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Germline mutations of the DNA repair pathways in uterine serous carcinoma

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HIGHLIGHTS

- We report a high frequency of gremlin mutations in DNA repair genes in patients with USC.
- PARP inhibition may benefit select USC patients with HR gene mutations.
- Mutations in HR genes correlate with platinum sensitivity.

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ABSTRACT

Objective. Treatment options are limited for patients with uterine serous carcinoma (USC). Knowledge of USC's somatic mutation landscape is rapidly increasing, but its role in hereditary cancers remains unclear. We aim to evaluate the frequency and characteristics of germline mutations in genes commonly implicated in carcinogenesis, including those within homologous recombination (HR) and mismatch repair (MMR) pathways in patients with pure USC.

Methods. By using targeted capture exome sequencing, 43 genes were analyzed in a cohort of 7 consecutive patients with paired tumor and non-tumor USC samples in our institutional tumor repository. Mutations predicted to have damaging effects on protein function are validated by Sanger Sequencing.

Results. We found 21 germline mutations in 11 genes in our USC cohort. Five patients harbored 7 germline mutations (33.3%) within genes involved in the HR pathway, RAD51D being the most common. Four patients had 9 (42.8%) germline mutations in hereditary colon cancer genes, most commonly MLH. All patients (42.7%) who are platinum-sensitive had HR germline mutations (RAD50, NBN, ATM). Patients with HER2 overexpression (2/7, 28.6%) had germline HR mutations and were platinum-sensitive. Three patients in our cohort reported a personal history of breast cancer, one with HR germline mutation, and 2 in patients with germline mutations in HCC genes. In addition, 5 out of 7 patients had germline mutations in genes associated with growth factor signaling pathway.

Conclusions. A significant proportion of our cohort harbor germline mutations in DNA repair genes. This may be associated with the high rate of breast cancer in our patients and their family, and suggests a targeted cohort for genetic counseling. If validated in a larger cohort, our findings may allow clinicians to expand therapeutic options to include targeted therapies and inclusion of USC patient in preventative and genetic counseling.

1. Introduction

Endometrial cancer (EC) is the most common malignancy of the female reproductive tract in the United States, with an estimated 54,870 new cases and 10,170 deaths in 2015 [1]. It is traditionally separated into two histologically distinct subtypes (type 1 and 2) based on broad epidemiologic and molecular signatures. The vast majority of patients with EC have type 1 disease associated with favorable 5-year survival rates of 85.6%. Type 2 EC, consisting mostly of the highly aggressive subtypes such as uterine serous carcinoma (USC), has a much poorer prognosis and contributes up to 40% of all EC-related deaths, and half of all EC-related recurrences [2]. This phenomenon is likely attributable to

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the metastatic disease at diagnosis that is highly resistant to the platinum-based chemotherapy and/or radiotherapy that is currently the standard of care after initial surgery.

Defects in DNA repair are responsible for many genetic cancer syndromes as well as sporadic cancers. The mismatch repair (MMR) and homologous recombination (HR) are two examples of well-established DNA repair pathways with links to human cancer, including those originating from the breast, uterus and ovary [3–5]. Numerous studies have proposed epidemiologic and molecular associations between USC and breast and ovarian cancer [6–11]. Recently, the Cancer Genome Atlas (TCGA) analysis, which included 373 endometrial carcinomas, with 53 USC samples, focused on identifying somatic copy number variations in patients with endometrial carcinoma. The TCGA analysis showed that USC shares many similar genomic characteristics with basal-like breast and high grade serous ovarian cancers, implicating a genetic overlap between these cancer types [12]. Furthermore, microsatellite instability (MSI) testing performed on all samples found MSI in 40% of endometrioid tumors and 2% of serous tumors. Genomic instability resulting from the cell's inability to coordinate interstrand DNA repair, as in the case of HR, or inability to repair base-pair mismatches and insertion/deletion loops during DNA replication, as in the case of MMR, have therapeutic potentials [12]. Cells with mutations in genes involved in HR repair are hypersensitive to DNA damaging agents targeted at creating intrastrand DNA crosslinks such as platinum and other alkylating agents [3]. Conversely, a defective MMR pathway contributes to platinum-resistance in vitro [13,14].

Currently, USC is not universally recognized as an index cancer of any hereditary cancer syndromes, although germline mutations in HR [15] and MMR [15,16] genes have been reported in USC to varying degrees. Understanding the driving mutagenesis underlying this lethal disease may expand therapeutic options other than conventional chemotherapy and improve our ability to treat this tumor with greater precision. Based on the current literature, we identify 43 tumor suppressor genes, including BRCA1, BRCA2, the DNA mismatch repair genes (MLH1, MSH2, MSH6, PMS2), TP53, insulin-growth receptor pathway, and genes in the Fanconi anemia (FA)-BRCA pathway that have been shown to have significant putative mutations in gynecologic cancers, specifically in patients with USC (Table 1) [15,17]. We hypothesize that inherited germline defects in these pathways underlies a portion of USC cases that is associated with cancer susceptibility syndromes. In this pilot study of a prospective cohort of treatment-naïve patients with pure USC, we determine the frequency of germline mutations and correlate their genomic profiles with clinical and pathologic characteristics in hopes to identify potential new therapies or biomarkers of outcome.

2. Materials and methods

2.1. Study subjects

After IRB approval, we extracted tumor (T) and non-tumor (NT) tissue from consecutive patients with USC designated as having pure

Table 1
Targeted exonic regions on relevant genes for exome capture in uterine serous carcinoma.

Gene	Name	Chr	Chromosome location	Base pairs	Exons
ANGPT1	Angiopoietin-1	8	108,257,710–108,514,254	256,544	9
ATM	Ataxia Telangiectasia Mutated	11	108,089,559–108,243,826	154,267	63
BARD1	BRCA1 Associated RING Domain 1	2	215,589,275–215,678,428	89,153	11
BRCA1	Breast Cancer 1 early onset	17	41,192,312–41,281,500	89,188	22
BRCA2	Breast Cancer 1 early onset	13	32,885,617–32,977,809	92,192	27
BRIP1	BRCA1 Interacting Protein C-terminal helicase 1	17	59,752,547–59,944,920	192,373	20
CDH1	Cadherin 1	16	68,767,195–68,873,444	106,249	16
CHEK2	Checkpoint Kinase 2	22	29,079,731–29,141,822	62,091	16
EGF	Epidermal Growth Factor	4	110,830,040–110,938,118	108,078	23
EGFR	Epidermal Growth Factor Receptor	7	55,082,725–55,279,031	196,306	28
ErbB2	Receptor tyrosine-protein kinase erbB-2	17	37,852,254–37,888,915	36,661	30
ErbB3	Receptor tyrosine-protein kinase erbB-3	12	56,469,809–56,501,291	31,482	28
ErbB4	Receptor tyrosine-protein kinase erbB-4	2	212,236,442–213,407,352	1,170,910	28
FGFR2	Fibroblast Growth Factor Receptor 2	10	123,233,844–123,361,972	128,128	17
GRB2	Growth factor Receptor-Bound protein 2	17	73,310,157–73,405,790	95,633	6
HIF1alpha	Hypoxia Inducible Factor 1	14	62,158,119–62,218,977	60,858	14
IGF1R	Insulin-like Growth Factor 1 receptor	15	99,188,761–99,511,759	322,998	21
KDR	Kinase insert Domain Receptor	4	55,940,426–55,995,762	55,336	30
MAPK1	Mitogen-Activated Protein Kinase 1	22	22,109,947–22,225,970	116,023	9
MAPK3	Mitogen-Activated Protein Kinase 2	16	30,121,426–30,138,630	17,204	8
MAP2K1	Mitogen-Activated Protein Kinase Kinase 1	15	66,675,211–66,787,882	112,671	11
MAP2K2	Mitogen-Activated Protein Kinase Kinase 2	19	4,086,320–4,128,126	41,806	11
MLH1	MutL Homolog 1	3	37,030,841–37,096,337	65,496	19
MRE11A	Meiotic Recombination 11 homolog A	11	94,146,469–94,231,040	84,571	20
MSH2	MutS homolog 2	2	47,626,263–47,714,360	88,097	17
MSH6	MutS Homolog 6	2	48,006,221–48,038,092	31,871	10
NBN	Nibrin	8	90,941,564–91,000,899	59,335	16
PALB2	Partner and Localizer of BRCA2	16	23,610,483–23,656,678	46,195	13
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3- kinase	3	178,862,311–178,956,497	94,186	21
PIK3R1	Phosphoinositide-3-Kinase Regulatory subunit alpha	5	67,507,584–67,601,649	94,065	10
PIK3R2	Phosphoinositide-3-Kinase Regulatory subunit 2	19	18,260,016–18,285,343	25,327	16
PMS1	Postmeiotic Segregation increased 1	2	190,644,811–190,746,355	101,544	12
PMS2	Postmeiotic Segregation increased 3	7	6,008,870–6,052,737	43,867	15
PTEN	Phosphatase and Tensin homolog	10	89,619,195–89,732,532	113,337	9
RAD50	DNA repair protein RAD50	5	131,888,616–131,984,313	95,697	25
RAD51C	DNA repair protein RAD51 homolog 3	17	56,765,963–56,815,692	49,729	9
RAD51D	DNA repair protein RAD51 homolog 4	17	33,422,811–33,437,500	14,689	10
RAF-1	v-raf-1 murine leukemia viral oncogene homolog 1	3	12,621,100–12,709,700	88,600	17
STK11	Serine/Threonine Kinase 11	19	1,201,798–1,232,434	30,636	10
TEK	TEK tyrosine kinase	9	27,105,147–27,234,172	129,025	23
TGF-alpha	Transforming Growth Factor alpha	2	70,670,412–70,785,147	114,735	6
TP53	Tumor Protein p53	17	7,567,720–7,594,863	27,143	11
VEGFalpha	Vascular Endothelial Growth Factor A	6	43,733,946–43,758,223	24,277	7