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Distinct homologous recombination gene expression profiles after neoadjuvant chemotherapy associated with clinical outcome in patients with ovarian cancer

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HIGHLIGHTS

- Homologous recombination (HR) pathway is associated with outcome in ovarian cancer.
- Some HR pathway genes are associated with survival before and after chemotherapy.
- Different genes are associated with outcome before and after chemo.

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ABSTRACT

Objective. The expression of homologous recombination (HR) genes in high grade ovarian cancer (HGOC) samples from debulking surgeries were correlated to outcomes in patients selected for chemotherapy treatment regimens.

Study design. RNA was extracted from 96 fresh frozen tumor samples from debulking surgeries from chemotherapy naïve patients with HGOC (primary derived surgeries (PDS), $n = 55$) or following neoadjuvant chemotherapy treatment (NACT), $n = 41$). The samples were selected for high tumor content by a gynecological pathologist, and cancer cell content was further confirmed using a percent tumor content covariate, and mutation score covariate analysis. Gene expression analysis was performed using a tailored NanoString-based Pancancer Pathway Panel. Cox proportional hazard regression models were used to assess the associations between the expression of 19 HR genes and survival.

Results. In the PDS group, over-expression of six HR genes (C11orf30, NBN, FANCF, FANCC, FANCB, RAD50) was associated with improved outcome, in contrast to the NACT group where four HR genes (BRCA2, TP53, FANCB, RAD51) were associated with worse outcome. With the adding extent of debulking as a covariate, three HR genes (NBN, FANCF, RAD50), and only one HR gene (RAD51) remained significantly associated with survival in PDS and NACT groups, respectively.

Conclusion. Distinct HR expression profiles define subgroups associated with overall outcome in patients that are exposed to neoadjuvant chemotherapy and not only chemotherapy-naïve patients.

1. Introduction

High-Grade Serous Cancer (HGSC), the most common and deadly histological subtype of ovarian cancer [1], is also the most lethal gynecologic cancer [1]. Recent findings point to the significant clinical and molecular heterogeneity of HGSC [2,3]. In addition to the conventional

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prognostic factors such as stage, grade, histological type, and residual disease after surgery [3], recent studies have assessed the utility of predictive molecular biomarkers [4–7]. Despite these efforts, no clinically validated predictive biomarkers are available that complement individual patient stratification.

The homologous recombination (HR) DNA repair pathway is essential to preserve genomic integrity, by allowing accurate repair of double-strand DNA breaks, and has been associated with the response to treatment and the overall outcome of patients with HGSC [8]. Germline and somatic molecular genetic defects in the HR pathway occur in up to 50% of HGSC cases [2]. Mutations in *BRCA1* and *BRCA2* (germline or somatic), which include gene silencing due to hypermethylation of BRCA1 promoter [9], account for the most frequent genetic deficiencies in HR pathway in HGSC tumors [2], and are associated with better overall responses to standard of care platinum-based chemotherapy and longer progression-free survival relative to non-mutated BRCA1/2 cases [10,11]. Somatic genetic defects in other HR pathway genes, such as *RAD51* and *BRIP1*, have also been correlated with response to treatment [2,12,13]. The high frequency of loss of function somatic mutations in these other HR-related genes in HGSC tumors led to the concept of "BRCAness", which refers to the presence of molecular genetic phenotypes similar to that observed in *BRCA1/2*-mutated cells [14–16].

The advent of new drugs, such as poly ADP ribose (PARP) inhibitors takes the advantage of the HR deficiency of HGSC tumors and improved progression free survival (PFS) has been shown in clinical trials of HGSC patients [17]. As PARP inhibitors are introduced into clinical settings, it is important to further define molecular biomarkers of BRCAness associated with improved response rates and survival, leading to a personalized approach for the treatment of HGSC. Towards this goal, this study has correlated the expression of HR pathway genes with clinical outcomes in patients with HGOC that were selected based on exposure to chemotherapy at debulking surgery.

2. Materials and methods

2.1. Cohorts

HGOC tumors were obtained from a cohort of 96 patients who underwent staging and debulking surgery either upfront (PDS, $n = 55$) or after neoadjuvant therapy (NACT, $n = 41$) platinum-based combination chemotherapy. The study was approved by the Jewish General Hospital Research Ethics Board (protocol #15-070). All patients participating in this study gave informed written consent and tissue samples were kept in the gynecologic oncology tumor bank (ethics board protocol #03-041).

For each patient, we collected information regarding age, body mass index (BMI), histologic type and tumor grade, FIGO stage, extent of cytoreduction and residual disease after surgery, type of chemotherapy used in first line of treatment, serum CA 125 levels, and clinical response to treatment.

During the surveillance period, as part of routine clinical practice, follow-up examinations including serum CA125 levels, were performed at four-month intervals during the first two years from diagnosis, followed by every six months for up to five years and then yearly. Imaging was only performed if clinically indicated.

2.2. Gene expression assays

Prior to RNA extraction, 8–12 mm sections from the fresh frozen surgical sections were cut and stained with hematoxylin and eosin (H&E). A gynecological pathologist verified each sample for histology and selection for high tumor cell content. There was no different in tumor cell content between the two groups (PDS and NACT). RNA was then extracted from the cancer cells using the Qiagen RNeasy kit (Qiagen, Toronto, ON). RNA concentration and purity was assessed spectrophometrically using a NanoDrop ND-100 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). For the gene expression analysis, we used the NanoString-based Pancancer Pathway Panel (nanoString Technologies, Seattle, WA, USA). This panel has 770 genes shown to be implicated in various cancer types, curated from data in The Cancer Genome Atlas (TCGA). Gene expression for each sample was captured using the nCounter SPRINT Profiler (nanoString Technologies, Seattle, WA, USA). Details about the normalization procedure of the resulting data can be found in the supplementary material. For our subsequent analysis, we focused on the nineteen homologous recombination pathway genes measured in the panel: *C11orf30, RAD50, BRCA1, BRCA2, ATR, RAD51C, RAD51, FANCA, TP53, CHEK1, ATM, FANCE, CHEK2, NBN, FANCF, BRIP1, FANCC, PTEN, FANCB.* These 19 genes were selected based on previous publications showing their importance and clinical relevance in the HRD pathway [8,18].

2.3. Estimating cancer content of each sample

Tumors represent a heterogeneous mix of cancer and stromal cells, and gene expression in tumor specimens can be strongly affected by the proportion of the non-cancerous cells. We evaluated percent cancer cells present in a tumor specimen by a gynecological pathologist, and selected samples for both groups with over 80% cancer cells, as determined by the pathologist. In addition, using mutational data available for each sample, we estimated the cancer cell content of each sample by assaying somatic mutations, and including the results as variable in our analyses. DNA was extracted from the cancer samples using the DNeasy Blood and Tissue Kit (Qiagen, Toronto, ON, Canada). DNA concentration and purity was assessed using the NanoDrop ND-100 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Hotspot Next Generation Sequencing was performed using the Illumina MiSeq platform (Illumina Inc., San Diego, CA)[1]. The list of the 400 targeted regions can be found in the supplementary files (Supplementary File 1). The library was prepared using the Nimblegene TruSeqLT preparation kit (Illumina Inc., San Diego, CA). We estimated the frequency of the alternative allele using the somatic variant caller provided by Illumina in the "MiSeq Reporter Software." Where multiple variants were identified in the same gene of the same sample, we used the maximum frequency value. Variants were filtered for read depth and quality: all sequencing reads below or above two standard deviations from the mean were removed as well as reads with Quality Scores < 30. Quality Scores are a prediction of probability of a false positive variant call generated by the Reporter Software, and the cutoff of 30 represents an error probability of 1 in 1000. For the remaining variants, we performed principal component analysis on the alternative allele frequencies for all variants and found the eigenvector of the first principal component, which was then used as our estimate of the percent cancer content (percent_cancer).

2.4. Mutations in the HR pathway

Mutations affecting transcriptional activity might mediate gene expression levels. Therefore, to control for somatic mutations within the HR pathway that may affect gene expression levels and also be related to survival time, we also included an estimate of the overall mutation load in our analyses to control for this confounder. To obtain an estimate of the total somatic mutation load within the HR pathway, we created a weighted score (HR_score) based on the genes where an alternative allele was present as well as the type of mutations seen. That is, for each sample, we created a weighted sum

$$HR_{score} = \sum_{G \in HR} w_G I(AA_G)$$

where $I(AA_G) = 1$ if an alternative allele is present, and $I(AA_G) = 0$ otherwise. We coded the weights $w_G = 0.5$ for silent, synonmous and

[1] http://www.illumina.com/systems/miseq/software/miseq-reporter.html

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