HHS Public Access

Author manuscript

J Pathol. Author manuscript; available in PMC 2017 February 01.

Published in final edited form as:

J Pathol. 2016 February ; 238(3): 381–388. doi:10.1002/path.4675.

Uterine adenosarcomas are mesenchymal neoplasms

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Abstract

Uterine adenosarcomas (UA) are biphasic lesions composed of a malignant mesenchymal (i.e. stromal) component and an epithelial component. UAs are generally low-grade and have a favourable prognosis, but may display sarcomatous overgrowth (SO), which is associated with a worse outcome. We hypothesized that, akin to breast fibroepithelial lesions, UAs are mesenchymal neoplasms where clonal somatic genetic alterations are restricted to the mesenchymal component. To characterize the somatic genetic alterations in UAs and to test this hypothesis, we subjected 20 UAs to a combination of whole-exome (n=6), targeted capture (n=13) massively parallel sequencing (MPS) and/or RNA-sequencing (n=6). Only three genes, *FGFR2*, *KMT2C* and *DICER1*, were recurrently mutated, all in 2/19 cases; however, 26% (5/19) and 21% (4/19) of UAs harboured *MDM2/CDK4/HMGA2* and *TERT* gene amplification, respectively, and two cases harboured fusion genes involving *NCOA* family members. Using a combination of laser capture microdissection and *in situ* techniques, we demonstrated that the somatic genetic alterations detected by MPS were restricted to the mesenchymal component. Furthermore, mitochondrial DNA sequencing of microdissected samples revealed that epithelial and mesenchymal components of UAs were clonally unrelated. In conclusion, here we provide evidence that UAs are genetically heterogeneous lesions and mesenchymal neoplasms.

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Conflict of interest: The authors have no conflicts of interest to declare.

AUTHORS CONTRIBUTIONS

BW, RAS, BPR and JSR-F conceived and supervised the study. DAL, BPR and RAS provided the samples; RAS performed the histological review. JSR-F, ADP and AMS performed the sample microdissection; KAB, CKYN, MRDF and IdB performed the bioinformatic analysis; SP, GSM, LGM and RAI carried out experiments and analyzed data. SP, CKYN and FCG wrote the first draft that was revised by JSR-F and BW. All authors reviewed and approved the final version of the manuscript.

Keywords

Uterine adenosarcoma; massively parallel sequencing; RNA-sequencing; FISH

INTRODUCTION

Uterine adenosarcomas (UAs) comprise a group of mixed epithelial-mesenchymal tumours that account for 5% of uterine sarcomas [1, 2]. UAs typically present as a polypoid mass and are characterized histologically by an admixture of low-grade malignant mesenchymal (i.e. stromal) and neoplastic but benign/atypical epithelial components, with a typical phyllodes tumour-like architecture [2, 3]. The mesenchymal component of UAs generally shows mild-to-moderate nuclear atypia, with variable mitotic rates, while a minority presents with heterologous elements such as immature cartilage or skeletal muscle [1–3]. Sex cord-like differentiation may be present within the mesenchymal component [3]. Most UAs are of low grade and low stage (FIGO stage I), and have low recurrence and mortality rates after surgery (15%–25%)[3, 4]. Approximately 25% of UAs, however, have sarcomatous overgrowth (SO), which is often associated with lymphovascular and/or deep myometrial invasion and high recurrence rates (up to 70%)[2–4].

The genomic features of UAs have yet to be fully characterized. Recently, Howitt *et al.*[5] employed targeted massively parallel sequencing (MPS) of 275 genes and identified mutations in PI3K/AKT/PTEN pathway members, *ATRX* and *TP53* in 72%, 17% and 11% of UAs analyzed, respectively [5]. Copy number analysis (CNA) revealed recurrent amplifications affecting *MDM2*/*CDK4*/*HMGA*2 (28%) and *MYBL1* (22%)[5].

Breast fibroepithelial tumours are biphasic neoplasms with epithelial and mesenchymal components [6], some of which (i.e. phyllodes tumours) bear histological resemblance to UAs. Breast fibroepithelial tumours have been shown to harbour somatic genetic alterations (i.e. *MED12* mutations) exclusively in the mesenchymal component, suggesting that these tumours are primarily mesenchymal neoplasms [7, 8]. Furthermore, clonality analyses revealed that the mesenchymal component but not the epithelium is monoclonal in most cases [9].

We hypothesized that, akin to breast fibroepithelial lesions, UAs are mesenchymal neoplasms with clonally unrelated mesenchymal components and epithelium. Here we sought to characterize the landscape of somatic genetic alterations in UAs and to determine whether these alterations are restricted to the mesenchymal component or present in both mesenchymal and epithelial cells of these neoplasms, using a combination of whole-exome or targeted MPS, RNA-sequencing, *in situ* hybridization and laser capture approaches.

MATERIALS AND METHODS

Case selection

Seven frozen and 13 formalin-fixed paraffin-embedded (FFPE) UAs were retrieved from the pathology files of Memorial Sloan Kettering Cancer Center (MSKCC), New York, NY and the Cleveland Clinic, Cleveland, OH and centrally reviewed by a pathologist with expertise

in gynecological pathology (RAS) following the WHO criteria [2](Supplementary Methods). Cases were graded following the criteria outlined in the Supplementary Methods. The study was approved by the Institutional Review Board (IRB) from the respective authors' institutions. Consent was obtained according to the IRB-approved protocols.

Exome and targeted capture MPS

Tumour and germline DNA from six frozen UAs and from 13 FFPE UAs were subjected to whole-exome sequencing (WES)[10] and targeted capture MPS using the Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) platform [11], respectively (Table 1). WES and targeted MPS were performed at the MSKCC Integrated Genomics Operation (IGO) following validated protocols [10–12](Supplementary Methods).

Massively parallel RNA-sequencing

Six frozen UAs for which sufficient RNA was available were subjected to RNA-sequencing using a validated protocol employed at the MSKCC IGO [10](Supplementary Methods).

Additional molecular methods

Details of the reverse transcription PCR (RT-PCR)[12], fluorescence *in situ* hybridization (FISH) with *NCOA2* and *NCOA3* break-apart probes [13, 14], quantitative PCR (qPCR) for gene copy number analysis of *TERT* and *MDM2*, laser capture microdissection and mitochondrial DNA D-loop region (GAMDDL) analysis [15] are available in the Supplementary Methods.

RESULTS AND DISCUSSION

Histopathological review of the 20 UAs revealed that 14 (70%), four (20%) and two (10%) cases were of low-, intermediate- and high-grade, respectively (Table 1). All high-grade (2/2), 75% (3/4) of intermediate-grade and 7% (1/14) of low-grade UAs displayed SO. Three samples had heterologous elements, two displaying rhabdomyoblastic and one osseous differentiation, and two cases had sex cord-like features (Figure 1, Table 1).

UAs are a molecularly heterogeneous group of tumours

WES and MSK-IMPACT yielded a median depth of coverage of 246x (range 268–402x) and 453x (range 163–1624x), respectively (Supplementary Table S1). Somatic mutation analysis revealed that UAs are heterogeneous, with a median of 32.5 non-synonymous mutations (range 9–75) in the whole-exome, and a median of 1.5 non-synonymous mutations (range 0–4) in MSK-IMPACT (Supplementary Table S2). RNA-sequencing and Sanger sequencing resulted in the validation of 99.9% of the somatic mutations tested (Supplementary Figure S2, Supplementary Table S2). Only three genes, *FGFR2, KMT2C* and *DICER1*, were found to be recurrently mutated, all in 10.5% (2/19) of case (Figure 2A; Supplementary Table S2). Interestingly, both cases with *DICER1* hotspot mutations displayed rhabdomyoblastic differentiation. Although these cases were re-reviewed by pathologists (RAS, JSR-F) blinded to the results of the sequencing analysis, who favoured a diagnosis of UA with rhabdomyoblastic differentiation, excluding a diagnosis of uterine rhabdomyosarcoma is remarkably challenging. Further studies to investigate whether the mesenchymal

heterologous elements may be associated with, or underpinned by, specific somatic genetic alterations are warranted. No associations between SO, present in 6 cases, and specific genetic alterations were found. Likely pathogenic mutations in *bona fide* cancer genes such as a *FGFR2* N549K hotspot mutation, a *SMARCB1* mutation coupled with loss of heterozygosity (LOH) of the wild-type allele, and a *TP53* truncating mutation coupled with LOH of the wild-type allele (Figure 2A, Supplementary Table S2) were identified. PI3K/AKT/mTOR pathway and *ATRX* mutations, which were previously reported in >70% and 17% of UAs[5], respectively, were found in only 26% and 5% of the cases studied here.

CNA analysis revealed amplifications of *MDM2*/*CDK4*/*HMGA2* (12q14.1-15) and *MYBL1* (8q13.1) in 26% (5/19) and 5% (1/19) of UAs, respectively, in agreement with Howitt *et al.* [5]. In addition, we identified a previously unreported recurrent *TERT* (5p15.33) gene amplification in 21% (4/19) of UAs (Figures 2A–B). Amplification of *TERT* and *MDM2*/*CDK4*/*HMGA2* were validated using qPCR and FISH (Figure 2C, Supplementary Figures S1, S3).

To determine whether a highly recurrent fusion gene would drive UAs, we subjected six UAs to RNA-sequencing. deFuse [16] and ChimeraScan [17] revealed a total of 26 and 46 in-frame fusion transcripts, respectively, including eight unique fusion transcripts identified by both algorithms (Supplementary Table S3). Fusion transcripts with known associated functions and/or with intact functional domains were prioritized for further investigation. Two expressed and RT-PCR-validated in-frame fusion transcripts involved the promoter region and the first three exons of *ESR1* and the last ten exons of either *NCOA2* or *NCOA3*. In both fusion genes, the nuclear receptor coactivator domains of NCOA2 and NCOA3 were preserved (Figure 2D). *NCOA2* and *NCOA3* gene rearrangements, which have been documented in other cancers [18, 19], were confirmed using break-apart FISH probes (Figure 2D, Supplementary Table S4). These fusion genes may provide a functional link between the pathogenesis of UAs and oestrogen signaling; it should be noted, however, that these fusion genes were restricted to their respective index cases.

These findings demonstrate that UAs are molecularly heterogeneous and unlikely to be driven by a highly recurrent expressed in-frame fusion gene. Their repertoire of somatic genetic alterations includes recurrent amplifications of *MDM2*/*CDK4*/*HMGA2* and *TERT* and few recurrently mutated genes, which may vary according to the histological features of the sarcomatous component.

The mesenchymal but not the epithelial components of UAs harbour clonal somatic genetic alterations

Given that UAs are classified by the WHO as mixed epithelial-mesenchymal tumours [2], we hypothesized that, similar to breast fibroepithelial tumours, the somatic alterations would be restricted to the mesenchymal component and absent in the epithelium. To test this hypothesis, three samples harbouring well-defined mesenchymal and epithelial components were subjected to laser capture microdissection. DNA was extracted from each component separately (Figure 3A). Two truncal somatic mutations identified by MPS in each case were selected and tested in the different components using Sanger sequencing, all of which were found only in the respective mesenchymal component (Figure 3A). Consistent with these

findings, FISH analyses demonstrated that *TERT* and *MDM2* amplifications, as well as *NCOA2* and *NCOA3* rearrangements were found exclusively in the mesenchymal component of cases harbouring these alterations (Figures 2C, 3B). We next sought to define whether the two components were clonally-related by means of GAMDDL sequencing. This analysis revealed that sequences of the mitochondrial-DNA D-loop regions isolated from the epithelial elements consistently differed from the sequences obtained from the mesenchymal component (Figure 3C), supporting the contention that the two components are unlikely to be clonally-related. Our findings provide evidence that UAs are mesenchymal neoplasms and that the epithelium is independent and clonally unrelated to the mesenchymal component. The presence of the non-neoplastic epithelium may be the result of the almost invariable polypoid growth of UAs. One could hypothesize that the neoplastic sarcomatous component arises within the endometrial stromal compartment, and as the stromal component grows it may eventually result in entrapment of non-neoplastic endometrium. With tumour progression, interactions with the mesenchymal neoplastic component may promote the expansion of the non-neoplastic endometrium, and the non-neoplastic endometrium, itself, may also elicit factors that stabilize the relationship between the epithelium and the neoplastic mesenchymal component or even support the growth of the latter.

UAs and carcinosarcomas display distinct landscapes of somatic mutations

The mixed epithelial-mesenchymal tumours defined by the WHO include adenofibromas, UAs and carcinosarcomas. A comparison of the repertoire of somatic genetic alterations found in carcinosarcomas [20] and UAs revealed that nearly all recurrently mutated genes in carcinosarcomas were found not to be affected by mutations in this cohort of UAs (Supplementary Table S5). Furthermore, *TP53* (73%), *PIK3CA* (41%) and *PIK3R1* (41%), the most frequently mutated genes in carcinosarcomas [20], were significantly less frequently mutated in UAs (5%, 5% and 5%, respectively, $p<0.05$, Fisher's exact test). These findings are consistent with the notion that UAs and carcinosarcomas are unlikely to be closely related lesions.

Our study, albeit limited by its relatively small sample size, demonstrates that UAs are a genetically heterogeneous group of lesions, where clonal genetic alterations are found in the sarcomatous but not in the epithelial components, supporting the contention that UAs are mesenchymal neoplasms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

SP is funded by a Susan G Komen Postdoctoral Fellowship Grant (PDF14298348), AMS by a stipend from the German Cancer Aid (Dr. Mildred Scheel Stiftung) and GSM by CAPES (#BEX 5714/14-1). Research reported in this publication was supported in part by a Cancer Center Support Grant of the National Institutes of Health/ National Cancer Institute (Grant No. P30CA008748). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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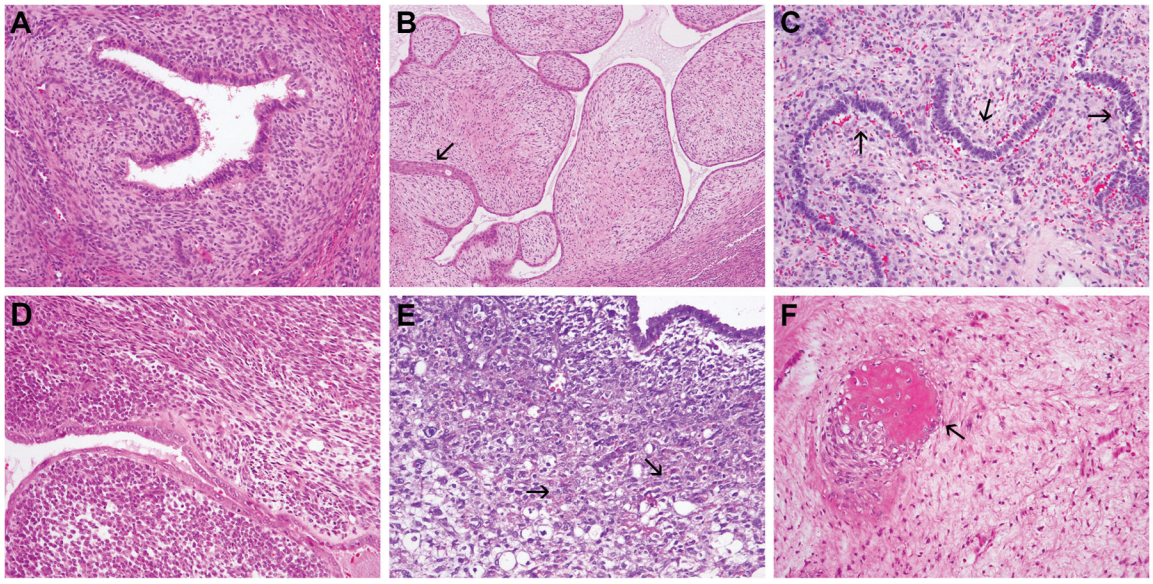

Figure 1. Histological features of uterine adenosarcoma

A, Low-grade uterine adenosarcoma (case BAS10); **B**, Low-grade uterine adenosarcoma with squamous differentiation (arrow; case BAS7); **C**, Low-grade uterine adenosarcoma with sex cord-like features (arrows; case AS7); **D**, Intermediate-grade uterine adenosarcoma with stromal overgrowth (case BAS9); **E**, High-grade uterine adenosarcoma with stromal overgrowth and heterologous rhabdomyoblastic differentiation (arrows; case AS3); **F**, Intermediate-grade uterine adenosarcoma with heterologous osseous differentiation (arrow; case AS6). Representative haematoxylin and eosin-stained sections are shown. Magnification A, C-F 100X, B 40X.

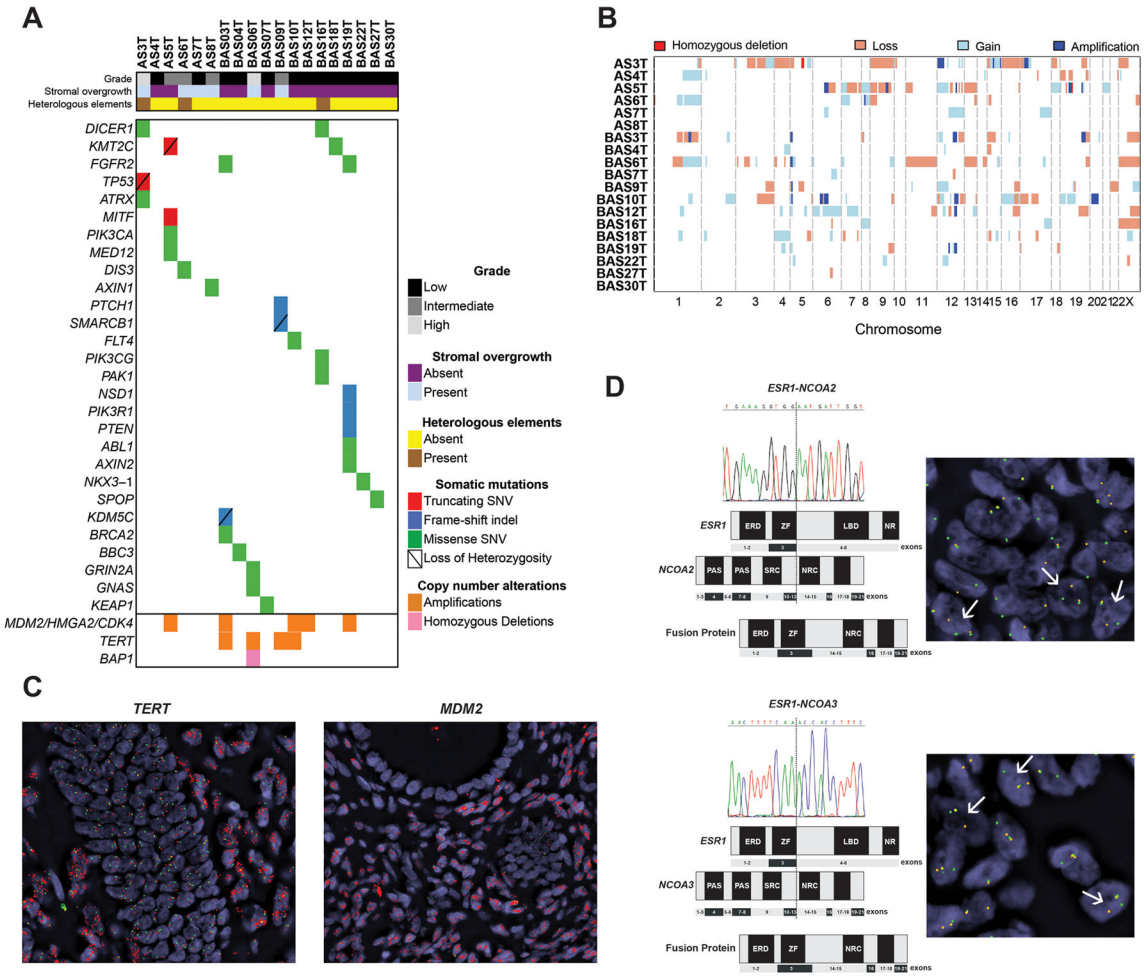

Figure 2. Somatic mutations, copy number alterations and expressed fusion genes in uterine adenosarcomas

A, Non-synonymous somatic mutations, amplifications and homozygous deletions identified in the uterine adenosarcomas analyzed. Each column represents one sample; altered genes are reported in rows. Only the 341 genes present on our smallest targeted panel are included. Alteration types are colour-coded according to the legend. SNV, single nucleotide variant; Indel, small insertion/deletion. **B,** Copy number profiles of uterine adenosarcomas. Samples are represented in rows, chromosomes are represented along the x-axis. Dark red: homozygous deletion; orange: copy number loss; white: copy number neutral; light blue: copy number gain; dark blue: amplification. **C,** Representative micrographs of amplified *TERT* (left panel) and *MDM2* (right panel) using fluorescent *in situ* hybridization (FISH). FISH analysis using two-colour probes for *MDM2* and *hTERT* full-length sequence (red) and internal control (green); **D,** Schematic representation of *ESR1-NCOA2* (upper panel) and *ESR1-NCOA3* (lower panel) fusion genes in the index cases. Both rearrangements were validated using RT-PCR and FISH using break apart probes. FISH analysis using two-color break-apart probes for *NCOA2* and *NCOA3*, with 5′ *NCOA2* and *NCOA3* green, 3′ *NCOA2* and *NCOA3* orange. Split signals are indicated by white arrows. ERD, estrogen

receptor domain; LBD, ligand-binding domain; NR, nuclear receptor; NRC, nuclear receptor coactivator; SRC, steroid receptor coactivator; ZF, zinc finger.

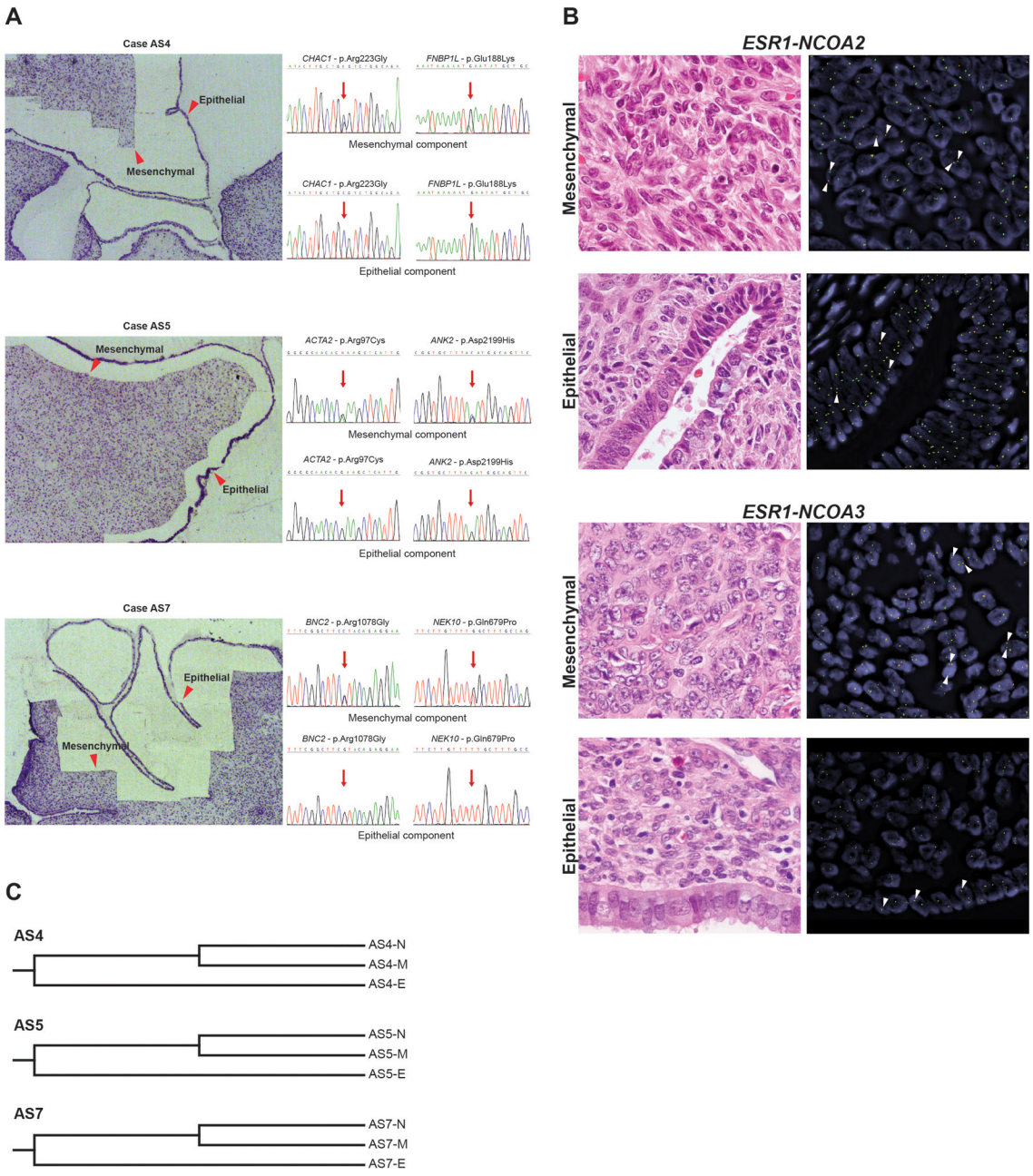

Figure 3. The presence of somatic genetic alterations in the mesenchymal component but not in the epithelial component of uterine adenosarcomas

A, Representative micrographs of negative microdissection, and the associated Sanger sequencing traces for selected mutations in the separately microdissected mesenchymal and epithelial components of three uterine adenosarcomas. The regions from which DNA was extracted are indicated by red arrows. Note that the mutations were restricted to the mesenchymal components in each case. **B**, Representative micrographs (haematoxylin and eosin left, fluorescence *in situ* hybridization (FISH), right) of the mesenchymal and epithelial components of uterine adenosarcomas AS8 (top) and AS4 (bottom). The presence or absence of the rearrangements using break-apart probes are indicated by white arrows.

FISH analysis using two-colour break-apart probes for *NCOA2* and *NCOA3*, with 5′ *NCOA2* and *NCOA3* green, 3′ *NCOA2* and *NCOA3* orange. Note that *NCOA* rearrangements are restricted to the mesenchymal components in both cases. **C**, Phylogenetic clustering of mitochondrial DNA D-loop regions in mesenchymal and epithelial components, and matched normal tissue of 3 cases (AS4, AS5 and AS7). The relative phylogenetic distances between matched normal tissue (N), mesenchymal component (M) and epithelial component (E) were determined using the neighbour-joining method.

Table 1

Histological characteristics of the uterine adenosarcomas included in this study, and sequencing analyses performed

Case ID	Grade	Heterologous element	Stromal overgrowth	Tissue type	Whole-exome MPS	Targeted capture MPS	RNA-Sequencing
AS1	Low	Absent	Absent	Frozen			Yes
AS3	High	Present (rhabdomyoblastic differentiation)	Present	Frozen	Yes		Yes
AS4	Low	Absent	Absent	Frozen	Yes		Yes
AS5	Intermediate	Absent	Absent	Frozen	Yes		Yes
AS6	Intermediate	Present (osseous differentiation)	Present	Frozen	Yes		
AS7	Low	Absent (sex cord-like features)	Present	Frozen	Yes		Yes
AS8	Intermediate	Absent (sex cord-like features)	Present	Frozen	Yes		Yes
BAS03	Low	Absent	Absent	FFPE		Yes	
BAS04	Low	Absent	Absent	FFPE		Yes	
BAS06	High	Absent	Present	FFPE		Yes	
BAS07	Low	Absent	Absent	FFPE		Yes	
BAS09	Intermediate	Absent	Present	FFPE		Yes	
BAS10	Low	Absent	Absent	FFPE		Yes	
BAS12	Low	Absent	Absent	FFPE		Yes	
BAS16	Low	Present (rhabdomyoblastic differentiation)	Absent	FFPE		Yes	
BAS18	Low	Absent	Absent	FFPE		Yes	
BAS19	Low	Absent	Absent	FFPE		Yes	
BAS22	Low	Absent	Absent	FFPE		Yes	
BAS27	Low	Absent	Absent	FFPE		Yes	
BAS30	Low	Absent	Absent	FFPE		Yes	

FFPE, formalin-fixed, paraffin-embedded; MPS, massively parallel sequencing.