Background: Uterine carcinosarcoma, a rare gynecological malignancy, often presents at the advanced stage with a poor prognosis because current therapies have not improved rates of survival. Genetic characterization of this tumor may lead to novel, specifically targeted drug targets to provide better treatment options for patients with this malignancy.

Methods: We present a case of a woman aged 61 years with uterine carcinosarcoma and retrospectively analyzed 100 study patients with uterine carcinosarcoma. From this group, 9 study patients underwent targeted sequencing of 1,321 genes.

Results: All 9 study patients had at least 1 mutation in JAK2, KRAS, PIK3CA, CTNNB1, PTEN, FBXW7, TP53, and ERBB2; of these, TP53 was the most frequently mutated gene (6/9). In addition, ARID1A and KMT2C, which have been described and identified as part of a set of chromatin-remodeling genes, were also found in our analyses. From our 100-person cohort clinical analyses, study patients with stage 1 cancer had a median survival rate of 33 months (95% confidence interval, 19–109) compared with a median survival rate of 6 months (95% confidence interval, 3–12) in those with stage 4 disease.

Conclusions: Disease stage alone predicted the rate of clinical survival. Up to 50% in the study group were identified at having early stage disease (stage 1/2), indicating improved rates of overall detection compared with previously reported data. Our mutational analysis findings add to the number of tumors in which these mutations have been found and suggest that chromatin-remodeling dysregulation may play a role in the tumorigenesis of carcinosarcoma.

Introduction

Uterine carcinosarcomas are rare gynecological tumors that make up fewer than 5% of all uterine malignancies; however rare, these tumors account for a disproportionate percentage of associated mortality. Their classic presentation has been described as a triad of symptoms: pain, severe vaginal bleeding, and the passage of necrotic tissue through the vagina. More than one-half of patients (53%) present with advanced stage disease, and 20% of patients with localized disease at presentation are upstaged during laparotomy. Staging is the most significant prognostic indicator and reflects a poor overall, 5-year, disease-specific survival rate (36.4% for all stages; 62.3% for stage 1 and 0% for stages 2 to 4). Risk factors are similar to those seen in endometrial carcinoma and include nulliparity, advanced age, obesity, exposure to exogenous estrogens, history of pelvic irradiation, and the long-term use of tamoxifen.

The histology of the tumor is mixed with epithelial and mesenchymal elements, thus designating it a carcinosarcoma. The tumor is classified based on the sarcomatous portion as either homologous (consisting of mesenchymal elements found in the female genital tract) or heterologous (consisting of mesenchymal elements foreign to the female genital tract). Within the uterus, carcinosarcomas commonly arise from the posterior wall of the uterine body near the fundus. The mass usually grows to fill and distort the uterus, and most carcinosarcomas are visualized as exophytic lesions. The extent of myometrial invasion of these tumors is sometimes controversial, but it is generally accepted that the degree of myometrial invasion is a poor prognostic indicator and likely a predictor of metastasis.

The origin of the tumor is thought to follow the embryological development path of the Müllerian ducts and is most likely derived from a pluripotent stem cell that differentiates into both epithelial and mesenchymal cell types. Further characterization of the tumor histol-
ogy supports the “conversion” theory, which states that an epithelial-to-mesenchymal transition occurs because the sarcomatous portion of the tumor exhibits markers consistent with an epithelial origin.\textsuperscript{8}

Historically, this tumor was considered to be uterine sarcoma, and treatment was directed at the sarcomatous element. However, the carcinoma portion of the tumor is now favored as the primary determinant of tumor aggressiveness, with recent advances in treatment directed toward those elements.\textsuperscript{9} Regardless, standards of therapy (surgical debulking, lymphadenectomy, and adjuvant chemotherapy) have not improved overall survival rates, particularly in those with advanced disease. Therefore, improving our understanding of tumor biology (molecular profile and oncogenic drivers) should be a priority.

In that regard, somatic mutations (eg, \textit{KRAS}, \textit{PI3K-CA}) known to be carcinogenic drivers in other tumors (eg, lung adenocarcinomas, colon adenocarcinomas) have been demonstrated in carcinosarcomas.\textsuperscript{10} This increased our curiosity on a more detailed molecular analysis of these tumors. Thus, we present a case patient with uterine carcinosarcoma and report on our clinical and molecular analysis findings for a cohort of patients with this disease.

**Case Report**

**Presentation**

A 61-year-old postmenopausal, gravida 3, para 3 woman with uncontrolled type 2 diabetes mellitus, hypertension, and a 32-pack-year smoking history (quit 12 years prior) but no history of gynecological cancer or exogenous estrogen exposure presented with urinary retention for 2 days and weight loss of 24 pounds during the previous 2 to 3 months. She denied abdominal pain, hematuria, abnormal vaginal bleeding, changes in her bowel pattern, night sweats, shortness of breath, and cough. She had undergone tubal ligation after her last pregnancy and also reported abnormal results on a Papanicolaou test 6 months before her presentation. Findings on physical examination were benign, except for a palpable but nontender mass above the pubic symphysis. Serum levels of cancer antigen 125, cancer antigen 19-9, and carcinoembryonic antigen were all within normal limits.

Computed tomography (CT) revealed a large, 16-cm heterogeneous intrapelvic mass with no invasion into the surrounding structures, no mesenteric or retroperitoneal lymphadenopathy, and no ascites. Secondary to the large pelvic mass, external compression of the bilateral distal ureters was present that resulted in severe bilateral hydroureteronephrosis and urethral obstruction. Based on these findings, the diagnosis of uterine malignancy was suggested and surgical debulking was recommended for diagnostic and therapeutic purposes to relieve the urethral obstruction and bilateral hydroureteronephrosis.

Exploratory laparotomy, modified radical hysterectomy, bilateral salpingo-oophorectomy, and tumor debulking were performed. Physical examination under anesthesia revealed a fixed mass involving the entire uterus and gross tumor spillage in the vagina. Midline laparotomy confirmed a grossly necrotic tumor in the cul-de-sac, arising from the uterus, and all visible tumors were excised and submitted for pathology.

**Results**

Histopathology with uterine carcinosarcoma was consistent with the diagnosis of high-grade primary uterine heterologous carcinosarcoma. The carcinoma component was endometrioid adenocarcinoma and the sarcomatous component was rhabdomyosarcoma (Fig 1).

Fluorodeoxyglucose positron emission tomography/CT was obtained of the skull base to the midthigh and revealed at least 5 metabolically active pulmonary nodules (2 nodules on the right and 3 nodules on the left; Fig 2). CT angiography of the chest showed pulmonary thromboembolism in the left lower lobe and also confirmed bilateral pulmonary metastases (Fig 3). The patient was started

![Fig 1A–B. — Histopathological features of uterine carcinosarcoma. (A) Adenocarcinoma. (B) Rhabdomyosarcoma.](image)
Fig 2. — Postoperative positive emission tomography obtained for the case patient.

Fig 3. — Postoperative computed tomography obtained for the case patient.
on carboplatin and paclitaxel therapy but did not respond. Subsequently, she died from complications of progressive disease.

Cohort Analysis

Clinical

We accessed clinical and tissue data from the Total Cancer Care protocol, which was approved by the Institutional Review Board of the H. Lee Moffitt Cancer Center & Research Institute (Tampa, Florida); patients also provided prospectively written informed consent. Analysis of our study patient cohort included staging by either clinical or pathological staging, with the composite stage being the higher of the 2 scores. We used the composite stage to describe our cohort.

Deidentified clinical data, including age, race, sex, smoking status, stage of disease, histology, and type of treatment, were obtained through an “honest broker” web-based system designed by Moffitt Cancer Center to protect confidential patient information following the regulations set forth by the Health Insurance Portability and Accountability Act. Tissue samples had been collected using common protocols for tissue preservation, processing, and data annotation.

Molecular

Tumor samples from Total Cancer Care were subject to genomic capture and massively parallel sequencing. Sequences were aligned to the hs37d5 human reference with the Burrows-Wheeler Alignment tool. Insertion/deletion realignment, quality score recalibration, and variant identification were performed with the Genome Analysis ToolKit (Broad Institute, Cambridge, Massachusetts). Sequence variants were annotated with ANNOVAR (http://annovar.openbioinformatics.org). Additional contextual information was incorporated, including allele frequency in other studies (eg, 1000 Genomes, National Heart, Lung, and Blood Institute’s “Grand Opportunity” Exome Sequence Project), in silico function impact predictions, and observed impacts from select databases such as ClinVar (www.ncbi.nlm.nih.gov/clinvar) and the Collection of Somatic Mutations in Cancer (COSMIC; version 61; Wellcome Trust Sanger Institute, Cambridge, UK). Mutation frequencies were compared with data from The Cancer Genome Atlas (TCGA) using the cBioPortal for Cancer Genomics tool (Memorial Sloan Kettering Cancer Center, New York, New York). A single KRAS G12D mutation was also identified.

To enrich for somatic mutations, we excluded any positions observed in 1,000 genomes or at a frequency larger than 5% in a local normal population. We eliminated any variants with a variant quality score recalibration tranche level equal to 100 and required a genotype quality of at least 10 to include the variant of a particular sample.

Results

Clinical Analysis: The Total Cancer Care cohort included 100 study patients diagnosed with uterine carcinosarcoma seen at Moffitt Cancer Center or an affiliated hospital between 1996 and 2012. Most study patients at presentation were between the ages of 60 and 79 years, female, white, and diagnosed in an early composite stage (1/2). Tumor grade was most often poorly differentiated. The first course of treatment was distributed between surgery alone, surgery and chemotherapy, and combination surgery, chemotherapy, and radiotherapy. The strongest predictor of survival was stage at presentation, with stage 1 having a median survival rate of 33 months (95% confidence interval, 19–109) and stage 4 having a median survival rate of 6 months (95% confidence interval, 3–12; Fig 4).

Genetic Mutation Analysis: Nine of the 100 study patients were part of a targeted sequencing study covering 1,321 genes. We examined their mutation profiles, comparing observed frequencies to 31 patients with carcinosarcoma in a previous study of 15 cancer genes. We focused on those positions seen more than 5 times in COSMIC. Mutations in JAK2, KRAS, PIK3CA, CTNNB1, PTEN, FBXW7, TP53, ARID1A, and ERBB2 were identified (Fig 5; Supplemental Table). All 9 samples had at least 1 mutation in these genes, with TP53 being the most commonly mutated (6 of 9). If TP53 is removed, then 8 of the 9 samples had at least 1 mutation in the remaining genes, suggesting that these pathways play an important role in carcinosarcoma. Many of these genes are mutated at recurrent positions, including PIK3CA (R88Q, H1047R/Y), FBXW7 (R465H, R658X), ERBB2 (V842D), JAK2 (V617F), and CTNNB1 (S37F, T41A). Truncating mutations in PTEN (R233X, Q97X), TP53 (2 different nonsense mutations, 1 splice-altering mutation), KMT2C (1 nonsense mutation), and ARID1A (2 nonsense mutation) were also observed, consistent with previous observations of truncating mutations in these genes in different tumor types (TCGA via cBio Portal). A single KRAS G12D mutation was also identified.

Many of these genes (PIK3CA, KRAS, TP53, PTEN, and CTNNB1) were observed in an earlier study and were part of the 15-gene panel used by Penson et al. Many of these genes were observed to be commonly mutated in uterine carcinosarcoma (TCGA via cBio Portal): ARID1A at 18% and FBXW7 at 39%. However, although we observed 1 mutation in PPP2R1A (the most significantly mutated gene in uterine carcinosarcoma via the cBio Portal, as of publication), this exact mutation was not observed in TCGA data. Furthermore, CTNNB1 mutations were observed in our study as well as in Penson et al but not in TCGA data.

A single sample, DS–90250, had many mutations
across these commonly mutated driver genes (Fig 5; see Supplemental Table). Upon further examination, we identified a POLE proofreading-domain mutation, V411L, in this sample. After reviewing uterine carcinosarcoma data from TCGA, we identified a single sample with a POLE mutation in the same region, P286R; the sample also had a high number of mutations.16

Thus, although these mutations are uncommon (ie, less common than those reported in uterine corpus endometrial carcinoma19), our findings suggest that POLE mutations can be observed in uterine carcinosarcoma.

Discussion

The patient discussed in our case report presented in the sixth decade of life with stage 4 disease and more than 50% myometrial invasion, so her prognosis was poor. She was referred to our gynecological oncology clinic and received chemotherapy with carboplatin and paclitaxel. She tolerated 2 cycles of chemotherapy before she died from her disease.

Most women in our cohort were white and their stages of presentation were equally distributed throughout the group. This is in contrast to other epidemiological studies, which have included higher percentages of black or non–white women and those likely to present with advanced-stage disease.20,21

In our examination of mutations in 1,321 genes thought to be associated with cancer in a small cohort of individuals diagnosed with uterine carcinosarcoma (n = 9), we reproduced some earlier mutations. Direct comparisons of frequency between studies are limited by small sample size, but general similarities exist. Therefore, we can conclude that mutations in PIK3CA, KRAS, PTEN, TP53, ARID1A, KMT2C, and FBXW7 are important drivers of uterine carcinosarcoma. However, some differences in mutation frequencies are apparent, perhaps reflecting the pathological heterogeneity

Fig 4. — Characteristics of the 100-person cohort. TNM = tumor, node, metastasis.

Fig 5. — Mutations in commonly mutated cancer-related genes. Mutation matrix shows missense (blue) and truncating (black) mutations in each study patient (n = 9) for the listed genes.
of this disease. The large number of potential driver genes suggests that uterine carcinosarcoma is a genetically heterogeneous disease.

Genetic investigation of additional samples will be needed to more accurately determine incidence, to identify lower-frequency genetic events, and to associate status with clinical phenotype. However, we observed a mutation in at least 1 well-characterized oncological gene for every sample analyzed, suggesting that, although uterine carcinosarcoma may be driven by heterogeneous events, these drivers are already known to be important for the development of other cancer types.

Given poor overall survival rates for uterine carcinosarcoma, genetic characterization offers the potential to improve our understanding and treatment of this rare type of cancer. Our preliminary investigation demonstrated that pathways altered in other cancer types are also altered here, suggesting that therapeutic options (including targeted therapies) may also be effective in uterine carcinosarcoma. We also confirmed the presence of mutations in chromatin-remodeling genes in this disease, suggesting a possible therapeutic strategy via histone deacetylase inhibitors.

In addition, we observed a POLE proofreading-domain mutation resulting in a large mutational load in uterine carcinosarcoma. Large mutation load has been linked to durable response to immune checkpoint–inhibitor therapy in non–small-cell lung cancer and melanoma, suggesting another possible strategy for this disease. Future studies will also need to incorporate clinical outcome information to further understand the progression and prognosis of this malignant disease.

Conclusions

Given the genetic heterogeneity reported here, knowledge of the mutational profile of any given tumor could be linked to a differential prognosis or therapeutic response, and mutation profiles described here and elsewhere suggest new therapeutic options. Potential exists for personalized understanding and treatment options for patients with uterine carcinosarcoma; however, given the rarity of the disease, collaborative efforts will be critical to understanding the relationships between molecular alterations and clinical features.

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References