Mullerian adenosarcoma: clinicopathologic and molecular characterization highlighting recurrent BAP1 loss and distinctive features of high-grade tumors

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INTRODUCTION
Mullerian adenosarcoma is an uncommon gynecologic neoplasm, often found in the lower uterus and cervix, and accounts for 5–7% of uterine sarcomas. It can also arise in ovaries or peritoneum, in some cases, associated with endometriosis. As its name implies, adenosarcoma is a biphasic tumor composed of epithelial and mesenchymal components, with somatic genetic alterations confined to the latter. The histomorphologic appearance is characterized by periligandular condensation and stromal expansion, imparting a leaf-like architecture, closely resembling Phyllodes tumor of the breast.

Most adenosarcomas are low-grade mesenchymal neoplasms, comprising non-specific fibroblastoid spindle stroma or resembling endometrial stroma. These tumors tend to have indolent behavior and are typically curable with surgery. In some adenosarcomas, there is predominance of the mesenchymal component, termed “sarcomatous overgrowth” when pure sarcoma comprises over 25% of the tumor. Areas of sarcomatous overgrowth are often composed of markedly atypical tumor cells with high mitotic activity, and high-grade sarcomatous overgrowth is associated with advanced stage disease and poor prognosis. However, sarcomatous overgrowth can rarely be encountered in low-grade adenosarcomas, which in this context, is of unknown prognostic significance. Conversely, high-grade tumor cells, particularly when present only focally, can be observed in adenosarcomas lacking sarcomatous overgrowth. A recent study suggests that even a minor component of high-grade histology may be associated with increased risk of recurrence, though the data on such rare cases are limited.

Approximately a quarter of adenosarcomas contain heterologous elements, most commonly in the form of rhabdomyosarcomatous differentiation. Heterologous elements are more commonly seen in high-grade adenosarcomas with sarcomatous overgrowth.

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Molecular genetic profiling of adenosarcomas has been performed in several studies, which revealed these adenosarcomas to be genetically heterogeneous, but with recurrent pathogenic driver alterations identified, including rare cases with ESR1-NCOA2/3 fusions. DICER1 mutations are among the most common and of particular interest, as they have also been implicated in uterine and cervical embryonal rhabdomyosarcomas, which may show morphologic overlap with adenosarcoma. In addition, a subset of high-grade adenosarcomas harbor TP53 genetic alterations with associated aberrant p53 immunohistochemical expression. Mutations of genes within the PI3K/akt/PTEN pathway, ATRX, FGFR2, and KMT2C have been reported, as well as amplifications of the MDM2/CDK4 loci and BAP1 deletions.

As adenosarcomas are relatively uncommon, our understanding of this entity is based on small series, mostly of uterine tumors. Herein, we describe the clinicopathologic and molecular features of a cohort of 27 adenosarcomas, including uterine and extraterine primary sites, and enriched for high-grade tumors. The main goals of this study were to uncover oncogenic drivers of high-grade adenosarcoma and to identify recurrent alterations which may lead to development of clinically useful diagnostic or prognostic markers.

MATERIALS AND METHODS

Case selection and review

Following institutional review board approval, 27 uterine or extraterine Mullerian adenosarcomas were identified from our institutional database of tumors subjected to clinical targeted massively parallel sequencing of up to S05 cancer genes using the Memorial Sloan Kettering Cancer Center - Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) platform (from 2014 to 2020). Demographic and clinicopathologic data were extracted from electronic medical records. Of note, none of these cases were previously reported in the prior study of Mullerian adenosarcomas from our institution.

Only cases with slides of the initial primary tumor available were included in this study and diagnoses were confirmed by a gynecologic pathologist (MHC). Histomorphologic review was performed on all available primary and recurrent tumors from each patient. In addition, the following features were evaluated: mitotic rate, presence/absence of sarcomatous overgrowth, high-grade component, and heterologous elements (and type, if present). As there is no established system for assigning tumor grade in adenosarcoma, we adapted criteria previously described by Hodgson et al. Only cases with slides of the initial primary tumor available were included in this study and diagnoses were confirmed by a gynecologic pathologist (MHC). Histomorphologic review was performed on all available primary and recurrent tumors from each patient. In addition, the following features were evaluated: mitotic rate, presence/absence of sarcomatous overgrowth, high-grade component, and heterologous elements (and type, if present). As there is no established system for assigning tumor grade in adenosarcoma, we adapted criteria previously described by Hodgson et al. Low-grade adenosarcomas displayed monotonous, small ovoid nuclei, resembling those seen in low-grade endometrial stromal sarcoma. High-grade atypia was defined as enlarged nuclei with coarse chromatin, exhibiting marked pleomorphism and prominent nucleoli, identifiable at low power magnification, as previously described. We further subclassified the extent of high-grade histology as "focal" when it comprised <10% of a tumor that is predominantly low-grade.

The number of H&E-stained slides of tumor available for review for each case ranged from 2 to 25 (median 12, mean 12). Of note, for the focally high-grade tumors (n = 3), all tumor slides were reviewed (14–25 slides). For the 2 low-grade adenosarcomas that recurred as high-grade sarcoma, only a limited number of tumor slides of primary tumor were available (2 and 5, respectively), as these were received in consultation from other institutions.

Targeted next generation sequencing

Targeted panel sequencing of matched tumor and blood-derived normal DNA was performed using MSK-IMPACT, a hybridization capture-based next-generation sequencing assay targeting all exons and selected intronic regions of 410–505 cancer-related genes. Sequencing data were analyzed as previously described. Variants were annotated by Oncork. Fraction of genome altered by copy number alterations and tumor mutational burden were derived from MSK-IMPACT data. Total and allele-specific copy number was estimated using FACETS.

Targeted RNA-sequencing

For the tumor with ESR1-NCOA3 gene rearrangement identified by MSK-IMPACT, targeted RNA-sequencing was performed using the MSK Solid Fusion assay (v3), for orthogonal validation. The assay incorporates the Archer™ FusionPlex™ and a custom designed Gene Specific Primer Pool kit, designed to target specific exons in 62 genes known to be involved in chromosomal rearrangements.

Immunohistochemistry

Immunohistochemistry for p53 and BAP1 was performed on all cases, while ATRX, ARID1A, PTEN, mismatch repair protein immunohistochemistry was only performed on tumors harboring the corresponding genetic alterations. Immunohistochemical stains were performed on tissue sections from the same tissue blocks that were used for sequencing. The following antibodies, at the specified dilutions, were used: BAP1 (C-4; Santa Cruz, 1:500), ATRX (HPA001906; Sigma, 1:500), ARID1A (HPA005456; Sigma, 1:400), PTEN (1363G6, Cell Signaling, 1:200), MLH1 (ES05, Leica, 1:250), PMS2 (A16–4, BD Pharmingen, 1:500), p53 (DO7, Ventana, pre-diluted). All immunohistochemical stains were performed on the BOND RX platform (Leica), using the BOND Epitope Retrieval Solution 2 (Leica) and BOND Polymer Detection DAB kit (Leica).

Microsatellite instability and MLH1 promoter methylation

For MA04, which harbored an MLH1 deletion, microsatellite instability (MSI) was assessed using the Idylla MSI™ Test. MLH1 promoter hypermethylation status was determined using the Illumina MethylationEPIC 850 K bead array platform.

Statistical analysis

For categorical data, comparisons between groups were analyzed by the Fisher’s exact test. Group comparisons of continuous data were performed using the Mann-Whitney test. All statistical tests were two-tailed, with the threshold for statistical significance set at p < 0.05.

RESULTS

Clinical and histopathologic features of adenosarcomas

Primary sites of the 27 adenosarcomas included in this study were uterine corpus (n = 19), cervix (n = 3), ovary (n = 4), and pelvic peritoneum (n = 1; Table 1). The mean age at diagnosis was 56 years (range: 23–76 years). Apart from 1 patient with a biopsy diagnosis only, all patients underwent primary surgical resection. All tumors showed peri-glandular stromal cuffing, stromal hypercellularity and atypia, and at least focal Phylloides-like architecture, manifested by polyoid growth of stromal cells protruding into glands (Fig. 1A–I). Mitotic activity was variable, ranging from 1 to 58 (median: 5) per 10 high-powered fields. The glandular component showed variable degrees of proliferation, with atypical endometrial hyperplasia present in five cases. Tumors arising in the cervix (n = 3) were lined by benign endocervical mucinous epithelium. Of the 27 adenosarcomas, at the time of initial presentation, 9 were low-grade and 14 were high-grade, three were focally high-grade in background of low-grade adenosarcoma, and 1 was predominantly low-grade, but with an area of indeterminate grade (nuclear irregularities and hyperchromasia, with mitotic activity, but lacking severe nuclear pleomorphism, Fig. 1E, F). Sarcomatous overgrowth was observed almost exclusively in high-grade adenosarcomas (12/17, 71%), tumors with a high-grade component vs 1/10, 10%, low-grade tumors, p = 0.004; Fig. 1C, D). Heterologous differentiation was present in seven cases, all high-grade adenosarcomas (p = 0.03), and consisted of rhabdomyosarcoma (n = 6) and chondrosarcoma (n = 1).

The median length of clinical follow-up, from the date of primary surgical resection, was 29 months (range: 1–69 months; Table 1). Two patients had extensive disease which could not be completely resected, and seven patients developed subsequent recurrence (all of whom achieved complete gross resection at primary surgery) and 18 patients remained disease-free at last follow-up. Sites of disease recurrence included abdomen, colonic serosa, vagina, pelvis, and chest wall. Median time to recurrence was 15 months (range: 1–52 months). For the recurrent cases, the
Table 1. Clinicopathologic features of Mullerian adenosarcomas.

| Case | Age | Grade | Anatomic site | Size (cm)a | Sarcomatous overgrowth | Heterologous elements | Glandular component | FIGO Stage | Clinical follow-up |
|------|-----|-------|---------------|------------|------------------------|-----------------------|---------------------|------------|-------------------|
| MA01 | 44  | Low   | Ovary, arising from endometriosis | 7.8        | No                     | No                    | Non-atypical hyperplasia | N/A        | NED (25 months)   |
| MA02 | 72  | Low   | Rectovaginal septum | 5.5        | No                     | No                    | Atrophic endometrium    | N/A        | DOD (high-grade recurrence in colonic serosa at 28 months; death at 44 months) |
| MA03 | 58  | Low   | Uterine corpus | 2.0        | No                     | No                    | Inactive endometrium     | IA         | NED (29 months)   |
| MA04 | 52  | Low   | Cervix        | 4.4        | No                     | No                    | Endocervical/ tubal metaplasia | IA         | NED (25 months)   |
| MA05 | 53  | Low   | Uterine corpus | 5.3        | No                     | No                    | Atypical hyperplasia     | IB         | NED (49 months)   |
| MA06 | 49  | Low   | Uterine corpus | 3.2        | No                     | No                    | Inactive endometrium     | IA         | NED (35 months)   |
| MA07 | 60  | Low   | Uterine corpus | 0.5        | No                     | No                    | Proliferative endometrium | IB         | NED (17 months)   |
| MA08 | 71  | Low   | Uterine corpus | 5.7        | No                     | No                    | Disordered proliferative endometrium | IB         | DOD (high-grade abdominal recurrence at 52 months; death at 69 months) |
| MA09 | 51  | Low   | Uterine corpus | 4.1        | Yes                    | No                    | Atypical hyperplasia     | IA         | AWED (pelvic recurrence at 5 months; last followup at 8 months) |
| MA10 | 44  | High  | Uterine corpus | 3.0        | No                     | No                    | Inactive endometrium     | IA         | NED (25 months)   |
| MA11 | 65  | High  | Ovary        | 15         | Yes                    | No                    | Atypical hyperplasia     | IIB        | NED (49 months)   |
| MA12 | 60  | High  | Ovary        | >30 (fragmented) | Yes                    | No                    | Atrophic endometrium     | IIBb       | DOD (10 months)   |
| MA13 | 39  | High  | Uterine corpus | N/A (biopsy only) | No                    | No                    | Atypical hyperplasia     | IIAb       | AWED (41 months)  |
| MA14 | 31  | High  | Uterine corpus | 5.5        | Yes                    | No                    | Secretory endometrium    | IIAb       | DOD (7 months)    |
| MA15 | 59  | High  | Uterine corpus | 4.0        | Yes (chondrosarcm)     | Yes                    | Atrophic endometrium     | IA         | NED (52 months)   |
| MA16 | 66  | High  | Ovary        | 12.0       | Yes                    | Yes (rhabdo)           | Atrophic endometrium     | IIB        | DOD (pelvic, vaginal, colonic recurrence at 28 months, death at 33 months) |
| MA17 | 23  | High  | Uterine corpus | 6.8        | Yes                    | Yes (rhabdo)           | Inactive endometrium     | IB         | NED (44 months)   |
| MA18 | 59  | High  | Uterine corpus | 3.8        | Yes                    | No                    | Inactive endometrium     | IA         | NED (30 months)   |
| Case  | Age | Grade | Anatomic site | Size (cm) | Sarcomatous overgrowth | Heterologous elements | Glandular component | FIGO Stage | Clinical follow-up |
|-------|-----|-------|--------------|-----------|-----------------------|---------------------|---------------------|-------------|--------------------|
| MA19  | 40  | High  | Uterine corpus | 3.5       | Yes                   | Yes (rhabdo)         | Proliferative endometrium | IVA        | AWED (vaginal recurrence within 1 month; last followup at 12 months) |
| MA20  | 68  | High  | Uterine corpus | 17.1      | Yes                   | Yes (rhabdo)         | Atrophic endometrium    | IIB        | DOD (abdominal, peritoneal recurrence at 2 months; death at 8 months) |
| MA21  | 63  | High  | Uterine corpus | 6.5       | No                    | No                  | Atrophic endometrium    | IA         | NED (7 month) |
| MA22  | 56  | High  | Uterine corpus | 1.8       | Yes                   | Yes (rhabdo)         | Inactive endometrium    | IA         | NED (1 month) |
| MA23  | 76  | High  | Uterine corpus | 5.5       | Yes                   | No                  | Inactive endometrium    | IB         | NED (1 month) |
| MA24  | 48  | Focal high | Uterine corpus | 2.4       | No                    | No                  | Inactive endometrium    | IB         | NED (12 months) |
| MA25  | 43  | Focal high | Cervix       | 1.2       | No                    | Yes (rhabdo)         | Endocervical            | IA         | NED (34 months) |
| MA26  | 41  | Focal high | Cervix       | 4.5       | Yes                   | No                  | Endocervical            | IB         | DOD (vaginal, chest wall recurrence at 15 months, death at 43 months) |
| MA27  | 60  | Focal indeterminate | Uterine corpus | 7.0       | No                    | No                  | Atypical hyperplasia    | IA         | NED (46 months) |

NED no evidence of disease, AWED alive with evidence of disease, DOD died of disease, LFU lost to follow-up.

aLargest dimension of tumor, determined from gross examination.
bIncomplete primary resection.
members of the PI3K pathway (were observed. Other notable genetic alterations included missense and truncating mutations. Recurrent gene amplifications, including MDM2 (frameshift mutations, n = 2, homozygous deletion, n = 1, missense mutations, n = 2, K1344I and R1093M, both classified as VUS). MA07 harbored an in-frame ESR1-NCOA3 fusion involving exon 5 of ESR1 and exon 15 of NCOA3, which was confirmed by targeted RNA-sequencing. An MLH1 homozygous deletion was detected in MA10.

Somatic genetic alterations
Targeted next-generation sequencing was performed on the primary tumor in 26 cases and the recurrent tumor in 1 case (MA08; Fig. 2). Unless otherwise stated, all somatic genetic variants described henceforth were annotated as pathogenic. The most frequently genetic alterations involved BAP1 (homozygous deletion, n = 4; missense mutation, n = 1, I675F, classified as a variant of unknown significance/pathogenicity, VUS). DICER1 mutations were present in 4 cases, all with at least 1 mutation within the RNase III domain; a second DICER1 mutation was identified in 3 of 4 DICER1-mutated adenosarcomas, which included a splice site mutation (n = 1), a frameshift mutation (n = 1) and a missense VUS (Y936C, n = 1). TP53 mutations (n = 2) included indels, missense and truncating mutations. Recurrent gene amplifications, including MDM2 (n = 2), CDK4 (n = 2) and CCNE1 (n = 2) were observed. Other notable genetic alterations included members of the PI3K pathway (PTEN, n = 3; PIK3CA, n = 4; AKT1, n = 2), MAPK pathway (KRAS, n = 4, BRAF, n = 2), ARID1A (n = 3), TERT (promoter mutation, n = 2; amplification, n = 1), and ATRX (frameshift mutations, n = 2, homozygous deletion, n = 1, missense mutations, n = 2, K1344I and R1093M, both classified as VUS). MA07 harbored an in-frame ESR1-NCOA3 fusion involving exon 5 of ESR1 and exon 15 of NCOA3, which was confirmed by targeted RNA-sequencing. An MLH1 homozygous deletion was detected in MA10.

Associations between histomorphologic and molecular features of Mullerian adenosarcomas
There was clear separation of low-grade and high-grade tumors with respect to the fraction of genome altered by copy number alterations (FGA; low-grade, median: 0.8% vs. high-grade, median: 17%; p = 0.002, Fig. 3). MA02 was an outlier amongst low-grade adenosarcomas, which demonstrated low-grade morphology, but displayed a high FGA. This patient subsequently developed a high-grade sarcoma recurrence. Tumors with only a focal high-grade component had low FGA values, similar to the low-grade group, likely attributable to only low-grade tumor or predominantly low-grade tumor present in the sample extracted for molecular analysis. Total mutation counts did not differ significantly across tumor grade. MA27, a predominantly low-grade adenosarcoma which focally showed nuclear atypia of indeterminate grade had a low FGA, but harbored the highest number of mutations across the cohort.
There were no statistically significant associations between any specific genetic alteration and tumor grade, sarcomatous overgrowth or heterologous elements, though statistical analysis may not be meaningful, as each individual gene was altered in only up to a maximum of four cases. Nevertheless, there were some notable observations. All TP53-mutated tumors were high-grade (n = 4), two of which also displayed sarcomatous overgrowth, and were exemplified by high chromosomal instability (median FGA, TP53-mutated: 38% vs TP53-wildtype: 3%, p = 0.01). Immunohistochemistry confirmed the aberrant p53 expression in tumors harboring TP53 mutations, and a wildtype expression pattern in those lacking TP53 genetic alterations, including the two high-grade tumors with MDM2 amplification. Notably, in MA24, p53 immunohistochemical analysis demonstrated aberrant diffuse overexpression restricted to the focal high-grade area present only in the biopsy specimen (Fig. 4A–C). Molecular analysis performed on available tumor tissue from the hysterectomy specimen, which consisted only of low-grade tumor, did not detect a TP53 mutation. Adenosarcomas with CCNE1 amplification (n = 2) were also high-grade.
Two patients with somatic ATRX frameshift mutations (MA02, MA08) initially presented with low-grade adenosarcoma, but subsequently recurred with high-grade sarcoma and died of disease (Fig. 4D–F). Another case of high-grade adenosarcoma (MA23, Fig. 4G–I) harbored an ATRX homozygous deletion and the tumor also displayed foci of low-grade adenosarcoma, compatible with a low-grade origin. Immunohistochemical analysis confirmed loss of ATRX expression in evaluable tumors from all 3 patients (primary low-grade tumor for MA02, recurrent high-grade tumor for MA08, both low-grade and high-grade components for MA23). In MA01 and MA24, which harbored an ATRX VUS, immunohistochemical staining revealed intact ATRX expression.

Of the four adenosarcomas with DICER1 mutations, three showed rhabdomyosarcomatous differentiation: one (MA16) showed extensive sarcomatous overgrowth by undifferentiated sarcoma, focally admixed with pleomorphic rhabdomyoblasts, while 2 (MA17 and MA25) displayed features of embryonal rhabdomyosarcoma. For the latter 2 cases, the presence of focal areas with Phyllodes architecture, peri-glandular cuffing by stromal cells with low-grade fibroblastoid morphology were consistent with origin of the rhabdomyosarcomatous component from a pre-existing adenosarcoma (Fig. 1G–I). Of note, rhabdomyosarcomatous elements were also observed in three adenosarcomas without DICER1 mutations and consisted of large, pleomorphic rhabdomyoblasts in areas of sarcomatous overgrowth (Fig. 1C, D).

MA07, with the ESR1-NCOA3 fusion, was a 0.5 cm endometrial-based tumor with superficial (1 mm) myometrial invasion, which exhibited typical morphologic features of low-grade adenosarcoma (Fig. 1B), with mitotic activity reaching up to 3 per 10 high-powered fields. With exception of the fusion, no other somatic mutations, copy number alterations or structural variants were found in this tumor. Of note, ESR1-NCOA2/3 fusions have previously been reported in uterine tumors resembling ovarian sex cord tumor (UTROSCTs)20, however, we did not observe evidence of sex-cord differentiation in this case.

Given the frequent occurrence of endometrial glandular hyperplasia in adenosarcoma, we performed immunohistochemical analysis on cases with PTEN, ARID1A, and MLH1 genetic alterations to determine whether loss of expression was seen in the neoplastic mesenchymal component or the benign/hyperplastic glandular component (Fig. 5A–F). For cases harboring PTEN mutations, loss of PTEN expression was restricted to benign proliferative glands only in MA01 but was observed in the mesenchymal component in MA12 and MA22. For cases with ARID1A mutations detected by sequencing, loss of expression was detected in atypical hyperplasia only in MA27, with retained expression in the mesenchymal component. MA12 also showed retained expression in the mesenchymal component, which comprised the entirety of the sample submitted for sequencing. MA10 harbored a homozygous MLH1 deletion, and showed loss of MLH1 and PMS2 expression, confined to the stromal component.
and to our knowledge, is the first reported case of a mismatch repair protein-deficient adenosarcoma. MLH1 promoter hypermethylation was negative. MSI analysis, however, demonstrated the tumor to be microsatellite stable, suggesting that MLH1 deletion may have occurred late in tumor progression and probably not a significant pathogenic driver. This tumor also harbored a concomitant BAP1 deletion.

**BAP1 loss in Mullerian adenosarcomas and other mesenchymal neoplasms of gynecologic tract**

BAP1 homozygous deletion was identified in four adenosarcomas (high-grade, n = 3, and low-grade, n = 1; Fig. 6A, B). Immunohistochemical analysis of BAP1 in 24 adenosarcomas with available tissue confirmed loss of protein expression in 6 cases (25%), including all 4 tumors with BAP1 deletions, and 2 tumors lacking BAP1 genetic alterations (MA25 and MA14, with focal weak retained expression in the latter). MA08, which harbored an ID75F VUS, showed retained expression, and hence this likely represents a non-pathogenic passenger mutation.

The relative prevalence of BAP1 genetic alterations was interrogated in 169 other mesenchymal neoplasms of gynecologic origin (primary uterine, n = 134, cervical, n = 6, ovarian, n = 4, vulvovaginal, n = 12, and pelvic, n = 13) subjected to molecular profiling by MSK-IMPACT, comprised of leiomyosarcomas (n = 78), endometrial stromal sarcomas (n = 27), rhabdomyosarcomas (n = 21), PEComas (n = 13), undifferentiated/unclassifiable sarcomas (n = 25), and other rare sarcomas (epithelioid sarcoma, n = 3, radiation-associated sarcoma, n = 1, angiosarcoma, n = 1). None of these other mesenchymal neoplasms harbored a BAP1 homozygous deletion, which appeared to a genetic feature specific to a subset of adenosarcomas (4/27, 15%), of adenosarcomas vs 0/169, 0%, of other mesenchymal neoplasms of gynecologic origin, P = 0.0003; Fig. 6C).

**DISCUSSION**

Mullerian adenosarcomas have a heterogeneous genomic landscape. Despite the lack of a pathognomonic molecular feature10,13, recent recurrent genetic alterations have been identified. Many of these are commonly mutated cancer genes that are not specific to adenosarcoma, including PI3K and MAPK pathway gene alterations, TP53 mutations, and MDM2/CDK4 amplification. As adenosarcomas are uncommon, study cohorts are generally small (less than 30 cases). Therefore, multiple studies of independent cohorts are needed to comprehensively characterize the spectrum and frequencies of genetic alterations in this disease. Our present study confirms prior findings, provides new insights on the molecular features distinguishing low-grade and high-grade adenosarcomas, and evidence supporting BAP1 deletion as a distinctive feature of a subset of adenosarcomas.

While sarcomatous overgrowth is well recognized as a poor prognostic feature in adenosarcoma, the clinical significance of histologic grading has not been addressed until relatively recently. Hodgson et al. demonstrated that adenosarcomas could be subdivided based on nuclear grade, independent of sarcomatous overgrowth, and that high-grade tumors have distinct clinical, morphologic and molecular characteristics3. In that study, high-grade morphology was associated with large tumor size, high mitotic index, sarcomatous overgrowth, and presence of TP53 mutations (observed in 6/9 cases). These tumors had aggressive clinical behavior, characterized by widespread metastasis and early recurrence, which was observed even in cases with a minor (<25%) high-grade component. In our cohort, almost all tumors with sarcomatous overgrowth were high-grade and heterologous elements were exclusive to high-grade adenosarcomas. All seven deaths were from high-grade disease: 2 of these were associated with exclusively low-grade adenosarcoma at initial presentation, and 1 had only a focal high-grade component.

We observed a few characteristic molecular features of high-grade adenosarcomas. Aside from TP53 mutations (n = 4), MDM2 amplification (n = 2) may serve as an alternative mechanism to cause p53 inactivation. CCNE1 amplification (n = 2) is known to induce chromosome instability, through centrosome amplification and chromosome missegregation3.

The significantly higher FGA in high-grade compared to low-grade adenosarcomas, is consistent with chromosomal instability being a characteristic feature of high-grade tumors. Indeed, this is in keeping with the marked nuclear pleomorphism, and is analogous to other high-grade TP53-mutated tumors, such as high-grade serous carcinomas or uterine leiomyosarcomas. Interestingly, the only low-grade adenosarcoma with high FGA subsequently recurred as an overtly high-grade sarcoma. While total mutation counts did not vary significantly between low-grade and high-grade tumors, it is notable that a case displaying nuclear irregularities and increased mitotic activity, but lacking pleomorphism, had a particularly high number of mutations.
Fig. 6  BAP1 homozygous deletion is a distinctive feature of a subset of Mullerian adenosarcomas. A, B Representative adenosarcomas with BAP1 homozygous deletions: A MA10, which also harbors an MLH1 deletion (see Fig. 5 for MLH1/PMS2 immunohistochemistry) and B MA11. For each case, log2 copy number ratio plots across the whole genome (top) and chromosome 3, containing the BAP1 locus at 3p21 (middle, right), are presented. Estimated copy number plots for chromosome 3 (middle, left), with total (black line) and minor allele copy number (red line), demonstrate focal homozygous deletion of BAP1. Immunohistochemical staining for BAP1 (bottom) shows loss of expression in the neoplastic mesenchymal component. C BAP1 copy number alterations across the spectrum of gynecologic mesenchymal neoplasms.
Overall, our results support the contention that tumor nuclear morphology reflects the extent of genomic instability. While most of high-grade adenosarcomas showed high-grade morphology throughout, several tumors were predominantly low-grade with only a focal high-grade component, suggesting that at least a subset of high-grade adenosarcomas evolve from a pre-existing low-grade neoplasm. One of these showed aberrant diffuse P53 overexpression restricted to the high-grade area (though unfortunately, tissue was not available for molecular confirmation of a TP53 genetic alteration).

Interestingly, there were two cases with ATRX frameshift mutations, and both were observed in the patients with low-grade adenosarcomas who subsequently developed high-grade sarcoma recurrence. In MA02, this was detected in the primary tumor, which showed typical morphologic features of low-grade adenosarcoma. In MA08, as only the recurrent high-grade sarcoma was sequenced, it is unknown whether the ATRX mutation was present in the primary tumor. A third case with ATRX homozygous deletion (MA23) was a high-grade adenosarcoma with a residual low-grade component. Overall, these findings suggest that ATRX dysfunction may drive high-grade transformation of low-grade adenosarcomas, which may occur independent of TP53 genetic alterations. This contention is in line with the known biologic functions of ATRX in regulating chromatin structure, chromosome stability and telomere maintenance. In corroboration with our findings, in the series of high-grade adenosarcomas by Hodgson et al., two cases had ATRX mutations (one with insertion/deletion and the other with a missense mutation); in these cases, the high-grade component reportedly comprised 75% and 10% of the tumor area, respectively. Howitt et al. also reported ATRX mutations in three adenosarcomas, all associated with stromal overgrowth (though grade was not assessed); only one of these tumors showed concomitant loss of ATRX expression by immunohistochemistry. Future studies are needed to determine whether ATRX genetic alterations and/or immunohistochemical loss of staining in low-grade adenosarcomas could predict for subsequent high-grade recurrence.

A major aim of this study was to identify characteristic genetic alterations that may aid in the distinguishing Mullerian adenosarcoma from other entities with morphologic overlap. Embryonal rhabdomyosarcoma has overlapping morphologic features with adenosarcoma and is often an important diagnostic consideration. Previous work has established DICER1 mutations to be almost universally present in embryonal rhabdomyosarcoma, but almost are also found in uterine adenosarcomas, albeit at lower frequencies (ranging from 10–42%, median 22%, across various studies). Consistent with the study by Bean et al., in our cohort, the presence of rhabdomyosarcomatous differentiation was more frequently seen in, but are not exclusive to, adenosarcomas harboring DICER1 mutations. Notably, in our cohort, rhabdomyosarcomatous elements consisted of large and pleomorphic rhabdomyoblasts in adenosarcomas that lacked DICER1 mutations, whereas embryonal rhabdomyosarcoma-like features were only observed in the context of DICER1 mutations. The presence of a DICER1 mutation cannot distinguish between adenosarcoma and embryonal rhabdomyosarcoma, as both entities (including adenosarcomas lacking any rhabdomyosarcomatous elements) can harbor this alteration. It is debatable whether some DICER1-mutated “adenosarcomas” may be better considered as embryonal rhabdomyosarcomas with areas displaying an adenosarcoma-like growth pattern.

Indeed, given the molecular heterogeneity of Mullerian adenosarcoma, it is possible that the morphologic diagnosis of adenosarcoma comprises a variety of different neoplastic entities. Aside from embryonal rhabdomyosarcoma, just discussed, other tumors including endometrial stromal sarcoma, NTRK-fusion cervical sarcoma, SMARCA4-deficient uterine sarcoma, to name a few, could all exhibit adenosarcoma-like architectural features, and some of these may have even been included in published cohorts of adenosarcoma.

In this context, our work highlights BAP1 homozygous deletion as a unique molecular feature in Mullerian adenosarcoma, and not identified in other gynecologic mesenchymal neoplasms. BAP1 (BRCA1-associated protein 1) is a tumor suppressor with growth inhibitory functions in cells via regulation of cell cycle, cell differentiation and DNA damage response. Germline and somatic BAP1 mutations or deletions are found in various human cancers, most frequently in mesothelioma, cutaneous melanoma, and uveal melanoma. In Mullerian adenosarcoma, BAP1 deletions have been reported at frequencies ranging from 5–17%.

Including the present study, this amounts to a cumulative total of 15 of 114 (13%) adenosarcomas across various studies. Loss of nuclear BAP1 immunohistochemical staining confirms functional inactivation of BAP1 and was observed in all four cases with homozygous deletion in our cohort, and also in two other cases without BAP1 genetic alterations (with one of these showing focal retained weak expression), a phenomenon which has been previously reported in other tumors, such as gallbladder carcinoma. The loss of BAP1 expression in cases without any identifiable genetic alterations may be due to epigenetic silencing or deep intronic splice variants not identified by our targeted sequencing panel.

A particular strength of the present study is the use of matched tumor-normal sequencing data, which enabled us to confirm the specificity of BAP1 homozygous deletion for adenosarcomas, while only heterozygous losses or copy neutral loss-of-heterozygosity were observed in a handful of other gynecologic mesenchymal neoplasms. In contrast, analysis of tumor genetic alterations against a pooled normal control, as done in most studies, precludes accurate distinction of single copy versus homozygous deletions.

Since we did not perform BAP1 immunohistochemistry on this cohort of other gynecologic mesenchymal neoplasms, we cannot comment on whether some of these may potentially show loss of BAP1 expression though an epigenetic mechanism, as seen in two adenosarcomas lacking BAP1 deletions. Future studies investigating BAP1 staining patterns on a larger cohort of gynecologic sarcomas of various subtypes are needed to establish the specificity of BAP1 loss for adenosarcoma and the prognostic impact of this feature.

Another limitation of this study is that for some cases, only a subset of H&E-stained slides were available for histomorphologic review. Hence, for the low-grade adenosarcomas that subsequently recurred as high-grade sarcoma, we cannot exclude the possibility of a high-grade component in the primary tumor that was unsampled or not represented on the slides that were reviewed. In summary, the present study confirms and extends prior observations on the molecular heterogeneity of Mullerian adenosarcoma. High-grade adenosarcomas, characterized by chromosomal instability, exhibit recurrent deleterious genetic alterations in TP53 and ATRX, with the latter typically seen in the context of a pre-existing low-grade component. Furthermore, BAP1 deletion is a recurrent driver and distinctive feature of a subset of adenosarcomas.

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