Acquisition of APOBEC Mutagenesis and Microsatellite Instability Signatures in the Development of Brain Metastases in Low-Grade, Early-Stage Endometrioid Endometrial Carcinoma

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INTRODUCTION

Low-grade, early-stage endometrioid endometrial cancer (EEC) is considered a highly treatable malignancy with less than 5% recurrence rates and greater than 95% 5-year overall survival.¹ When recurrences do occur, these are generally confined to the vaginal cuff or the regional lymph nodes; distant metastases in stage I EECs are rare (1.4%), and are found most commonly in the upper abdomen, lungs, or liver.^{1,2} Brain metastases in this patient population are exceedingly rare. We recently performed a clinical review of a large series of low-grade EECs with brain metastases (n = 23), and we found that 65% of these patients had stage I disease at diagnosis.³ The outcome of patients with EEC with brain metastases is poor, and despite multimodal aggressive treatment, median overall survival after diagnosis of brain metastasis was only 5.8 months.³ No consistent histologic features distinguishing low-grade EECs that eventually metastasize to the brain were identified,³ and the biology and genetic underpinning of these tumors has yet to be understood. Here, we sought to characterize the landscape of genomic alterations and mutational signatures of two International Federation of Gynecology and Obstetrics (FIGO) stage I, low-grade primary EECs and their matched pelvic recurrences and brain metastases.

METHODS

After institutional review board approval and written informed consent from the two patients included in this study, representative formalin-fixed paraffin-embedded tissues of the primary tumors, matched recurrences, brain metastases, and normal myometrium were obtained and were subjected to microdissection and DNA extraction, as described.^{4,5} High-depth whole-exome sequencing (WES) data (median coverage, 292x; range, 176-387x tumors; 99x and

105x normal tissues) were analyzed for the identification of somatic mutations, copy number alterations, and clonality/cancer cell fractions of mutations using validated bioinformatics tools.^{6,7} Mutational signatures were inferred using deconstructSigs,⁸ and microsatellite instability (MSI) using MSIsensor,⁹ as described previously.¹⁰ β -catenin immunohistochemistry (IHC) was performed on representative tissue sections of case 1.¹¹ For case 2, MLH1, MSH2, MSH6, and PMS2 IHC were performed, and *MLH1* promoter methylation was clinically assessed, as described previously.^{12,13}

CASE 1

A 64-year-old woman was diagnosed with a 3.5 cm stage IA FIGO grade 1 EEC displaying lymphovascular space invasion. The patient underwent staging surgery, received intravaginal radiation therapy, and developed metastatic recurrent disease 15 months later, including disease in the pelvis, upper abdomen, and lungs. After multiple lines of chemotherapy and endocrine therapy, 18 months after the first recurrence, the patient was found to have recurrent lung metastases and multiple brain metastases, and underwent a craniotomy and excision of the cerebellar metastases. Histopathologic review showed that the glandular structure of the primary tumor was retained in the pelvic recurrence and brain metastasis (Fig 1A).

WES analysis of the microsatellite-stable primary EEC (C1-P) revealed the presence of 58 nonsynonymous somatic mutations, including a clonal *ARID1A* loss-of-function mutation (p.P2114Rfs*21) associated with loss of heterozygosity of the wild-type allele, a clonal *KRAS* p.G12V hotspot mutation, and clonal *PTEN* mutations (p.R130Q/p.I33del; Fig 1B, Supplementary Table 1). Several copy number gains and losses, including gains of chromosomes 1q, 10, and 12 and loss

ASSOCIATED CONTENT

Appendix

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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FIG 1. Genomic analysis of case 1. (A) Micrographs of representative hematoxylin and eosin–stained sections of the primary endometrioid endometrial cancer (EEC), pelvic recurrence, and brain metastasis. (B) Nonsynonymous somatic mutations identified by whole-exome sequencing in the primary tumor (C1-P), pelvic recurrence (C1-R), and brain metastasis (C1-BM). Mutation types (left) and cancer cell fraction (CCF) of mutations identified (right) are color coded according to the legend. Selected pathogenic mutations are shown on the left. The numbers of mutations are shown on the right. (C) Copy-number alterations of the primary tumor (C1-P), pelvic recurrence (C1-R), and brain metastasis (C1-BM). Copy-number log₂ ratios are shown on the y-axis according to the chromosomes on the x-axis. Arrow, homozygous deletion. (D) Representative micrographs of the β -catenin immunohistochemical analysis of the primary tumor (top) and the brain metastasis (bottom). Note the cytoplasmic accumulation in the brain metastasis sample. (E) Mutational signature analysis and relationship among samples. Pie charts represent the mutational signatures identified in mutations shared between primary tumors and metastases (trunk [T]), in mutations private to the primary tumor (C1-P), the recurrence (C1-R), and the brain metastasis (C1-BM), color coded according to the legend. The length of the branches is proportional to the number of somatic mutations that are shared/unique to a given lesion, and the number of all mutations is shown alongside the branches. Selected pathogenic somatic mutations, as well as the number of all mutations (and non-synonymous mutations), are shown along their corresponding branches. APOBEC, apolipoprotein B mRNA editing enzyme catalytic polypeptide-like; MMR-D, DNA mismatch repair deficiency; NA, not assigned; SNV, single-nucleotide variant.

of chromosome 18q, were present in the primary EEC (Fig 1C).

In the progression of the primary tumor to the pelvic recurrence (C1-R) and brain metastasis (C1-BM), we observed the acquisition of additional genomic alterations. The pelvic recurrence harbored 60 nonsynonymous somatic mutations, five of which were private including a mutation in the metastasis-related *GPNUMB* gene, and a homozygous deletion on 11q23 not present in the other two samples (Figs 1B and 1C). Furthermore, subclonal mutations in the primary EEC became clonal in the pelvic recurrence and the brain metastasis (Fig 1B). The brain metastasis was found to harbor a gain of chromosome 2, not present in the other samples, as well as 79 nonsynonymous somatic mutations, of which 24 were unique to the brain metastasis. The brain metastasis acquired two subclonal *CTNNB1* gain-of-function hotspot mutations, p.D32N and p.S37C (Fig 1B) and displayed immunohistochemical β -catenin overexpression with cytoplasmic accumulation (Fig 1D). Mutational signature analysis of the mutations shared among the primary tumor, pelvic recurrence, and brain metastasis revealed a dominant aging mutational signature 1. In contrast, the mutations restricted to the brain metastasis showed dominant hypermutation-associated apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) signatures 2 and 13 (Fig 1E).

CASE 2

A 64-year-old woman was initially diagnosed after surgical staging with a 2 cm stage IA, FIGO grade 2 EEC. Given the lack of lymphovascular space invasion or other risk factors, no adjuvant therapy was administered after surgery. Twenty months after diagnosis, the patient presented with recurrent disease metastatic to the peritoneum and liver. After optimal cytoreduction and chemotherapy, 14 months after the first recurrence, multiple brain lesions were detected, which, despite radiation and chemotherapy, progressed, and a frontal brain lesion was resected.

The homogeneous endometrioid architecture and cytologic features were, akin to those of case 1, preserved throughout progression (Fig 2A). All three samples lacked MLH1 expression (Fig 2A) due to *MLH1* promoter hypermethylation, were MSI high by sequencing (MSIsensor scores ≥ 3.5 ; Fig 2B), and, apart from a chromosome 1q gain, had stable genomes (Fig 2C). A stepwise increase in the mutational burden was observed: the primary EEC (C2-P) harbored 84; the pelvic recurrence (C2-R), 659; and the brain metastasis (C2-BM), 915 nonsynonymous somatic mutations (Fig 2B). Forty-nine clonal nonsynonymous somatic mutations were shared across the three lesions. In addition, 20 subclonal mutations in the primary EEC, including a *MSH6* F1088Dfs*2 mutation, became clonal in the peritoneal recurrence and brain metastasis. Furthermore, the *PIK3CA* p.C410R, *STK11* p.Q170*, and *RNF43* p.G659Vfs*41/p.I104T mutations were among the 364 clonal mutations

shared between the pelvic recurrence and brain metastasis (Figs 2B and 2D; Data Supplement); frameshift mutations in chromatin remodeling genes, including *KDM5A*, *KMT2D*, and *KMT2A*, were restricted only to the brain metastasis. To establish the presence of intratumor genetic heterogeneity, WES of two spatially distinct areas of the primary tumor and pelvic recurrence was performed. This analysis revealed that $> 80\%$ of total mutations were shared between the two primary tumor areas and between the two areas of the pelvic recurrence (Appendix Fig A1). Mutational signature analysis revealed that the primary tumor had a dominant aging signature 1 as well as the MSI-related signatures 6 and 15. In contrast, the peritoneal and brain metastases primarily harbored dominant MSI-related signatures, including 6, 20, and 26, accompanied by an increase in the percentage of small insertions and deletions, from 4% in the primary EEC to 13% and 21% in the peritoneal and brain metastases, respectively (Fig 2D).

DISCUSSION

Here, we genomically characterized the evolution of two low-grade, early-stage EECs from primary uterine-confined malignancies treated with standard-of-care therapy to brain metastasis. In both cases, we observed branched evolution, where the primary EEC, pelvic recurrence, and brain metastasis of a given case shared a common ancestor, with independent evolution at each site. Furthermore, we found subclonal mutations in the primary EECs, which became clonal during progression to the pelvic recurrences and brain metastases. In both cases, the metastatic sample displayed increased mutational burden compared with the respective primary tumor; one was a result of the acquisition of APOBEC mutagenesis (case 1), whereas the second displayed dysfunctional DNA mismatch repair (case 2). Despite the change in the genomic make-up and the mutational signatures during progression, the brain metastases of both cases maintained their histologic features. Neither of the two primary tumors harbored *CTNNB1* hotspot or *TP53* mutations, genetic alterations previously associated with increased recurrence risk in low-risk EECs.^{14,15} Instead, both EECs harbored chromosome 1q gains, a molecular change associated with adverse outcome in favorable histologies of other cancer types.¹⁶⁻¹⁸

The recurrences and/or brain metastases in both cases studied were found to harbor additional pathogenic mutations, consistent with previous reports on the genomics of brain metastases from other tumor types.¹⁹ In case 1, the brain metastasis acquired two *CTNNB1* hotspot mutations coupled with β -catenin IHC overexpression not present in the primary tumor/recurrence. Exon 3 *CTNNB1*/Wnt pathway alterations have been associated with resistance to chemotherapy and immunotherapy in other cancer types.²⁰⁻²⁴ In case 2, the recurrence and brain metastasis acquired a *PIK3CA* hotspot gain-of-function mutation; whereas the clinical usefulness of this mutation in

FIG 2. Genomic analysis of case 2. (A) Micrographs of representative hematoxylin and eosin- and MLH1-stained sections of the primary endometrioid endometrial cancer (EEC), pelvic recurrence, and brain metastasis. (B) Nonsynonymous somatic mutations identified by whole-exome sequencing in the primary tumor (C2-P), pelvic recurrence (C2-R), and brain metastasis (C2-BM). Mutation types (top) and cancer cell fraction (CCF) of mutations identified (bottom) are color coded according to the legend. The numbers of mutations are shown at the bottom and the MSIsensor scores on the right. (C) Copy-number alterations of the primary tumor (C2-P), pelvic recurrence (C2-R2), and brain metastasis (C2-B). Copy number $\log_2$ ratios are shown on the y-axis according to the chromosomes on the x-axis. (D) Mutational signature analysis and relationship among samples. Pie charts represent the mutational signatures identified in mutations shared between primary tumors and metastases (trunk [T]), in mutations private to the primary tumor (C2-P), recurrence (C2-R), and brain metastasis (C2-BM), color coded according to the legend. The length of the branches is proportional to the number of somatic mutations that are shared/unique to a given lesion, and the number of all mutations (and nonsynonymous mutations) is shown alongside the branches. The percentage of small insertions and deletions (indels [I]) is included. Selected pathogenic somatic mutations are shown alongside their corresponding branches. MMR-D, DNA mismatch repair deficiency; NA, not assigned; POLE, DNA polymerase epsilon, catalytic subunit; SNV, single-nucleotide variant.

EECs is currently unknown, the PI3K inhibitor alpelisib is Food and Drug Administration approved for the treatment of *PIK3CA*-mutant hormone receptor-positive advanced breast cancer.²⁵

In both cases analyzed, we observed a shift from aging mutational signatures in the primary tumor to APOBEC or DNA mismatch repair signatures in the brain metastases. In a subset of primary endometrial cancers and their matched abdominopelvic metastases analyzed previously by our team, we observed a similar phenomenon, providing evidence to suggest that in the progression of primary endometrial cancer to metastatic disease, the acquisition

of additional genetic instability through defects in DNA repair mechanisms (eg, MSI) or of additional mutagenic processes (eg, APOBEC) may play a role.¹⁰ Both patients analyzed here, however, were heavily treated during their disease course, and it is possible that the hypermutation signatures identified in the brain metastasis are reflective of pathways resistant to the evolutionary pressure of radiation and chemotherapy. Our findings support the notion that in this era of precision medicine and availability of immunotherapy options for patients with hypermutated endometrial cancer, immunohistochemical and/or genetic analyses should be repeated in metastatic disease.

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APPENDIX

FIG A1. Analysis of intratumor genetic heterogeneity in case 2. (A) Nonsynonymous somatic mutations identified by whole-exome sequencing in two spatially distinct areas of the primary tumor (C2-P1 and C2-P2), two spatially distinct areas of the pelvic recurrence (C2-R1 and C2-R2), and the brain metastasis (C2-BM). Mutation types (top) and cancer cell fraction (CCF) of mutations identified (bottom) are color coded according to the legend. The numbers of mutations are shown at the bottom, and the numbers of mutations shared between the two different areas of the primary tumor and of the two recurrences are shown on the right. (B) Copy-number alterations of the two spatially distinct areas of the primary tumor (C2-P1 and C2-P2) and pelvic recurrence (C2-R1 and C2-R2) and the brain metastasis (C2-BM). Copy-number log₂ ratios are shown on the y-axis according to the chromosomes on the x-axis. SNV, single-nucleotide variant.