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The *FOXA2* transcription factor is frequently somatically mutated in uterine carcinosarcomas and carcinomas

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Abstract

Background—Uterine carcinosarcomas (UCSs) are a rare but clinically aggressive form of cancer. They are biphasic tumors consisting of both epithelial and sarcomatous components. Most uterine carcinosarcomas are clonal, with the carcinomatous cells undergoing metaplasia to give rise to the sarcomatous component. The objective of this study was to identify novel somatically mutated genes in UCSs.

Methods—We whole exome sequenced paired tumor and non-tumor DNAs from 14 UCSs and orthogonally validated 464 somatic variants using Sanger sequencing. Fifteen genes that were somatically mutated in at least two tumor exomes were Sanger sequenced in another 39 primary UCSs.

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Author Contributions: D.W.B., designed and directed the study, and wrote the first draft of the manuscript. M.L.R., M.E.U., and P.J.G. provided critical comments and edits on the manuscript. D.G.M. and P.J.G., contributed clinical specimens and clinicopathological interpretation. M.J.M., performed histologic review and determined neoplastic cellularity of tumor specimens obtained from CHTN. M.L.R., isolated DNA and RNA samples provided by CHTN, performed identity testing, and microsatellite instability analysis. NISC performed library construction and exome sequencing. NISC and N.F.H. performed variant calling in exomes. M.L.G., M.L.R., and M.E.U., curated and orthogonally validated exome sequencing data. M.L.G., M.L.R., M.E.U., and D.W.B. designed, performed, analyzed, and/or interpreted the mutation discovery and prevalence screens. M.E.U., isolated protein and DNA for unmatched primary uterine carcinosarcomas, performed immunoblotting, and cell culture. P.J.G. performed statistical analysis.

Results—Overall, among 53 UCSs in this study, the most frequently mutated of these 15 genes were *TP53* (75.5%), *PIK3CA* (34.0%), *PPP2R1A* (18.9%), *FBXW7* (18.9%), *CHD4* (17.0%), and *FOXA2* (15.1%). *FOXA2* has not previously been implicated in UCSs and was predominated by frameshift and nonsense mutations. One UCS with a *FOXA2* frameshift mutation expressed truncated FOXA2 protein by immunoblotting. Sequencing of *FOXA2* in 160 primary ECs revealed somatic mutations in 5.7% of serous, 22.7% of clear cell, 9% of endometrioid and 11.1% of mixed ECs, most of which were frameshift mutations.

Conclusions—Collectively, our findings provide compelling genetic evidence that *FOXA2* is a pathogenic driver gene in the etiology of primary uterine cancers including UCSs.

Keywords

Endometrial cancer; endometrial carcinoma; mutation; exome; uterine cancer; carcinosarcoma

Introduction

Uterine carcinosarcomas (UCSs), also referred to as malignant mixed Müllerian tumors of the uterus, are rare biphasic tumors consisting of both carcinomatous and sarcomatous elements. UCSs have an aggressive clinical course; one study reported 5-year relative survival rates of 53.7% at all AJCC (American Joint Committee on Cancer) stages, 73.7% at stage I, 43.3% at stage II, 26.2% at stage III, and 13.6% at stage IV [1]. Although early studies reported that the carcinomatous component determines tumor aggressiveness [2, 3], a recent meta-analysis of 906 UCS cases found that both the carcinoma and sarcoma components can influence prognosis [4].

Molecular studies that analyzed the carcinoma and sarcoma components of individual tumors separately, found evidence for shared alterations between each component in a majority of cases [5–10]. Thus, most UCSs are believed to be monoclonal in origin, resulting from a metaplastic transition of carcinoma cells to sarcoma cells [11]. The carcinoma component is often of serous, clear cell, undifferentiated, high grade endometrioid, or mixed histology; lower-grade endometrioid carcinomas are also observed although they are less common [4]. The sarcoma component can be of uterine ("homologous") or extra-uterine ("heterologous") origin including rhabdomyosarcoma, chondrosarcoma, or osteosarcoma [12].

Targeted gene sequencing and whole exome sequencing of UCSs have uncovered frequent somatic mutations in genes involved in the DNA damage response (*TP53*, *MLH1*), the PI3-kinase pathway (*PIK3CA*, *PTEN*, *PIK3R1*), chromatin remodeling (*ARID1A*, *MLL3*/*KMT2C*, *ARID1B*, *BAZ1A*), ubiquitin-mediated protein degradation (*FBXW7*, *SPOP*), signal transduction (*KRAS*, *NRAS*, *JAK2*, *ERBB2*), WNT signaling (*CTNNB1*) and protein dephosphorylation (*PPP2R1A*) [5, 10, 13–21]. Microsatellite instability (MSI) and loss of mismatch repair (MMR) protein expression, which are indicative of mismatch repair defects, have also been observed (reviewed in [22]). The recent integrated genomic analysis of 57 UCSs by The Cancer Genome Atlas (TCGA) further implicated the mutational disruption of *ARHGAP35*, *RB1*, *U2AF1*, and *ZBTB7B* in the molecular pathogenesis of UCS [8].

The objective of this study was to identify novel somatically mutated genes in UCSs. Here, we whole exome sequenced 14 primary UCSs and paired normal DNAs. Follow-up Sanger sequencing of 15 genes that were mutated in at least two discovery screen tumors was performed in another 39 UCSs. Our finding that *FOXA2* is somatically mutated in 15.1% of UCSs and is predominated by frameshift and nonsense mutations, strongly implicates *FOXA2* as a novel pathogenic driver gene in UCSs. We also report frequent *FOXA2* mutations in other histological subtypes of EC, further supporting *FOXA2* as a driver of uterine carcinomas.

Materials and Methods

Clinical specimens

The NIH Office of Human Subjects Research determined that this research was not "human subjects research" per the Common Rule (45 CFR 46), and therefore that no IRB review was required for whole exome sequencing or Sanger sequencing of these samples. Anonymized, matched tumor and non-tumor (normal) samples sequenced in the discovery and prevalence screen were obtained as fresh frozen tissues from the Co-Operative Human Tissue Network (CHTN), which is funded by NCI (Supplementary Table 1). Genomic DNA for 38 UCSs from Washington University in St. Louis (Supplementary Table 2) was also included in the prevalence screen. Endometrial carcinoma (EC) DNAs included in this study were previously isolated from 53 serous ECs, 22 clear cell ECs, 67 endometrioid ECs, and 18 mixed histology ECs [23–25]. Clinical follow-up information is not available for the 15 UCSs obtained from CHTN or for the 160 ECs in this study.

Estimation of neoplastic cellularity and genomic DNA extraction

An H&E section prepared for each frozen tumor specimen received from CHTN was evaluated by a pathologist (M.J.M) to assess histotype and demark regions of greater than 70% neoplastic cellularity for subsequent DNA isolation (Puregene DNA extraction kit, Qiagen) (Supplementary Table 1). Prior to whole exome sequencing, 14 paired tumor-normal DNAs were further purified by phenol-chloroform extraction. For Washington University cases, a gynecologic pathologist performed histologic review of the diagnostic slides from formalin-fixed paraffin embedded tumor tissue, as well as sections prepared from the fresh-frozen tumor tissue used for DNA isolation (Supplementary Table 2). Identity testing was performed on all tumor-normal DNA pairs using the Coriell Identity Mapping kit (Coriell) according to the manufacturer's instructions.

Whole exome capture and sequencing

Sequencing libraries of matched tumor and normal DNAs were generated using the Illumina TruSeqV2:28 capture kit (Supplementary Table 3). Enriched libraries were sequenced with the Illumina HiSeq 2000 platform until at least 85% of targeted bases had adequate coverage to produce a Most Probable Genotype (MPG) score of 10 [26]. The average depth of coverage for individual exomes is provided (Supplementary Table 3).

Read mapping, variant calling, and variant filtering

Short sequence reads were aligned to the UCSC human reference "hg19" using novoalign version 2.08.02, and genotypes were called using the program bam2mpg with the –qual_filter 20 option [26]. VarSifter [27] was used to identify coding and splice somatic variants with an MPG score ≥10 in the normal exome, an MPV score ≥10 in the tumor exome, and at least 5 reads covering the site in each sample. SNVs detected in any normal sample, probable false-positive variants consistent with sequencing artifacts or misaligned reads as determined by manual data curation, and variants annotated as non-clinical SNVs in dbSNP v135 were filtered out.

Primer design, PCR amplification and Sanger sequencing

Primer sequences and PCR conditions are available on request. Sanger sequencing and analysis were performed as previously described [25]. In the mutation prevalence screen, variant calling was restricted to exonic sequence; splice donor and acceptor sequences were not assessed. Exonic variants were discriminated as somatic or germline by sequencing independent PCR products generated from relevant tumor and matched normal DNAs.

Microsatellite instability analysis

Microsatellite instability (MSI) analysis of tumor-normal DNA pairs was performed using the Promega MSI Analysis System v 1.2, according to the manufacturer's instructions. MSI-high tumor genotypes (≥2 unstable mononucleotide markers) are reported here as MSI+. MSI-low (one unstable mononucleotide marker) and MSI-negative (no unstable mononucleotide markers) tumor genotypes are reported here as microsatellite stable (MSS).

Cell lines

The RL-95-2, HEC1A, HEC1B, KLE and AN3CA endometrial cancer (EC) cell lines were obtained from the American Type Culture Collection or the National Cancer Institute Developmental Therapeutics Program cell line repository. MFE-280 and MFE-296 were purchased from Sigma-Aldrich. HEC-59 was purchased from AddexBio. Dr. Alessandro A. Santin kindly provided ARK1, ARK2, ARK4, and ARK6 serous EC cell lines, Dr. Bo Rueda kindly provided the SK-UT-2 EC cell line, and Dr. John J. Risinger kindly provided the NCI-EC2 clear cell EC cell line.

Protein extraction, quantification and immunoblotting

Protein was extracted from seven primary UCSs (UCS1-7) using a protocol modified from Peña-Llopis and Brugarolas [28]. Briefly, tissue was homogenized in 10 volumes of RIPA buffer (Thermo Fisher Scientific) containing complete tablet protease inhibitors (Roche), 0.1mM NaVO4, and 1mM NaF (New England BioLabs). Lysate was centrifuged twice through a QIAshredder column (Qiagen), then cleared using centrifugation. EC cells were lysed in RIPA buffer (Thermo Fisher Scientific) containing complete tablet protease inhibitors (Roche), 0.1mM NaVO4, and 1mM NaF (New England Biolabs) 30 min on ice, then cleared using centrifugation. Protein was quantified using the Quick Start Bradford reagent (Bio-Rad) and equal amounts of total protein were denatured in LDS sample buffer (Thermo Fisher Scientific) at 80°C prior to SDS-PAGE and wet transfer to PVDF

membranes (Bio-Rad). Primary and HRP-conjugated secondary antibodies were: anti-FOXA2 (Cell Signaling #8186), β-actin (Sigma-Aldrich, A2228), goat anti-rabbit HRP (Cell Signaling) and goat anti-mouse HRP (Cell Signaling).

DNA and RNA extraction from frozen tissue sections

The following extractions were completed according to the manufacturers' protocols. DNA was extracted from frozen sections that corresponded to regions targeted for protein extraction using The Pinpoint Slide DNA Isolation System (Zymo Research). For tumor UCS-5, RNA was extracted with TRIzol® Reagent (Thermo Fisher Scientific) pipetted directly onto the frozen section corresponding to the region used for DNA and protein extraction. RNA was converted to cDNA using the Superscript III First strand synthesis kit (Thermo Fisher Scientific).

Accession code

Whole exome sequencing data have been deposited into dbGAP, under accession code phs001153.v1.p1.

Results

Mutation discovery screen by whole exome sequencing

Whole exome sequencing of 14 primary UCS tumor-normal pairs achieved an average depth of coverage of 77× for aligned reads. On average, 89% of targeted bases had sufficient coverage and quality for variant calling (Supplementary Table 3). After data filtering, we identified 882 exonic (614 nonsynonymous and 268 synonymous) and 21 splice-junction somatic variant calls among the 14 tumors (Supplementary Table 4). We could orthogonally assess 519 of the nonsynonymous and splice junction variants by Sanger sequencing; 89.4% (464 of 519) of assessed variants orthogonally validated as *bona fide* somatic mutations (448 nonsynonymous, and 16 splice-junction mutations) (Supplementary Table 4). There was a mean of 33 validated nonsynonymous/splice junction somatic mutations per tumor (range 13–71 mutations/tumor); validated mutations were distributed among 426 protein-encoding genes. Seventeen protein-encoding genes, including six consensus cancer genes, were somatically mutated in two or more UCS exomes (Supplementary Table 4). Two of the 17 genes (*TTN* and *DNAH2*) had relatively large open reading frames and were not analyzed further; the other 15 genes were Sanger sequenced in a mutation prevalence screen.

Mutation prevalence screen

We Sanger sequenced the coding exons of *TP53*, *FBXW7*, *PIK3CA*, *PPP2R1A*, *CHD4*, *RPS6KA3*, *FOXA2*, *INSR*, *KLF5*, *TAF1*, *PAPL*, *PLXNC1*, *C2CD2*, *ABCC9*, and *STAG2* in another 39 primary UCSs. *TP53*, *PPP2R1A*, and *PIK3CA* were previously sequenced by targeted next-generation sequencing in 36 of 39 prevalence screen tumors as reported by McConechy et al [13]. Given the high frequency of frameshift *FOXA2* mutations in the prevalence screen tumors (described below), and the known challenges in calling insertions and deletions by next generation sequencing [29], we subsequently also Sanger sequenced *FOXA2* in the 14 discovery screen tumors.

Among the 53 tumors in the combined discovery and prevalence screens, the most frequently mutated of the 15 genes were *TP53* (75.5% of UCSs somatically mutated), *PIK3CA* (34.0%), *PPP2R1A* (18.9%), *FBXW7* (18.9%), *CHD4* (17.0%), and *FOXA2* (15.1%) (Figure 1, Table 1, Supplementary Table 5, and Supplementary Figure 1). Of the six most frequently mutated genes, *FOXA2* is the only gene that has not previously been implicated in UCS. Most *FOXA2* mutations were frameshift or nonsense mutations predicted to encode truncated proteins that would disrupt one or more functional domains, including the carboxy-terminal transactivation domain (Figure 2a, and Table 1). Two missense mutations in *FOXA2* localized to the central DNA binding (Winged helix) domain (Figure 2a), which is highly conserved across proteins in the forkhead box family [30]. Both missense mutations are predicted to impact protein function by three *in silico* algorithms (Mutation Assessor, PolyPhen, and SIFT). Only one of eight *FOXA2*–mutated tumors (1241T; FOXA2-Met142Profs*8) was MSI+ and none were *MSH6*- or *POLE*-mutated (Figure 1). In terms of histology, *FOXA2* mutations were identified in UCSs for which the sequenced DNA was isolated from admixed carcinoma/sarcoma (three cases) or from predominantly carcinoma (five cases) (Supplementary Table 1 and Supplementary Table 2).

FOXA2 is somatically mutated in endometrial carcinomas (ECs)

Because most UCSs are believed to originate from ECs [11], we extended our study to Sanger sequence the coding exons of *FOXA2* from 160 primary ECs of diverse histotypes (53 serous, 22 clear cell, 67 endometrioid, 18 mixed). We uncovered somatic *FOXA2* mutations in 5.7% of serous ECs, 22.7% of clear cell ECs, 9.0% of endometrioid ECs, and 11.1% of mixed histology ECs (Table 1). Differences in the mutation frequency of *FOXA2* in serous, clear cell, and endometrioid ECs were not statistically significant (p=0.07, Chi-Square test). Five frameshift mutations in ECs and one frameshift mutation in a UCS formed a *FOXA2* mutation cluster, resulting from overlapping genomic deletions in which the first positions of the frameshift were located within amino acids 346–349 (Figure 2b). All but one of 16 *FOXA2* mutations in ECs are predicted to prematurely truncate FOXA2 (Figure 2a), and most *FOXA2*-mutated ECs (13 of 16 cases) were microsatellite stable (Supplementary Table 6) consistent with our observations in UCSs (Figure 1).

A recurrent mutation in *FOXA2* encodes a truncated protein

We next sought to evaluate FOXA2 expression in EC cell lines and in primary UCSs. We performed immunoblotting for FOXA2 on protein lysates from 14 EC cell lines and seven primary UCSs. These UCSs lacked matched normal tissue and therefore were not included in our discovery or prevalence mutation screens. Immunoblotting showed variable levels of FOXA2 expression in EC cell lines (Figure 3a and Supplementary Figure 2). Specifically, FOXA2 protein expression was readily detected in seven of 14 EC cell lines, including three of four serous EC cell lines (ARK2, ARK4, and ARK6) (Figure 3a); the single clear cell EC cell line evaluated (NCI-EC-2) expressed lower levels of FOXA2 (Supplementary Figure 2). Three of the seven primary UCSs assessed by immunoblotting had readily detectable FOXA2 protein expression (Figure 3b). Strikingly, one tumor (UCS-5) exhibited a shorter form of FOXA2 than expected (Figure 3b); sequencing all coding exons of *FOXA2* from the genomic DNA of this tumor revealed that it harbored the A346Gfs*11 variant (Figure 3c). Matched normal DNA for UCS-5 was not available for sequencing but we predict that the

A346Gfs*11 variant in this tumor is more likely to be somatic than germline because this alteration also occurred as a somatic mutation in a serous EC in our study (Table 1), and because it occurred within the recurrently mutated N-terminal region of FOXA2 (Figure 2). Although the A346Gfs*11 variant in UCS-5 appeared to be heterozygous at the genomic level, only the variant allele was detectable in cDNA generated from this tumor (Figure 3c). No *FOXA2* exonic sequence variants were detected within the genomic DNA of UCS-1, -2, -4, -6, or -7; UCS-3 was not sequenced.

Discussion

UCSs are rare gynecologic malignances that are associated with a poor clinical outcome [1–3]. Here, using a combination of whole exome sequencing and targeted Sanger sequencing, we show that the *FOXA2* (*HNF3B*) “pioneer” forkhead transcription factor, which has established roles in uterine glandular epithelium development and uterine biology [31–35], undergoes frequent somatic mutations in UCSs. The occurrence of *FOXA2* mutations in 15.1% of UCSs in our study coupled with the fact that they are predominated by frameshift mutations predicted to prematurely truncate FOXA2 strongly suggests they are pathogenic driver mutations in some UCSs. *FOXA2* has recently been proposed to be a tumor suppressor gene in endometrioid ECs[36]. Our observation of *FOXA2* mutations in 9% of endometrioid ECs corroborates the recent report of *FOXA2* mutations in 9.4% of such tumors [36]. We extend these observations to further implicate *FOXA2* as a candidate driver gene in other EC histological subtypes: within our cohort, 22.7% of clear cell, 11.1% of mixed histology and 5.7% of serous ECs harbored *FOXA2* mutations.

The FOXA2 transcription factor has three major functional domains, a N-terminal transactivation domain (residues 1–52), a central winged-helix DNA binding domain (residues 157–257), and a C-terminal transactivation domain. All somatic frameshift/nonsense mutations we uncovered in UCSs and ECs are predicted to encode truncated proteins lacking the C-terminal transactivation domain. Twenty-five-percent (4 of 20) of somatic frameshift/nonsense mutations identified in our study are predicted to also disrupt the central winged-helix DNA-binding domain, and another 35% (7 of 20) of such mutations are initiated in the N-terminal forkhead region.

The genomic DNA sequences of most *FOXA2*-mutated tumors in our study also had evidence of a wildtype allele. One UCS that exhibited wildtype and mutated *FOXA2* in genomic DNA had only the mutated allele in the cDNA and exhibited only a truncated protein by immunoblotting. These observations suggest that this tumor is either homozygous for the mutated allele with the wildtype allele in the genomic DNA being contributed by admixed normal cells that do not express the FOXA2 protein, or that the tumor is heterozygous at the genomic level but expresses FOXA2 exclusively from the mutant allele. Further studies are required to determine whether other FOXA2-mutated UCSs and ECs exclusively express the mutant allele.

TP53, *PIK3CA*, *PPP2R1A* were somatically mutated in 75.5%, 34.0%, and 18.9% of our cohort of 53 UCSs. These genes were previously sequenced by targeted next-generation sequencing in 36 UCSs included in our study but matched normal DNAs were not

evaluated [13]. By Sanger sequencing these three genes, we found 43 of 46 variants reported by McConechy et al [13] to be somatic, and we identified 9 additional somatic mutations not previously noted among the 36 UCSs (Supplementary Table 7).

Our combined whole exome sequencing and targeted gene sequencing of UCSs also revealed frequent somatic mutations in *FBXW7* and *CHD4*. The incidence of somatic *FBXW7* mutations among the UCSs in our study (18.9%) is in keeping with the frequency at which this tumor suppressor gene is mutated in other UCS cohorts (10.7% to 39%) [5, 8, 20, 21]. All *FBXW7* mutations in the UCSs within our study localized to the substrate-binding WD repeats (Supplementary Figure 1), consistent with the *FBXW7* mutation spectrum in other cancers [37, 38], including endometrial cancers[25, 39]. We first nominated *CHD4* as a candidate cancer gene based on mutational observations we made in endometrial carcinomas [25]. Our finding that 17% of UCSs had somatically mutated *CHD4*, which encodes a catalytic subunit of the NuRD chromatin remodeling complex, is in line with recent reports of *CHD4* mutations in 5.9%–18% of UCS cohorts [5, 8, 19]. Most (6 of 10) *CHD4* mutations in our study localized to the catalytic ATPase-helicase and helicase domains (Supplementary Figure 1), and two of these mutations involved arginine-975 and arginine-1162, which are two prominent pan-cancer mutation hotspots in *CHD4* [40, 41].

In conclusion, using a combination of whole exome sequencing and targeted gene sequencing we have identified a high frequency of *FOXA2* frameshift mutations in UCSs. In addition, we also find frequent *FOXA2* frameshift mutations in uterine carcinomas including serous ECs and clear cell ECs. Collectively, these findings lead us to nominate *FOXA2* as a pathogenic driver gene in some of the most clinically aggressive forms of uterine cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Ries, LAG., Young, JL., Keel, GE., Eisner, MP., Lin, YD., Horner, M-J. Patient and Tumor Characteristics. Vol. 2007. National Cancer Institute; Bethesda, MD: 2007. SEER Survival Monograph: Cancer Survival Among Adults: U.S. SEER Program, 1988–2001. SEER Program, NIH Pub. No. 07-6215
2. Silverberg SG, Major FJ, Blessing JA, et al. Carcinosarcoma (malignant mixed mesodermal tumor) of the uterus. A Gynecologic Oncology Group pathologic study of 203 cases. Int J Gynecol Pathol. 1990; 9:1–19. [PubMed: 2152890]
3. Bitterman P, Chun B, Kurman RJ. The significance of epithelial differentiation in mixed mesodermal tumors of the uterus. A clinicopathologic and immunohistochemical study. Am J Surg Pathol. 1990; 14:317–328. [PubMed: 2157343]
4. Matsuo K, Takazawa Y, Ross MS, et al. Significance of histologic pattern of carcinoma and sarcoma components on survival outcomes of uterine carcinosarcoma. Ann Oncol. 2016; 27:1257–1266. [PubMed: 27052653]

5. McConechy MK, Hoang LN, Chui MH, et al. In-depth molecular profiling of the biphasic components of uterine carcinosarcomas. J Pathol Clin Res. 2015; 1:173–185. [PubMed: 27499902]
6. Abeln EC, Smit VT, Wessels JW, de Leeuw WJ, Cornelisse CJ, Fleuren GJ. Molecular genetic evidence for the conversion hypothesis of the origin of malignant mixed mullerian tumours. J Pathol. 1997; 183:424–431. [PubMed: 9496259]
7. Taylor NP, Zighelboim I, Huettner PC, et al. DNA mismatch repair and TP53 defects are early events in uterine carcinosarcoma tumorigenesis. Mod Pathol. 2006; 19:1333–1338. [PubMed: 16810312]
8. Cherniack AD, Shen H, Walter V, et al. Integrated molecular characterization of uterine carcinosarcoma. Cancer Cell. 2017; 31:411–423. [PubMed: 28292439]
9. Jin Z, Ogata S, Tamura G, et al. Carcinosarcomas (malignant mullerian mixed tumors) of the uterus and ovary: a genetic study with special reference to histogenesis. Int J Gynecol Pathol. 2003; 22:368–373. [PubMed: 14501818]
10. de Jong RA, Nijman HW, Wijbrandi TF, Reyners AK, Boezen HM, Hollema H. Molecular markers and clinical behavior of uterine carcinosarcomas: focus on the epithelial tumor component. Mod Pathol. 2011; 24:1368–1379. [PubMed: 21572397]
11. McCluggage WG. Uterine carcinosarcomas (malignant mixed Mullerian tumors) are metaplastic carcinomas. Int J Gynecol Cancer. 2002; 12:687–690. [PubMed: 12445244]
12. El-Nashar SA, Mariani A. Uterine carcinosarcoma. Clin Obstet Gynecol. 2011; 54:292–304. [PubMed: 21508698]
13. McConechy MK, Ding J, Cheang MC, et al. Use of mutation profiles to refine the classification of endometrial carcinomas. J Pathol. 2012; 228:20–30. [PubMed: 22653804]
14. Growdon WB, Roussel BN, Scialabba VL, et al. Tissue-specific signatures of activating PIK3CA and RAS mutations in carcinosarcomas of gynecologic origin. Gynecol Oncol. 2011; 121:212–217. [PubMed: 21168197]
15. Biscuola M, Van de Vijver K, Castilla MA, et al. Oncogene alterations in endometrial carcinosarcomas. Human Pathol. 2013; 44:852–859. [PubMed: 23199529]
16. Murray S, Linardou H, Mountzios G, et al. Low frequency of somatic mutations in uterine sarcomas: a molecular analysis and review of the literature. Mutat Res. 2010; 686:68–73. [PubMed: 20122944]
17. Cheung LW, Hennessy BT, Li J, et al. High frequency of PIK3R1 and PIK3R2 mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability. Cancer Discov. 2011; 1:170–185. [PubMed: 21984976]
18. Bashir S, Jiang G, Joshi A, et al. Molecular alterations of PIK3CA in uterine carcinosarcoma, clear cell, and serous tumors. Int J Gynecol Cancer. 2014; 24:1262–7. [PubMed: 25078343]
19. Jones S, Stransky N, McCord CL, et al. Genomic analyses of gynaecologic carcinosarcomas reveal frequent mutations in chromatin remodelling genes. Nat Commun. 2014; 5:5006. [PubMed: 25233892]
20. Hembree TN, Teer JK, Hakam A, Chiappori AA. Genetic investigation of uterine carcinosarcoma: Case report and cohort analysis. Cancer Control. 2016; 23:61–66. [PubMed: 27009459]
21. Zhao S, Santin AD. Mutational landscape of uterine and ovarian carcinosarcomas implicates histone genes in epithelial–mesenchymal transition. Proc Natl Acad Sci U S A. 2016; 113:12238–12243. [PubMed: 27791010]
22. Hoang LN, Ali RH, Lau S, Gilks CB, Lee CH. Immunohistochemical survey of mismatch repair protein expression in uterine sarcomas and carcinosarcomas. Int J Gynecol Pathol. 2014; 33:483–491. [PubMed: 25083964]
23. Rudd ML, Price JC, Fogoros S, et al. A unique spectrum of somatic PIK3CA (p110alpha) mutations within primary endometrial carcinomas. Clin Cancer Res. 2011; 17:1331–1340. [PubMed: 21266528]
24. Urick ME, Rudd ML, Godwin AK, Sgroi D, Merino M, Bell DW. PIK3R1 (p85alpha) is somatically mutated at high frequency in primary endometrial cancer. Cancer Res. 2011; 71:4061–4067. [PubMed: 21478295]

25. Le Gallo M, O'Hara AJ, Rudd ML, et al. Exome sequencing of serous endometrial tumors identifies recurrent somatic mutations in chromatin-remodeling and ubiquitin ligase complex genes. Nat Genet. 2012; 44:1310–1315. [PubMed: 23104009]
26. Teer JK, Bonnycastle LL, Chines PS, et al. Systematic comparison of three genomic enrichment methods for massively parallel DNA sequencing. Genome Res. 2010; 20:1420–1431. [PubMed: 20810667]
27. Teer JK, Green ED, Mullikin JC, Biesecker LG. VarSifter: visualizing and analyzing exome-scale sequence variation data on a desktop computer. Bioinformatics. 2012; 28:599–600. [PubMed: 22210868]
28. Pena-Llopis S, Brugarolas J. Simultaneous isolation of high-quality DNA, RNA, miRNA and proteins from tissues for genomic applications. Nat Protoc. 2013; 8:2240–2255. [PubMed: 24136348]
29. Zighelboim I, Mutch DG, Knapp A, et al. High frequency strand slippage mutations in CTCF in MSI-positive endometrial cancers. Hum Mutat. 2014; 35:63–65. [PubMed: 24130125]
30. Myatt SS, Lam EW. The emerging roles of forkhead box (Fox) proteins in cancer. Nat Rev Cancer. 2007; 7:847–859. [PubMed: 17943136]
31. Bazer FW. Uterine adenogenesis and pregnancy: multiple roles for Foxa2 in mice. Biol Reprod. 2010; 83:319–321. [PubMed: 20574051]
32. Filant J, Lydon JP, Spencer TE. Integrated chromatin immunoprecipitation sequencing and microarray analysis identifies FOXA2 target genes in the glands of the mouse uterus. FASEB J. 2014; 28:230–243. [PubMed: 24025729]
33. Jeong JW, Kwak I, Lee KY, et al. Foxa2 is essential for mouse endometrial gland development and fertility. Biol Reprod. 2010; 83:396–403. [PubMed: 20484741]
34. Kelleher AM, Peng W, Pru JK, Pru CA, DeMayo FJ, Spencer TE. Forkhead box a2 (FOXA2) is essential for uterine function and fertility. Proc Natl Acad Sci U S A. 2017; 11:E1018–E1026.
35. Yamagami K, Yamauchi N, Kubota K, et al. Expression and regulation of Foxa2 in the rat uterus during early pregnancy. J Reprod Dev. 2014; 60:468–475. [PubMed: 25262775]
36. Smith B, Neff R, Cohn DE, et al. The mutational spectrum of FOXA2 in endometrioid endometrial cancer points to a tumor suppressor role. Gynecol Oncol. 2016; 143:398–405. [PubMed: 27538367]
37. Moberg KH, Bell DW, Wahrer DC, Haber DA, Hariharan IK. Archipelago regulates Cyclin E levels in Drosophila and is mutated in human cancer cell lines. Nature. 2001; 413:311–316. [PubMed: 11565033]
38. Akhoondi S, Sun D, von der Lehr N, et al. FBXW7/hCDC4 is a general tumor suppressor in human cancer. Cancer Res. 2007; 67:9006–9012. [PubMed: 17909001]
39. Kandoth C, Schultz N, Cherniack AD, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013; 497:67–73. [PubMed: 23636398]
40. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012; 2:401–404. [PubMed: 22588877]
41. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013; 6:pl1. [PubMed: 23550210]

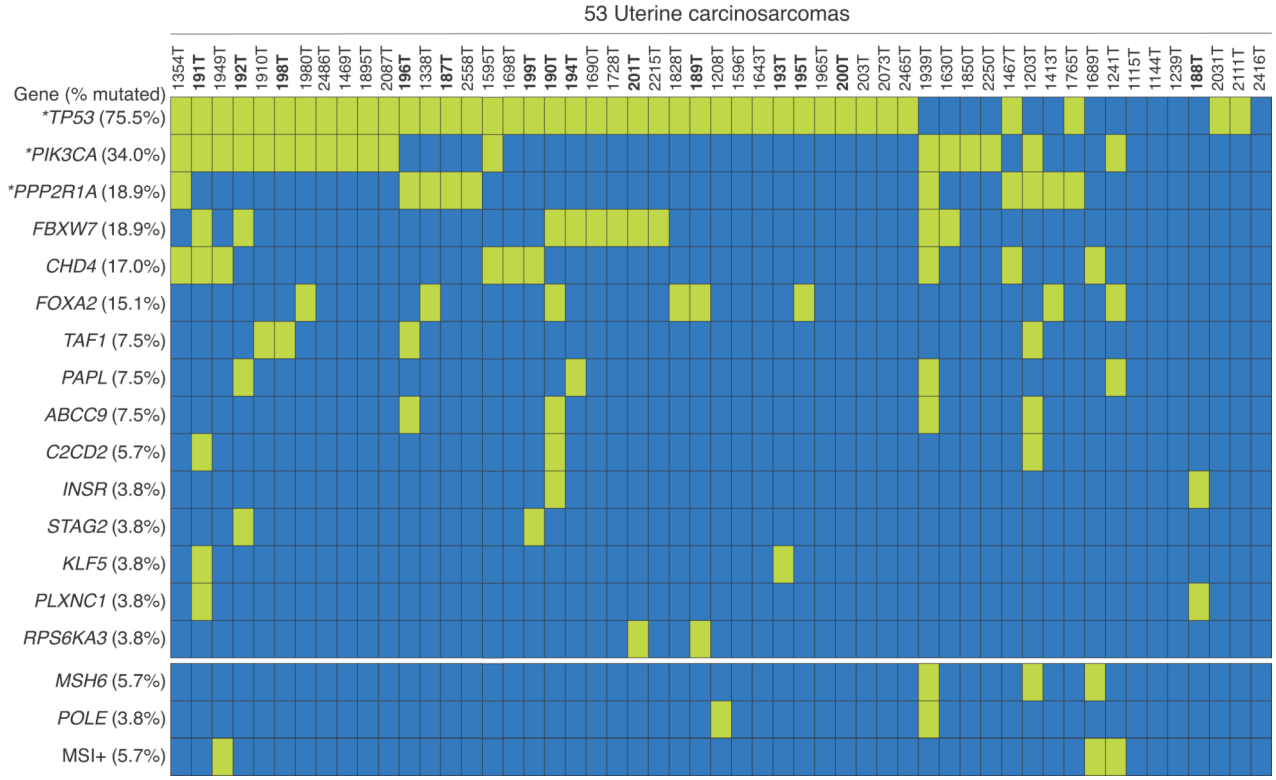

Figure 1. Oncoprint displaying the occurrence of somatic mutations in 15 prevalence screen genes and the MSI, *MSH6* and *POLE* status, of 53 primary UCSs forming the discovery and prevalence screens

The frequency (% of altered cases) of each aberration is shown (*left*). Coded tumor numbers are shown at top. Somatically mutated tumors are shown by the green bars; dark blue bars indicate that no somatic mutation was detected. Discovery screen tumors are indicated in bold. (*) Mutations in *TP53*, *PPP2R1A* and *PIK3CA* among 36 of 39 prevalence screen tumors were detected here by Sanger sequencing and previously detected in these tumors by targeted next generation sequencing (Supplementary Table 7) [13].

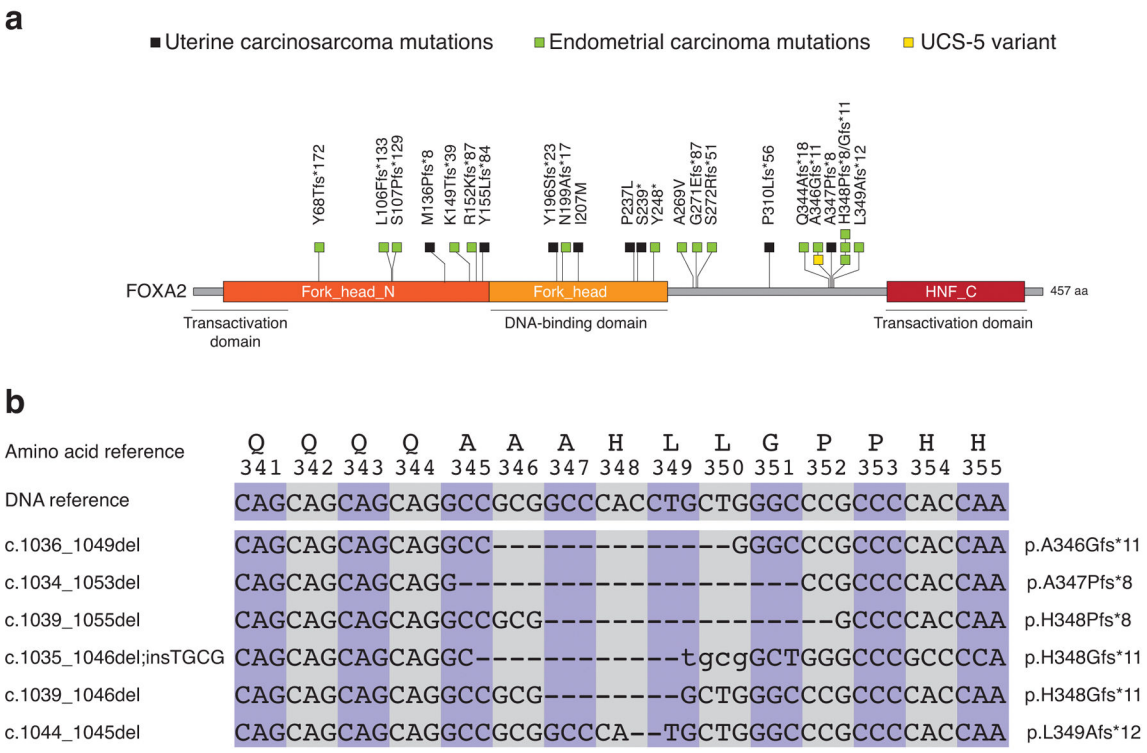

Figure 2. Localization of somatic *FOXA2* mutations uncovered in uterine carcinosarcomas and in endometrial carcinomas relative to functional domains of the encoded protein

(a) Location of somatic *FOXA2* mutations in endometrial carcinomas (green squares) and in uterine carcinosarcomas (black squares), relative to mapped functional domains of the protein; the A346Gfs*11 variant found in UCS-5, which did not have paired normal DNA available for analysis, is indicated (yellow square) (**b**) Overlapping nucleotide deletions result in a cluster of somatic frameshift mutations in the C-terminal region of *FOXA2*. Deleted nucleotides are indicated by dashes; inserted nucleotides are shown in lower case.

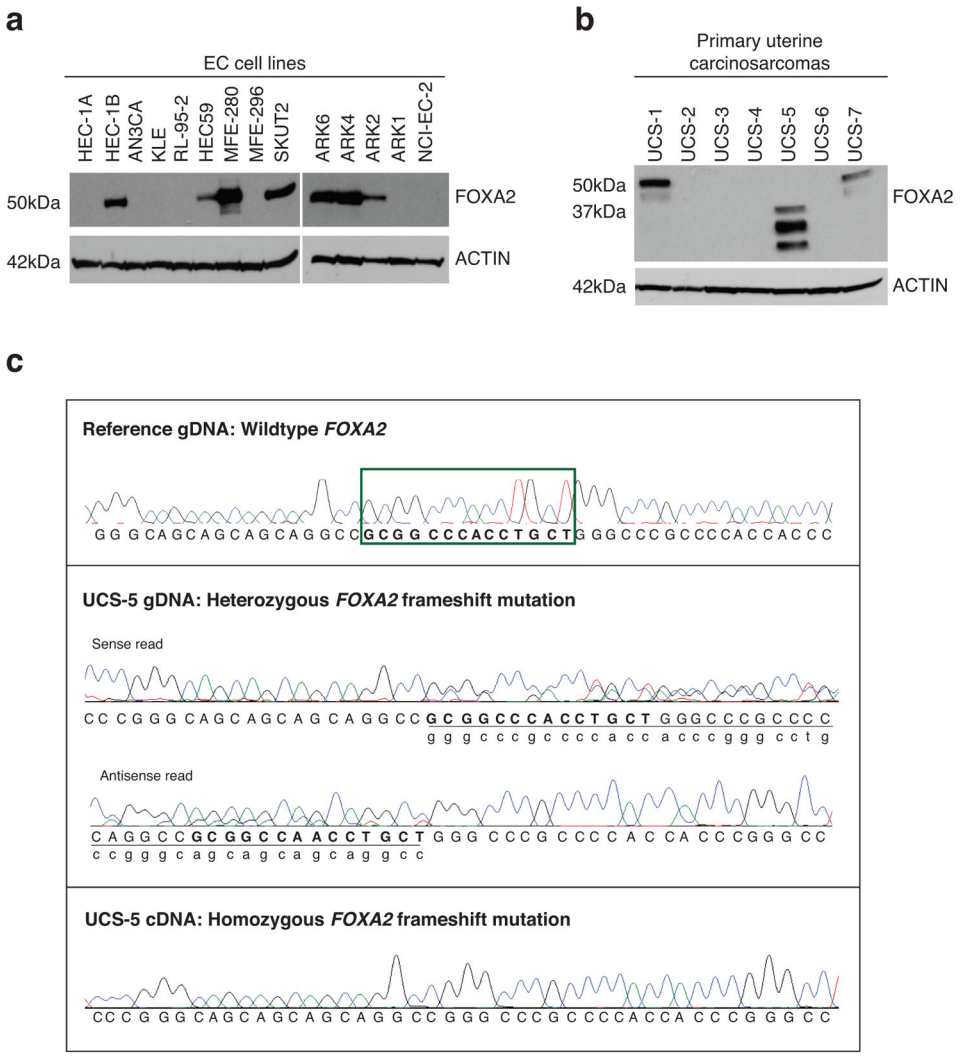

Figure 3. Variable expression of the FOXA2 protein in uterine cancer cell lines and expression of truncated FOXA2 in a UCS harboring a *FOXA2* frameshift mutation

Immunoblots showing variable FOXA2 protein expression levels among **(a)** endometrial carcinoma (EC) cell lines and **(b)** primary UCS tumors; a truncated FOXA2 protein (~30KDa) was detected in one tumor (UCS-5), **(c)** Partial *FOXA2* nucleotide sequence traces for tumor UCS-5 showing heterozygosity for a frameshift mutation (c. 1036_1049delGCGGCCCACCTGCT; p.A346Gfs*11) in the genomic DNA (gDNA) (middle panels) and homozygosity for this mutation in the cDNA (lower panel); an unmatched normal *FOXA2* sequence is shown for reference, the nucleotides boxed in green (upper panel) are deleted in UCS-5.

Table 1

Somatic mutations of *FOXA2* in uterine carcinosarcomas and endometrial carcinomas

Tumor ID	Tumor histology	Nucleotide change[§]	Somatic mutation type	Predicted protein change
Uterine carcinosarcoma (UCS) mutations				
189T	UCS	c.621C>G	Missense	p.I207M
190T	UCS	c.710C>T	Missense	p.P237L
195T[¶]	UCS	c.1034_1053delCCGCGGCCCACCTGCTGGGC	Frameshift	p.A347Pfs*8
1241T	UCS	c.401_402insCC	Frameshift	p.M136Pfs*8
1338T	UCS	c.587delA	Frameshift	p.Y196Sfs*23
1413T	UCS	c.462_463insT	Frameshift	p.Y155Lfs*84
1828T	UCS	c.716C>A	Nonsense	p.S239*
1980T	UCS	c.912_913insACTCGAGCGCCTT	Frameshift	p.P310Lfs*56
Endometrial carcinoma (EC) mutations				
84T	EEC	c.1039_1055delGCCCACCTGCTGGGCCC	Frameshift	p.H348Pfs*8
94T	EEC	c.445_446insCCCGCGCGCCCGCGACCCCA	Frameshift	p.K149Tfs*39
97T	EEC	c.201-202insACGG	Frameshift	p.Y68Tfs*172
124T	EEC	c. 1035_1046delCGCGGCCCACCTinsTGCG	Frameshift	p.H348Gfs*11
134T	EEC	c.318_325delGAGTCCCA	Frameshift	p.S107Pfs*129
146T	EEC	c.1039_1046delGCCCACCT	Frameshift	p.H348Gfs*11
43T	SEC	c.816delC	Frameshift	p.S272Rfs*51
49T	SEC	c.1044_1045delCC	Frameshift	p.L349Afs*12
75T	SEC	c.1036_1049delGCGGCCCACCTGCT	Frameshift	p.A346Gfs*11
24T	CCEC	c.453_454insA	Frameshift	p.R152Kfs*87
28T	CCEC	c.807_820delCGCCGGCAGCGGCAinsTGC	Frameshift	p.G271Efs*87
34T	CCEC	c.315_316insT	Frameshift	p.L106Ffs*133
46T	CCEC	c.743_756delACCTGCGCCGCCAG	Frameshift	p.Y248*
77T	CCEC	c.806C>T	Missense	p.A269V
15T	Mixed EC	c.594_604delGAACCAGCAGCinsA	Frameshift	p.N199Afs*17
176T	Mixed EC	c.1028_1029insG	Frameshift	p.Q344Afs*18

[§] *FOXA2* Transcript and protein accession numbers for variant annotation: NM153675 and NP710141

[¶] FOXA2 mutation detected in 195T was discovered by Sanger sequencing the 14 discovery screen tumors.

Abbreviations: UCS, uterine carcinosarcoma; EEC, endometrioid endometrial carcinoma; SEC, serous endometrial carcinoma; CCEC, clear cell endometrial carcinoma; mixed EC, mixed histology endometrial carcinoma.