Figures 3 and 4). The three cases that showed mixed features were subjectively classified into type II based on the more dominant nuclear features. Overall, 32 of our ALM cases were categorized as type I and 28 were categorized as type II. The patient age in type I ALM was younger (37.7 years) than in type II (43.7 years) (Table 1) ($P=0.012$), but that was the only statistically significant clinical demographic. Among the general histologic features,

type I ALM showed more diffuse distribution of atypia, a lower density of nuclear atypia, more staghorn vessels, higher rate of eosinophilic globules, and higher rate of prominent nucleoli than type II tumors (Table 3).

The molecular analysis of our ALM cases revealed that *MED12* mutations and *HMGA2* overexpression were present in type II cases but not in type I (Table 3). All of these findings suggest that ALM may represent two distinct disease entities. Overall, the histologic and nuclear features were often straightforward when subclassifying ALM, however, we did find three cases that presented with mixed patterns of type I and II. Thus, in clinical practice, careful analysis and additional tumor sections may be required to determine the dominant nuclear features and perhaps a small proportion of ALM cases may be unclassifiable by histology alone. Review of additional ALM cases and collaboration among different studies may help to improve this subclassification scheme.

This study was able to determine characteristic histologic features used to differentiate type I and type II ALM. These findings led us further review and compare type I ALM with other variants of uterine smooth muscle tumors. Reyes et al (12) described uterine smooth muscle tumors with fumarate hydratase alterations (SMT-FHs), which seem to show similar morphologic features to our type I ALM. In their study of 9 cases, SMT-FH showed slightly increased cellularity and staghorn vessels at low magnification. In all of their cases, the tumor cells had round to oval nuclei, fibrillary cytoplasm and eosinophilic globules. Most cases (8/9) also had large nucleoli with perinucleolar halos. Their findings are quite similar to the nuclear features we identified in type I ALM. Joseph et al reported similar findings in their study that evaluated ALM with fumarate hydratase gene aberrations (13). They identified five cases with fumarate hydratase mutations. Of these five cases, four of them showed the same characteristic nuclear features with prominent eosinophilic nucleoli and perinucleolar halos; the fifth did not. Our findings support the concept that a subgroup of ALM may be truly related to SMT-FH. Yet this requires additional genetic analysis of FH molecular alterations in all ALM to determine its relationship to type I ALM. Our molecular analysis of *FH* mutations is still ongoing.

Type II ALM also has unique features both histologically and molecularly. This subgroup of tumors usually presents with bizarre nuclei identified at 2X objective and smudgy, degenerative-appearing chromatin. Several previous studies have shown that these tumors occasionally have higher Ki-67 proliferation indices (3, 4) and harbor specific genomic alterations (5, 14). Almost two-thirds of our type II ALM (Table 3) showed either focal or multifocal distribution of atypia that was surrounded by usual-type leiomyoma (Figure 3). In addition, type II ALM harbored *MED12* and *HMGA2* mutations which are also commonly seen in usual-type leiomyomas. These findings suggest that type II ALM may develop from usual-type leiomyoma after gaining additional histologic and genetic alterations. More research is required to determine whether type II ALM is truly part of the degenerative process of usual-type leiomyoma or if it represents tumor progression. Furthermore, 28.6% of our type II ALM cases still showed diffuse patterns of atypia, so the histogenesis of these type II tumors requires further investigation.

This study analyzed the histologic features that define two subtypes of ALM. Type I ALM are composed of tumors with round nuclei, frequent eosinophilic globules and diffuse atypia. They are more often negative for p16 and p53 and do not show *MED12* mutations. They appear to be different histologically from both usual-type leiomyoma and leiomyosarcoma and some may be related to *FH* mutations. In contrast, type II ALM are tumors with focal or multifocal areas of atypia interspersed between areas with usual-type leiomyoma. They have large spindled nuclei, frequently show immunoreactivity for p16 and p53 and are often associated with *MED12* mutations. Their histogenesis supports the theory that they may arise from existing usual-type leiomyoma after additional molecular alterations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Type I atypical leiomyoma. A. Low magnification reveals a hypercellular smooth muscle tumor with diffuse cytologic atypia. B. Intermediate magnification shows large, pleomorphic and hyperchromatic nuclei. C and D. High magnification illustrates round and oval nuclei with smooth, distinct nuclear membranes as well as open, clumped chromatin. Many nuclei contain prominent cherry-red nucleoli with perinucleolar halos. Many intracytoplasmic and extracellular eosinophilic globules are present.

Figure 2.

Photomicrographs illustrate the histologic and cytologic features of 9 cases of type I ALM (case numbers shown in lower right corner). Although the density of atypia varies from case to case, the nuclear and cytologic features are quite similar.

Figure 3.

Type II atypical leiomyoma. A and B: Low magnification shows large areas of highly atypical smooth muscle cells surrounded by abundant extracellular matrix as well as areas of usual-type leiomyoma. C and D: High magnification reveals large spindled nuclei with irregular nuclear membranes and dark, smudgy, hyperchromatic chromatin.

Figure 4. Photomicrographs illustrate the histologic and cytologic features of 9 cases of type II ALM (case numbers shown in lower right corner). All tumors show a high density of large atypical cells with irregular, spindle-shaped nuclei.

Figure 5. Photomicrographs illustrate the immunohistochemical staining patterns for ER (B), p53 (C) and p16 (D) in a case of type I ALM.

Figure 6. Photomicrographs illustrate the immunohistochemical staining patterns for ER (B), p53 (C) and p16 (D) in a case of type II ALM.

Figure 7. Dotplot analysis of the immunohistochemical reactivity for the six selected immunomarkers in type I and II ALM. Each dot represents one ALM case, inverted triangles represent type I ALM, upright triangle represent type II ALM, and horizontal black bars represent the mean value.

Table 1

General clinical information for ALM and its subtypes

Clinical parameters	Measurement	Overall	Type I	Type II
No. Cases		60	32	28
Age (years)	Mean±sem	40.5±1.2	37.7±1.7	43.7±1.5
Hysterectomy (TAH)	N (%)	56% (27/48)	44% (11/25)	70% (16/23)
No. slides examined	Median (Range)	8.1 (2–20)	8.2 (3–19)	7.8 (2–20)
Tumor size (cm)	Mean±sem	7.6±0.5	8.5±0.8	6.6±0.7
Follow-up (months)	Mean (Range)	124 (48–258)	106 (37–232)	114 (37–258)
Survival	Recurrence (%)	7.0% (3/43)	9.5% (2/21)	4.5% (1/22)

Table 2

Nuclear features of type I and II ALM

Histologic features	Type I	Type II
Nuclear shape	round and regular	spindled, elongated and irregular
Nuclear membrane	distinct and smooth	irregular
Large nucleoli	prominent	small or pinpoint
Perinucleoli halos	may be present	absent
Chromatin pattern	open and coarse	dark and smudgy

Table 3

Histologic and molecular parameters for type I and II ALM

	Types	Low	Med	High	P value (I vs. II)
Cellularity	I	0% (0/32)	50% (16/32)	50% (16/32)	<i>0.163</i>
	II	11% (3/28)	46% (13/28)	43% (12/28)	
Density	I	3% (1/32)	84% (27/32)	13% (4/32)	< <i>0.001</i>
	II	7% (2/28)	36% (10/28)	57% (16/28)	
Eosinophilic Globules	I	Absent	Low	High	< <i>0.001</i>
	II	0% (0/32)	28% (9/32)	72% (23/32)	
Nucleoli	I	Absent	Pinpoint	Prominent	< <i>0.001</i>
	II	0%	25% (8/32)	75% (24/32)	
Patterns	I	Focal	Multifocal	Diffuse	< <i>0.001</i>
	II	39% (11/28)	46% (13/28)	14% (4/28)	
Staghorn Vessels	I	Present	Absent	Absent	<i>0.003</i>
	II	81% (26/32)	19% (6/32)	57% (16/28)	
HMG2	I	Negative	Positive	Positive	< <i>0.001</i>
	II	100% (25/25)	0%	22% (5/23)	
MED12	I	Wild type	Mutant	Mutant	<i>0.03</i>
	II	78% (18/23)	0%	26% (8/28)	
P53	I	Wild type	Mutant	Mutant	<i>0.229</i>
	II	94% (30/32)	6% (2/32)	19% (5/28)	
PTEN-DEL	I	Normal	Deletion	Deletion	<i>0.523</i>
	II	81% (22/28)	32% (8/32)	22% (10/28)	

Table 4

Comparison of clinical and histologic features in five ALM series

Study		Bell et al 1994	Downes & Hart 1997	Ly et al 2014	Croce et al 2014	Current study 2016
No. Cases		56	24	51	59	60
Age range	Yrs. (mean)	20–75 (40.0)	25–51 (40.7)	21–72 (42.5)	25–75 (45.0)	21–65 (40.5)
Tumor size	Cm. (mean)	0.7–20 (8.0)	1–14 (4.2)	0.7–14 (6.8)	0.4–29 (7.3)	1.5–22 (7.6)
Residual/ Recurrence		9% (4/46)	NA	5.9% (3/51)	1.7% (1/59)	7.0% (3/43)
Nuclear Atypia Distribution	Focal		13% (3/24)	12%	25% (15/59)	13% (8/60)
	Multifocal	N/A	37% (9/24)	29%	44% (26/59)	25% (15/60)
	Diffuse		50% (12/24)	59%	31% (18/59)	62% (37/60)
Nuclear Atypia Density	Low	0	33% (8/24)		47% (28/59)	5% (3/60)
	Med	45% (25/56)	25% (6/24)	N/A	32% (19/59)	62% (37/60)
	High	55% (31/56)	42% (10/24)		20% (12/59)	33% (20/60)
Mitoses per 10 HPF (high power fields)	1	21% (12/56)	13% (3/24)	73% (37/51)	63% (37/59) with <2/10	8% (5/60)
	1–5	61% (34/56) with 2 to 10/10	83% (20/24)	25% (13/51)	32% (19/59) with 2 to 5/10	82% (49/60)
	6–9		4% (1/24)		5% (3/59)	10% (6/60)
Tumor Cellularity	Low	11% (6/56)	21% (5/24)	N/A	2% (1/55)	5% (3/60)
	Med	55% (31/56)	58% (14/24)	N/A	53% (29/55)	48% (29/60)
	High	34% (19/56)	21% (5/24)	6% (3/51)	45% (25/55)	47% (28/60)
Vessels	Staghorn	N/A	21% (5/24)	N/A	34% (20/59)	63% (38/60)
Nucleoli	Prominent	N/A	N/A	N/A	32% (19/59)	47% (28/60)
Eosinophilic Globules		N/A	N/A	N/A	64% (38/59)	48% (29/60)
Growth Pattern	Pushing	70% (39/56)	96% (23/24)	100% (51/51)	98% (45/46)	92% (55/60)
	Infiltrating	30% (17/56)	4% (1/24)		2% (1/46)	8% (5/60)