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Comprehensive genomic profiling of epithelial ovarian cancer by next generation sequencing-based diagnostic assay reveals new routes to targeted therapies

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HIGHLIGHTS

- Using targeted NGS, 141 genomic alterations were identified in 48 ovarian epithelial carcinomas 67of which were actionable.
- Most common alterations were in TP53 (79%); MYC (25%); BRCA1/2 (23%); KRAS (16.6%) and NF1 (14.5%).
- NGS identifies an unexpectedly high frequency of genomic alterations that could influence targeted therapy selection for ovarian carcinoma.

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ABSTRACT

Objective. Targeted next generation sequencing (NGS) was evaluated for its ability to identify unanticipated targetable genomic alterations (GA) for patients with relapsed ovarian epithelial carcinoma (OC).

Methods. DNA sequencing was performed for 3320 exons of 182 cancer-related genes and 37 introns of 14 genes frequently rearranged in cancer on indexed, adaptor ligated, hybridization-captured libraries using DNA isolated from FFPE sections from 48 histologically verified relapsed OC specimens. The original primary tumor was sequenced in 26 (54%) of the cases and recurrent/metastatic tumor site biopsies were sequenced in 22 (46%) of the cases. Actionability was defined as: GA that predict sensitivity or resistance to approved or standard therapies or are inclusion or exclusion criteria for specific experimental therapies in NCI registered clinical trials.

Results. There were 38 (80%) serous, 5 (10%) endometrioid, 3 (6%) clear cell, 1 mucinous (2%) and 1 (2%) undifferentiated carcinomas. 141 GA were identified with an average of 2.9 GA (range 0–8) per tumor, of which 67 were actionable for an average of 1.4 actionable GA per patient (range 0–5). 33/48 (69%) of OC patient samples harbored at least one actionable GA. Most common GA were *TP53* (79%); *MYC* (25%); *BRCA1/2* (23%); *KRAS* (16.6%) and *NF1* (14.5%). One tumor featured an *ERBB2* point mutation. One of 3 (33%) of clear cell tumors featured *cMET* amplification validated by both FISH and IHC.

Conclusions. NGS assessment of therapy resistant OC identifies an unexpectedly high frequency of GA that could influence targeted therapy selection for the disease.

Introduction

Adenocarcinoma of the ovarian surface epithelium encompasses 90% of all ovarian malignant tumors and is the second most frequent gynecologic malignancy [1–3]. The incidence of ovarian carcinoma exceeded 22,000 new cases in the United States in 2012 and was responsible for approximately 14,000 cancer-related deaths [2,3]. Despite many attempts to develop methods and tests to detect the disease at an early stage, 85% of patients diagnosed with epithelial ovarian cancer present with advanced stage disease [1]. Although the use of radical surgery and cytotoxic chemotherapy in the last 3 decades has achieved a significant improvement in overall survival from 37% to 46% [3], ovarian cancer remains a major cause of morbidity and mortality for women both in the United States and around the world. After several decades of clinical trials in the 1990's, the

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Gynecologic Oncology Group (GOG) and other international study groups formally recommended a combination of platinum and taxane cytotoxic chemotherapy as the standard of care for epithelial ovarian cancer [4,5]. However, despite this regimen helping to improve the survival for this disease, the mortality rate for ovarian cancer patients with stages III and IV disease continues to be greater than 50% [1]. The inability to cure and, in many cases slow the progression of ovarian carcinoma has prompted investigators to search for potential new targets of systemic therapy that might improve the progression free and overall survival for the disease. In the following study, whose design contrasts with The Cancer Genome Atlas study [6] which was based on primary tumor assessment at the time of diagnosis, this comprehensive NGS-based test interrogating 182 cancer-related genes and 14 genes frequently rearranged in cancer was applied to 48 chemo-refractory relapsed/metastatic ovarian epithelial carcinomas to identify known and novel drug targets with the aim to personalize the therapy for patients with the advanced form of this life-threatening disease.

Methods

Next generation sequencing (NGS) was performed on hybridization-captured, adaptor ligation based libraries using DNA extracted from 4 FFPE sections cut at 10 μm from 26 (54%) primary OC and from 22 (46%) recurrent and metastatic tumor sites. The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin stained slides and all samples forwarded for DNA extraction contained a minimum of 20% tumor cells. Based on the submitted pathology reports that accompanied the tissue samples, of the 22 cases where a metastatic site was sequenced, 8 (36%) were obtained at the time of first surgical exploration, 10 (45%) were obtained at re-operation and 4 (18%) cases could not be established as to whether the metastatic site sample was synchronous with primary tumor surgery or obtained at a second operation or biopsy procedure. DNA sequencing was performed for 3320 exons of 182 cancer-related genes and 37 introns of 14 genes frequently rearranged in cancer (1.14 million total bps) on indexed, adaptor ligated, hybridization-captured (Agilent SureSelect custom kit) and fully sequenced using 49 bp paired reads on the Illumina HiSeq 2000 to at an average depth of 877× and evaluated for genomic alterations including base substitutions, insertions, deletions, copy number alterations (amplifications and homozygous deletions), and select gene fusions/rearrangements as previously described [7]. The bioinformatics processes used in this study included Bayesian algorithms to detect base substitutions, local assembly algorithms to detect short insertions and deletions, a comparison with process-matched normal control samples to detect gene copy number alterations and an analysis of chimeric read pairs to identify gene fusions.

Actionability classification

The genomic alterations identified were further divided into two main classes of actionability: 1) Genomic alterations that predict sensitivity or resistance to approved or standard therapies and 2) genomic alterations that are inclusion or exclusion criteria for specific experimental therapies in NCI registered clinical trials.

Results

Patients and tumors

The 48 ovarian cancer patients included in this study had a mean age at the time of genomic profiling of 55.7 years with a range of 23 to 76 years. There were a range of histologic phenotypes, with 38 (79%) papillary serous carcinomas, 5 (10%) endometrioid carcinomas, 3 (6%) clear cell carcinomas, 1 (2%) mucinous carcinoma and 1 (2%) undifferentiated carcinoma (Table 1). The majority of these tumors were high grade lesions, with only 2 (4%) FIGO grade 1 tumors, 6 (12.5%) FIGO grade II tumors and 40 (83%) FIGO grade III tumors. All of the patients had advanced stage disease at the time of genomic profiling (36 (71%) were stage III and 15 (29%) were stage IV.) In these 48 relapsed OC cases, the original primary tumor was sequenced in 26 (54%) of the cases and recurrent and metastatic tumor site biopsies were sequenced in 22 (46%) of the cases. Local site permissions to use clinical samples were used for this study.

A total of 141 genomic alterations were identified in the 48 OC with an average of 2.9 alterations per tumor (range 0–8) (Fig. 1). The most common alterations were *TP53* mutation (79% of tumors); *MYC* amplification (25% of tumors); *BRCA1/2* truncation (23%); *KRAS* mutation/amplification (16.6%) and *NF1* mutation truncating alterations (14.5%) (Supplementary Table 1). The *TP53* mutations were identified in many loci within the *TP53* gene and there was no significant recurrent locus or base substitution seen in this series of cases, as consistent with previous large-scale sequencing studies. The calculated *MYC* gene copy number gains in the 12 cases with amplifications varied from 6 to 16 with an average of copy number 9. When the alterations identified in the primary tumor specimens (which ultimately relapsed) are compared with the alterations identified in metastatic tumor samples, the findings were quite similar. Alterations in the *ARID1A* (5 patients); *PIK3CA* (4 patients) and *BRAF* (2 patients) genes were uniquely detected in the primary tumors and alterations in the *CCND2* (3 patients); *BRCA2* (3 patients); and *CCND1*; *ESR1*; *ERBB2*; *ERBB3*; and *ERBB4* (1 patient each) were restricted to the metastatic tumor samples. A larger follow up series that includes both primary and relapsed tumors from the same patient is required to determine whether these differences are biologically relevant.

Actionable genomic alterations

Sixty-seven genomic alterations identified in this series of 48 ovarian epithelial carcinomas (1.4 alterations per tumor) were potentially associated with clinical benefit of targeted therapies (Supplementary Table 2). Noteworthy genomic alterations potentially impacting the use of targeted therapy included: 11 (22.9%) tumors with either *BRCA1* or *BRCA2* mutations potentially sensitive to PARP inhibitors and DNA damaging agents. Eight of 8 (100%) of the alterations in *BRCA1* were frame shift truncations including 3 (38%) E23fs*17, 2 (25%) Q1756fs*74, 1 (13%) A17fs*14, 1 (13%) M1775fs*54 and 1 (13%) Y1522* mutation. Three (6%) of the tumors featured a *BRCA2* frame shift deletion. Of the 8 cases with *KRAS* genomic alterations potentially predicting resistance to anti-EGFR targeted therapies and sensitivity to MEK inhibitors, 6 (75%) were gene amplifications and 2 (25%) were point mutations including one G12D and one G13D mutation. Alterations in *NF1* that potentially predict responsiveness to mTOR inhibitors such as everolimus and temsirolimus included 2 nonsense mutations, 2 frame shift deletions, 1(14%) genomic truncation, 1 (14%) splice site modification mutation and 1 (14%) partial gene duplication predicted to be destructive. There were 4 (8%) tumors with two H1047R and two E545K *PIK3CA* mutations, and 1 (2%) *AKT3* mutation, also evoking the potential use of mTOR inhibitors (temsirolimus/everolimus). There was 1 (2%) tumor with a V842I *ERBB2* mutation raising potential for the use of multiple on the market anti-ERBB2 (HER2) targeted therapies and 1 (2%) tumor with a V104M *ERBB3* mutation also potentially treatable with anti-ERBB2 targeting agents. One (2%) tumor had an *ATM* mutation potentially sensitive to PARP inhibitors. In a single endometrioid case, an alteration in intron 11 leading to truncation in *PTCH1* was identified, suggesting potential treatment with a hedgehog pathway inhibitor such as vismodegib. A total of five OC harbored the amplifications of cell cycle regulatory genes including 1 case each for *CCND1*, *CCNE1* and *CDK4* and 3 cases with amplification of *CCND2*. The alterations in the cell cycle regulatory genes raise the potential for use of pazopanib, FGFR inhibitors and CDK4/6 inhibitors. Finally, 2 cases of ovarian carcinoma (one clear cell and one papillary serous) had *c-MET*