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

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Mullerian adenosarcoma is an uncommon mesenchymal tumor of the gynecologic tract. Most cases are low-grade, while high-grade adenosarcomas are rare and not well studied. Herein, we characterize the clinicopathologic and molecular features of 27 adenosarcomas of gynecologic origin, enriched for high-grade tumors subjected to targeted panel sequencing. Sarcomatous overgrowth was more frequently seen in high-grade compared to low-grade tumors (12/17, 71%, vs 1/10, 10%, $p = 0.004$) and heterologous elements were exclusive to high-grade cases ($n = 7$, $p = 0.03$). All deaths were from high-grade disease (advanced primary, $n = 2$, or recurrence, $n = 5$). Genetic alterations specific to high-grade adenosarcomas have known associations with chromosome instability, including *TP53* mutations ($n = 4$) and amplifications of *MDM2* ($n = 2$) and *CCNE1* ($n = 2$). Somatic *ATRX* frameshift mutations were found in 2 patients with high-grade recurrences following a primary low-grade adenosarcoma and *ATRX* deletion in 1 high-grade adenosarcoma with an adjacent low-grade component. The fraction of genome altered by copy number alterations was significantly higher in high-grade compared to low-grade adenosarcomas ($P = 0.001$). Other recurrent genetic alterations across the entire cohort included *BAP1* homozygous deletions ($n = 4$), *DICER1* mutations ($n = 4$), *ARID1A* mutations ($n = 3$), *TERT* promoter mutations ($n = 2$) and amplification ($n = 1$), as well as alterations involving members of the PI3K and MAPK signaling pathways. One tumor harbored an *ESR1-NCOA3* fusion and another had an *MLH1* homozygous deletion. Immunohistochemical analysis for BAP1 revealed loss of nuclear expression in 6/24 (25%) cases, including all four tumors with *BAP1* deletions. Notably, out of 196 mesenchymal neoplasms of gynecologic origin, *BAP1* homozygous deletion was only found in adenosarcomas ($P = 0.0003$). This study demonstrates that high-grade adenosarcomas are heterogeneous at the molecular level and are characterized by genomic instability and *TP53* mutations; *ATRX* loss may be involved in high-grade transformation of low-grade adenosarcoma; and *BAP1* inactivation appears to be a specific pathogenic driver in a subset of adenosarcomas.

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INTRODUCTION

Mullerian adenosarcoma is an uncommon gynecologic neoplasm, often found in the lower uterine corpus and cervix, and accounts for 5–7% of uterine sarcomas. It can also arise in ovaries or peritoneum, in some cases, associated with endometriosis[1–3]. As its name implies, adenosarcoma is a biphasic tumor composed of epithelial and mesenchymal components, with somatic genetic alterations confined to the latter[4,5]. The histomorphologic appearance is characterized by peri-glandular condensation and stromal expansion, imparting a leaf-like architecture, closely resembling Phyllodes tumor of the breast[6].

Most adenosarcomas are low-grade mesenchymal neoplasms, comprising non-specific fibroblastoid spindled 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[7–9]. Areas of sarcomatous overgrowth are often composed of markedly atypical tumor cells with high mitotic activity, and high-grade sarcomatous overgrowth is associated advanced stage disease and poor prognosis[2,7,8]. However, sarcomatous overgrowth can rarely be encountered in low-grade adenosarcomas[4,5], 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[9]. 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[9]. Approximately a quarter of adenosarcomas contain heterologous elements, most commonly in the form of rhabdomyosarcomatous differentiation[1]. Heterologous elements are more commonly seen in high-grade adenosarcomas with sarcomatous overgrowth[10].

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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[5,9,11–13]. *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[11,14]. In addition, a subset of high-grade adenosarcomas harbor *TP53* genetic alterations with associated aberrant p53 immunohistochemical expression[9]. Mutations of genes within the *PI3K/AKT/PTEN* pathway, *ATRX*, *FGFR2*, and *KMT2C* have been reported, as well as amplifications of the *MDM2/CDK4* locus and *BAP1* deletions[5,9,11–13].

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 extrauterine 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 extrauterine Mullerian adenosarcomas were identified from our institutional database of tumors subjected to clinical targeted massively parallel sequencing of up to 505 cancer genes using the Memorial Sloan Kettering Cancer Center - Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) platform[15] (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[5].

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.[9]. 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[9]. 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[15]. Sequencing data were analyzed as previously described[15,16]. Variants were annotated by OncoKB[17]. 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[18].

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)[19], 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 (136G6, Cell Signaling, 1:200), MLH1 (ES05, Leica, 1:250), PMS2 (A16–4, BD Pharmingen, 1:500), p53 (D07, 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 Phyllodes-like architecture, manifested by polypoid 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	IIIB[b]	DOD (10 months)
MA13	39	High	Uterine corpus	N/A (biopsy only)	No	No	Atypical hyperplasia	N/A (biopsy only)	AWED (41 months)
MA14	31	High	Uterine corpus	5.5	Yes	No	Secretory endometrium	IIIA[b]	DOD (7 months)
MA15	59	High	Uterine corpus	4.0	Yes	Yes (chondrosarc)	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)

Table 1. continued

Case	Age	Grade	Anatomic site	Size (cm)[a]	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	IIIB	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.
[a]Largest dimension of tumor, determined from gross examination.
[b]Incomplete primary resection.

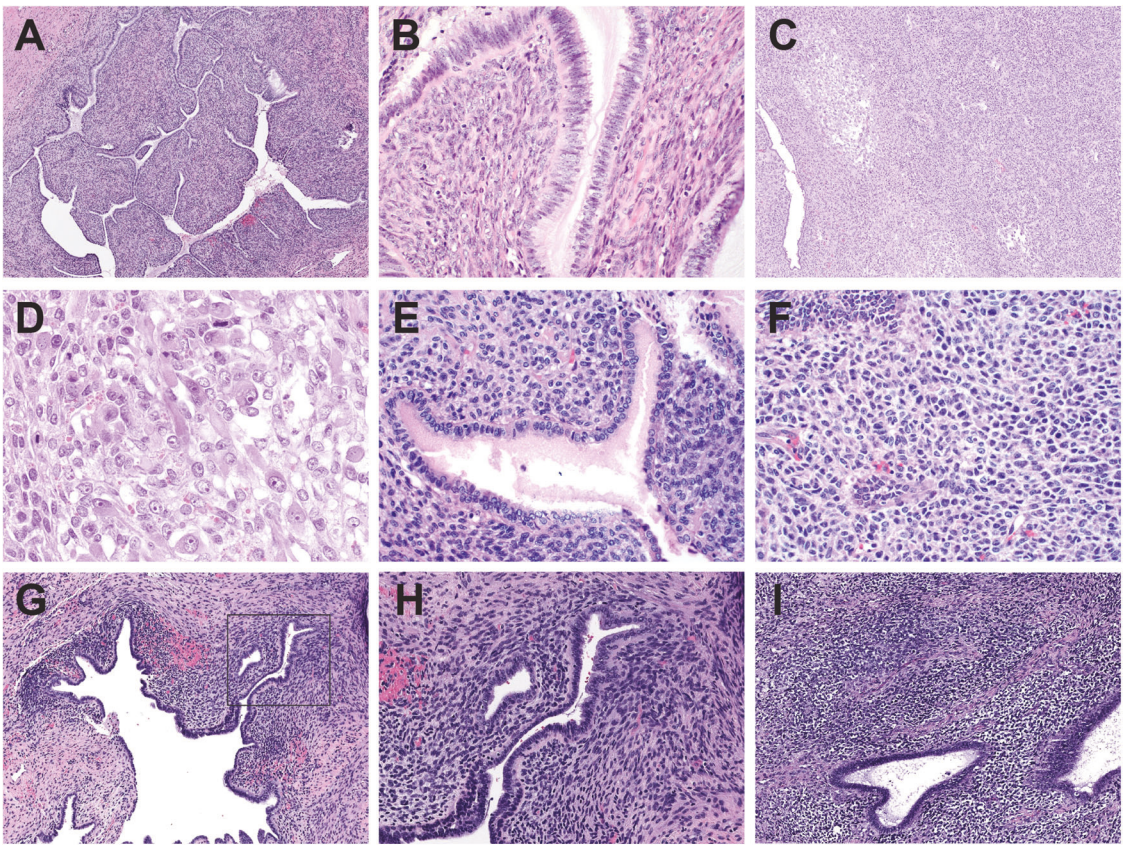

Fig. 1 Histomorphologic spectrum of Mullerian adenosarcomas. A MA04 (no specific genetic alterations), exhibiting characteristic leaf-life architecture; (**B**) MA7 (*ESR1-NCOA3* fusion), bland fibroblastic-like stroma with low-grade nuclear features. **C**, **D** MA22 (*TP53*-mutated), high-grade adenosarcoma with sarcomatous overgrowth. The tumor shows rhabdomyosarcomatous heterologous elements and marked nuclear pleomorphism with prominent nucleoli and increased mitotic activity. **E**, **F** MA27 (*DICER1*-mutated), a predominantly low-grade adenosarcoma (**E**) with focal area showing enlarged, hyperchromatic nuclei with irregular borders and increased mitotic activity, but lacking significant nuclear pleomorphism, and therefore, classified as indeterminate grade (**F**). No heterologous elements were present. **G**–**I** MA17 (*DICER1*-mutated), focal areas exhibit fibroblastoid morphology and architectural features of adenosarcoma (**G**), with area in box shown at higher magnification to highlight cytologic features (**H**); other areas of this tumor show sarcomatous overgrowth comprised of sheets of primitive cells, consistent with a heterologous embryonal rhabdomyosarcoma component (**I**).

original primary tumor was classified as low-grade ($n = 3$), focally high-grade ($n = 1$), or high-grade ($n = 3$); however, six of seven (86%) developed high-grade sarcomas at recurrence. Seven (26%) patients died of disease: five from recurrent high-grade sarcoma and two from extensive primary disease (both high-grade) that could not be completely resected.

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 ($n = 4$) 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.

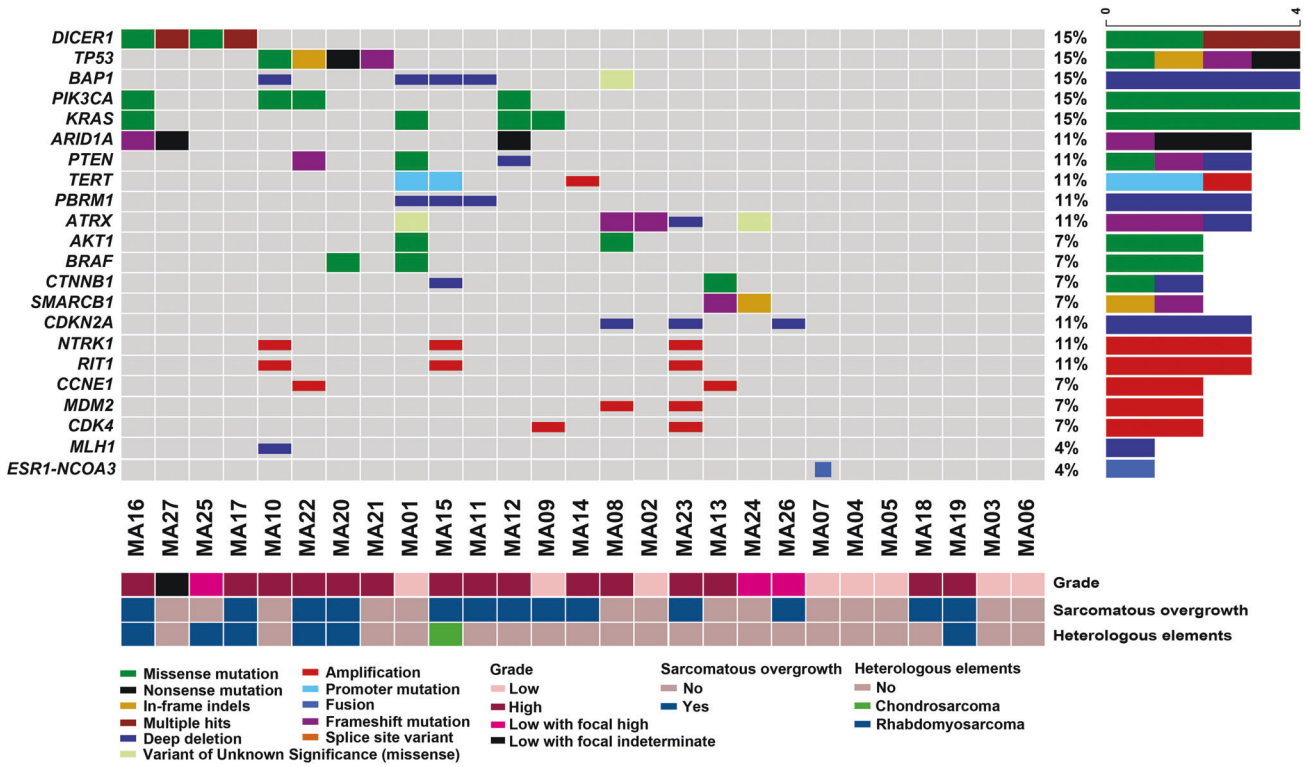

Fig. 2 Summary of somatic genetic alterations in Mullerian adenosarcomas. Recurrently altered genes in this cohort or reported in prior studies of adenosarcoma are presented, as well as the unusual finding of *MLH1* deletion ($n = 1$). With exception of MA08, for which only the high-grade recurrence (MA08) was available, molecular profiling was performed on the primary tumors.

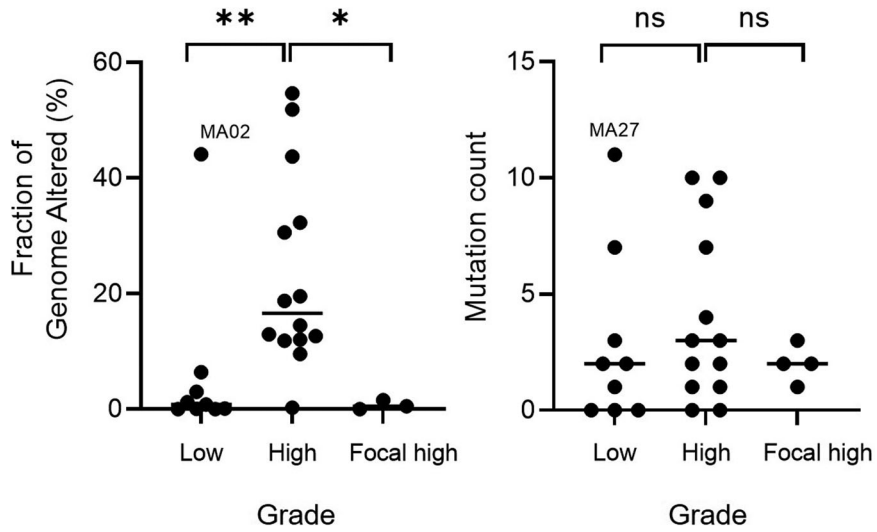

Fig. 3 Genomic instability and tumor mutational burden in adenosarcomas. Fraction of genome altered by copy number alterations (FGA) and total mutation counts are presented, stratified by histologic grade. Note that the low-grade group includes the tumor with focal indeterminate grade (MA27). MA08 (molecular analysis performed on recurrence) is excluded from these graphs. n.s. not significant, *$p < 0.05$, **$p < 0.01$, Mann-Whitney test.

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.

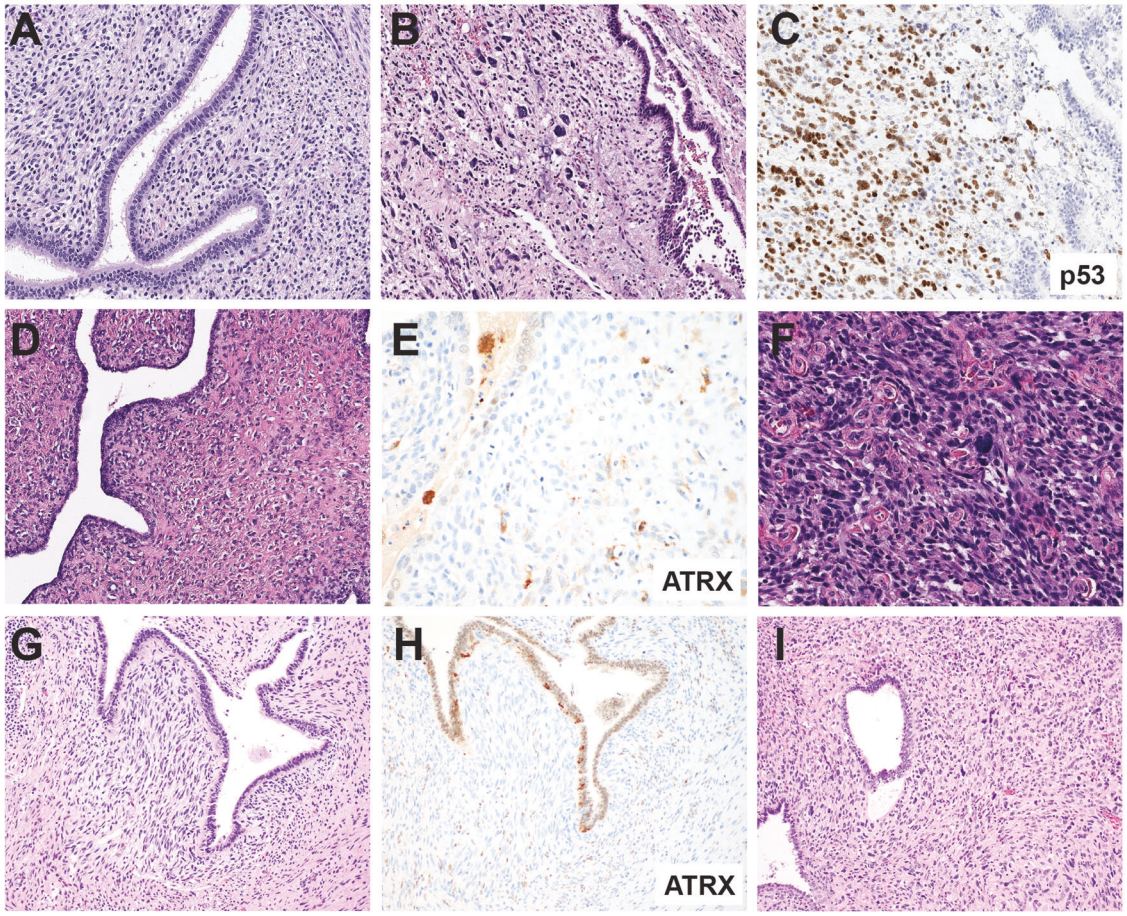

Fig. 4 Low-grade adenosarcoma with high-grade transformation. A–C MA23, low-grade adenosarcoma in hysterectomy specimen (**A**); focal high-grade area present only in the endometrial sampling (**B**), which demonstrates aberrant p53 overexpression (**C**). **D–F** MA02, low-grade adenosarcoma (**A**) with *ATRX* frameshift mutation and loss of ATRX expression (**B**). This patient subsequently developed a recurrent high-grade sarcoma (**F**). **G–I** MA23, high-grade adenosarcoma with *ATRX* homozygous deletion. The focal residual low-grade component (**G**) shows loss of ATRX expression (**H**). Most of the tumor consists of high-grade adenosarcoma (**I**), which also exhibits similar loss of ATRX by immunohistochemistry (not shown).

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 rhabdomysarcoma. 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 rhabdomysarcomatous 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,

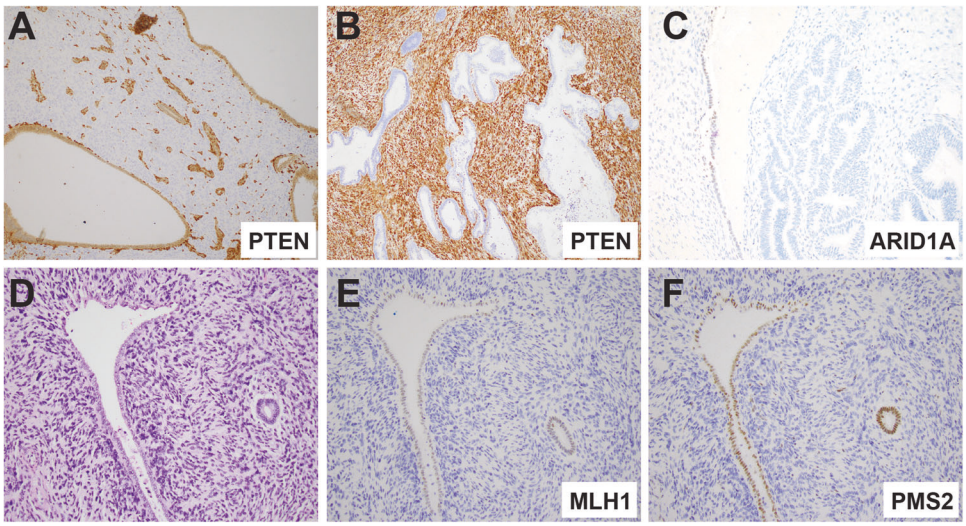

Fig. 5 PTEN and ARID1A expression patterns in adenosarcomas. A, **B** Immunohistochemical analysis of tumors harboring *PTEN* mutations demonstrates loss of PTEN expression in the neoplastic mesenchymal component in MA22 (**A**), but confined to the proliferative glands in MA01 (**B**). **C** In MA26, with *ARID1A* mutation, loss of expression is confined to endometrial glands exhibiting atypical hyperplasia. **D**–**F** MA10 with *MLH1* homozygous deletion, showing loss of MLH1 (**E**) and PMS2 (**F**) expression in the mesenchymal 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 I675F 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 feature[5,9,13], 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 characteristics[9]. 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 missegregation[21].

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.

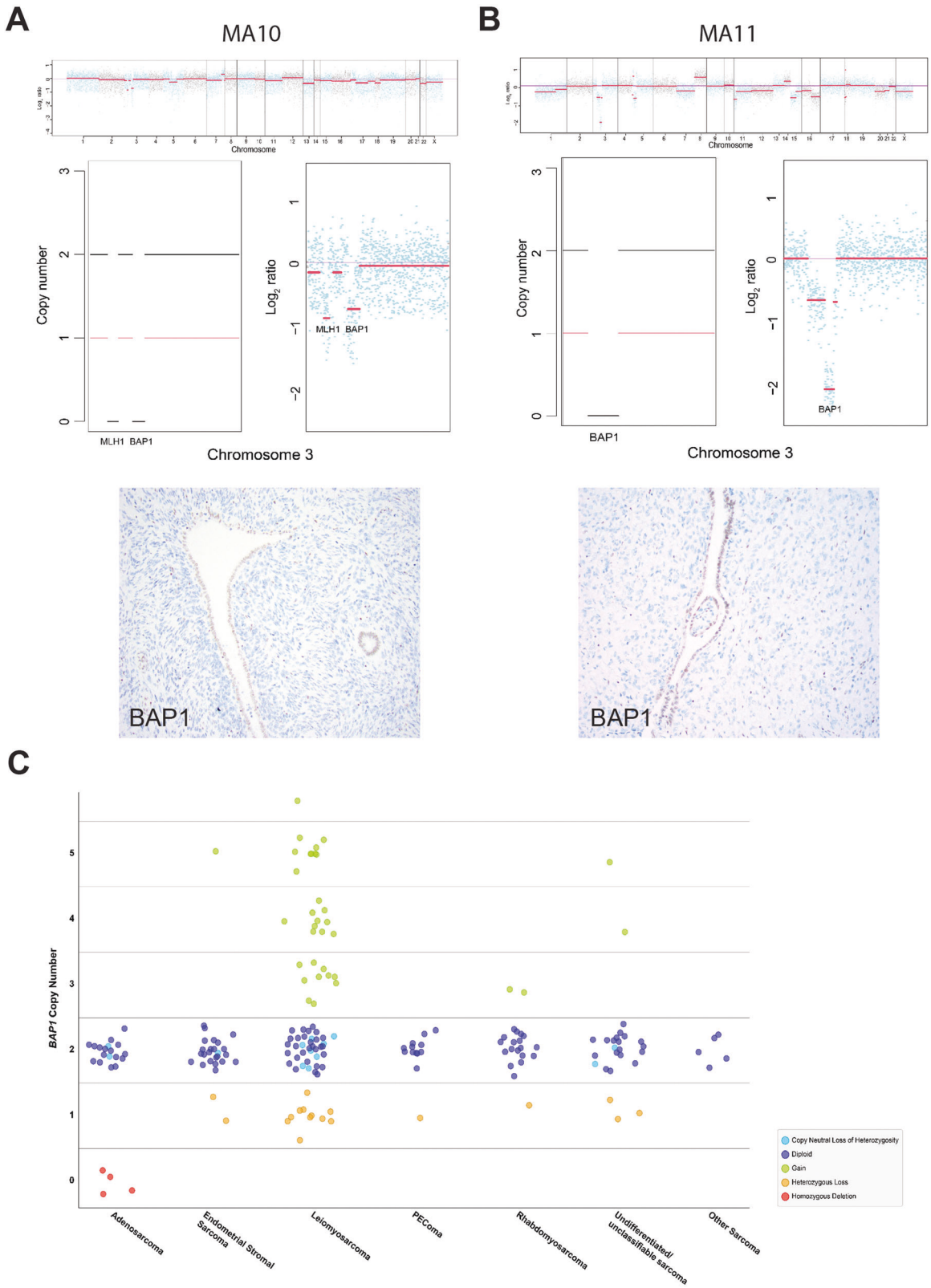

Fig. 6 ***BAP1* homozygous deletion is a distinctive feature of a subset of Mullerian adenosarcomas. A**, **B** Representative adenosarcomas with *BAP1* homozygous deletions: **A** MA10, which also harbors an *MLH1* deletion (see Fig. 5 for MLH1/PMS2 immunohistochemistry) and **B** MA11. For each case, 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[22].

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[9]. 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[12]. 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[23]. Previous work has established *DICER1* mutations to be almost universally present in embryonal rhabdomysarcoma, but are also found in uterine adenosarcomas, albeit at lower frequencies (ranging from 10–42%, median 22%, across various studies)[5,9,11,12,14]. Consistent with the study by Bean et al.[11], 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[11]. 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[23], just discussed, other tumors including endometrial stromal sarcoma, NTRK-fusion cervical sarcoma[24], SMARCA4-deficient uterine sarcoma[25], 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[26]. Germline and somatic *BAP1* mutations or deletions are found in various human cancers, most frequently in mesothelioma, cutaneous melanoma, and uveal melanoma[27–29]. In Mullerian adenosarcoma, *BAP1* deletions have been reported at frequencies ranging from 5–17%[5,9,11–13]. 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[30]. 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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AUTHOR CONTRIBUTIONS

AMB performed study design, collection, analysis and interpretation of data, and manuscript writing. EK, XW, and EMDS performed collection and analysis of data. SC, RAS, and BW performed data interpretation. MHC performed study conceptualization and design, collection, analysis and interpretation of data, and manuscript writing. All authors have read and approved the final paper.

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COMPETING INTERESTS

Dr Chui has served as a compensated consultant for Roche, outside of the present work. The other authors have no conflicts of interest to declare.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center. The study was performed in accordance with the Declaration of Helsinki.

ADDITIONAL INFORMATION

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