Proliferation pathway aberration frequencies differ in BRCA1- and BRCA2-mutated ovarian cancers

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INTRODUCTION

The majority of women with advanced stage ovarian cancer are diagnosed with high grade serous carcinomas and they are usually treated with combination platinum-taxane-based chemotherapy. Unfortunately, most patients recur and receive additional platinum-based chemotherapy, that is followed by other cytotoxic agents at each subsequent recurrence. Survival statistics are dismal with only 27% of the patients alive ten years after diagnosis.

Included in the small group of patients with more favorable prognoses are those with germline BRCA1 or BRCA2 mutations (1-3). Improved outcomes have been attributed to biological differences in these tumors as well as greater sensitivity to platinum treatment (2,3). Moreover, recent work suggests that patients with BRCA2 mutated cancers may do better than BRCA1 mutants and have greater genomic instability(4). BRCA1/2 carriers comprise 8-18% of the serous ovarian cancer population and an additional 3-5% of patients are reported to have somatic mutations of these genes. The increased responsiveness to platinum agents has been linked to defects in DNA repair associated with loss of BRCA1 and BRCA2 function since they are components of the homologous recombination pathway for repair of double strand breaks in DNA.

HYPOTHESIS and APPROACH

We hypothesized that other factors may contribute to the more favorable prognoses of patients with BRCA-related cancers, including aberrations in cell cycle and survival pathways.

As a first step to address this hypothesis, we used data produced by The Cancer Genome Atlas (TCGA) project team, who recently performed large-scale genomic analyses of 489 high-grade, advanced-stage serous ovarian cancers. Their published study revealed the presence of aberrations in genes comprising key proliferation and survival pathways (RB-E2F, RAS, PI3K) in the majority of tumors (5).

Mutation and copy number data for genes in each of these pathways (Table 1) were analyzed to investigate pathway associations with BRCA1/2, BRCA1, BRCA2, or WT tumors as well as with overall survival (OS) for patients in these groups.

Table 1, Genes and corresponding alterations scored for each pathway

Pathway	HUGO	Alteration Type
RB-E2F	RB1	MUT/HOMDEL
	CDKN2A	MUT/HOMDEL
	CCND1	AMP
	CCND2	AMP
	E2F3	AMP
	CCNE1	AMP
PI3K	PIK3CA	MUT/AMP
	PTEN	MUT/HOMDEL
	AKT1	AMP
	AKT2	AMP
RAS	KRAS	MUT/AMP
	BRAF	MUT/AMP
	NF1	MUT/HOMDEL

MATERIALS and METHODS

Genomic data. Mutation and copy number (GISTIC) for 316 TCGA-profiled serous ovarian tumors were downloaded (Nov/2011) from The cBio Cancer Genomics Portal (http://www.cbioportal.org/). Mutation (MUT), amplification (AMP; i.e., >+2), or deletion (HOMDEL; i.e., -1) calls were made by the TCGA. Tumors with BRCA2 AMP (n=3) and 3 BRCA1/2 double MUT (n=3) were excluded from the analyses.

Pathway gene selection and tumor scores. Genes comprising each pathway were selected by their (a) TCGA-reported association with serous ovarian cancer (5) and (b) known role as components of that pathway. Given the high frequency of CNV, only genes with demonstrated significant mRNA-DNA copy number correlations were included. Each tumor was scored as aberrant for a pathway if any gene in that pathway was MUT, AMP, or HOMDEL.

Clinical data and survival analyses. Clinical characteristics and survival data for 316 patients were obtained from TCGA supplementary data (5). Patients were predominantly stage IIIC and most were initially treated with platinum-taxane doublets.

Statistical analyses. The Fishers exact test was used to compare categorical variables for scored pathway alteration status with BRCA1/2 mutation status. Median survival was estimated using the survdiff function of the survival library in the R statistical software. Cox proportional hazards models were used to report p-values for survival analyses. P values < 0.05 were considered significant.

Two-thirds of the tumors have genetic alterations in at least one pathway and ~25% in at least two. RB-E2F pathway alterations were found in ~40% of the tumors (**Figure 1A**).

Most of the genetic alterations are copy number variations **(Figure 1B**).

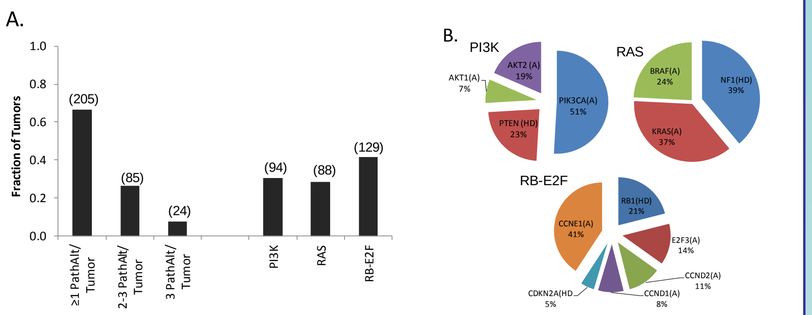

Figure 1. Pathway alteration (PathAlt) characteristics in 309 high grade serous ovarian carcinomas. A, Fraction of tumors with the indicated number of altered pathways/tumor. Parentheses, number of tumors/group. B, Alteration type and frequency for each gene in the PI3K, RB-E2F, and RAS pathway. Most frequent type of alteration for each gene: A, ampl; HD, homozygous deletion

Pathway alterations are significantly more frequent in BRCA1 than in BRCA2 mutant tumors (**Figure 2**).

There are no significant differences from WT if BRCA1 and BRCA2 tumors are grouped together.

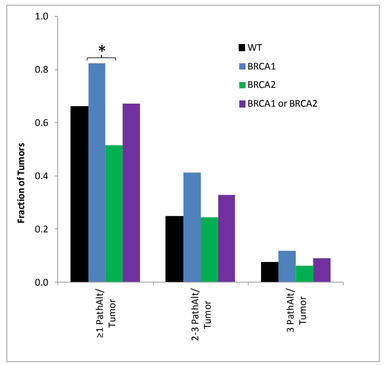

Figure 2. Pathway alteration frequency as a function of BRCA mutation status. 242 WT, 34 BRCA1, 33 BRCA2 mutant tumors were analyzed. Fisher's exact for 3-way analysis for one or more pathways: p= 0.027;
*2-way analyses: p < 0.01

Overall survival was not significantly associated with the presence of alterations in any pathway, RB-E2F, or RAS pathways (**Table 2**).

Alteration of the PI3K pathway was associated with improved outcomes in the entire patient cohort.

Pathway	Population	PathAlt Negative	PathAlt Positive	p-value
		Median (mos)	Median (Mos)	
PathAlt≥ 1	All	49.5	48.2	0.81
	WT	48.8	44.4	0.64
	BRCA1	35.1	68.7	0.13
	BRCA2	86.8	72.7	0.77
RAS	All	49.5	44.5	0.98
	WT	47.6	41.9	0.90
	BRCA1	66.5	58.9	0.64
	BRCA2	86.8	72.7	0.26
PI3K	All	44.9	53.9	0.02*
	WT	44.2	51.8	0.14
	BRCA1	49.2	NA	0.14
	BRCA2	86.8	53.9	1.00
RB-E2F	All	48.8	48.9	0.52
	WT	44.9	45.0	0.77
	BRCA1	66.5	68.7	0.68
	BRCA2	86.8	72.7	0.51

Table 2. Overall survival analysis for 254 patients. P-value from Cox proportional hazards model.

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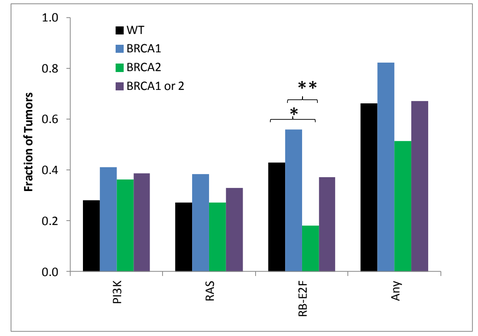
4. Yang D *et al* JAMA (2011) 306: 1557-1565.
5. TCGA Nature (2011) 474: 609- 615.

RB-E2F pathway is more frequently altered in WT and BRCA2 mutant tumors (**Figure 3A**).



Many tumors exhibit co-occurrence of alterations in at least two of these pathways (**Figure 3B**).

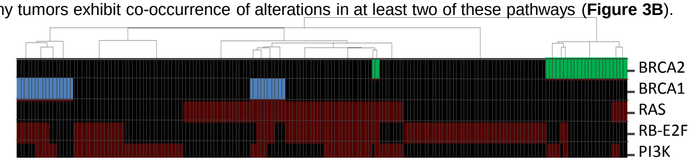


Figure 3. A. PI3K, RAS, RB-E2F pathway alteration frequency as a function of BRCA mutation status. Fisher's exact for 3-way analysis for RB-E2F: p= 0.004; 2-way analyses: *p < 0.01; **p <.005. B, Clustering analysis of 227 tumors based on pathway alteration status. Green, BRCA2; blue, BRCA1 mutant tumors; Red, tumors with alterations in RAS, PI3K, RB-E2F as indicated.

SUMMARY and CONCLUSIONS

1. Alteration of at least one gene in the RB-E2F, PI3K, or RAS pathways occurs in 2/3 of the 309 advanced stage serous carcinomas analyzed. Co-occurring alterations for more than one of these pathways occur in ~25% of those tumors.

2. There are few pathway alterations in BRCA2 mutant tumors. RB-E2F pathway alterations, in particular, were significantly more frequent in BRCA1 and WT than in BRCA2 mutant tumors.

3. No significant differences in OS were observed for patients with any pathway, RB-E2F or RAS pathway alteration. However, analysis of more data from this TCGA study as it matures, as well as another larger patient cohort is needed, particularly in the BRCA2 group, where these pathway mutations were infrequent.

4. BRCA2-mutated tumors have been reported to have a gene mutator phenotype (4) and greater overall genomic instability than BRCA1 tumors; despite such a high frequency of global DNA mutations, our results show a reduction in specific proliferation and survival pathway alterations in these tumors that may contribute to the more favorable prognoses of these patients.

5. Evidence for co-occurrence of >1 pathway alteration in some tumors is likely to impact responses to molecularly targeted therapies, particularly those directed towards these pathways, and should be considered in future efforts to identify response predictors for such drugs.