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LETTER TO THE EDITOR

Clinical impact of BRCA2 mRNA expression in high-grade serous ovarian cancer: validation using the TCGA cohort

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Introduction

Epithelial ovarian cancer (OC) is one of the leading causes of cancer death in women in the western world [1–3]. Approximately 10–15% of all epithelial OC are hereditary and the vast majority of them are due to germline mutations in *BRCA1* or *BRCA2* genes [4,5]. Furthermore, in a lower percentage of OC (5–7%) [6], so called somatic mutations in the one or the other of both *BRCA* genes occur. These aberrations are not hereditary but are restricted to cancer cells and are acquired during carcinogenesis. OC patients who carry a pathogenic germline or somatic *BRCA* mutation show better response to platinum based chemotherapy as well as to inhibitors of poly(ADP)-ribose polymerase (PARP) [7,8]. However, it is important to underline, that the role of *BRCA1* and *BRCA2* in DNA damage repair is different. While *BRCA1* is multifunctional, *BRCA2* is almost exclusively responsible for recruiting an essential homologous recombination protein RAD51C to double-strand break (DSB) sites, therefore the role of *BRCA2* in the DSB repair by homologous recombination (HR) is more direct and selective [9].

Besides *BRCA* mutation-status, the degree of expression of *BRCA1* and *BRCA2*, whose regulation is not completely understood so far, could contribute to the tumor pathogenesis and could influence responsiveness to platinum-based chemotherapy and treatment with PARP inhibitors and thus impact clinical outcome. In previous studies [10] we showed in 201 OC patients that low *BRCA1* expression was associated with favorable overall survival (OS) and low *BRCA2* expression with better progression-free survival (PFS) and OS. A subgroup analysis showed that these effects were confined to the *BRCA* wildtype cancers. Independency of all these findings was confirmed in multivariate Cox regression analysis.

The aim of the present confirmatory study was to validate and to underscore the pathophysiologic impact of *BRCA* expression in high-grade serous ovarian carcinoma (HGSOC) on a larger independent cohort.

Material and methods

Previous study and hypothesis

In our previous studies, we obtained ovarian tissue from 201 patients with OC at the time of primary debulking [10]. Total cellular RNA extraction, reverse transcription and PCR reactions were performed as previously described [11].

For survival analyses, patients were dichotomized into low and high mRNA-expression level groups by the optimal cut-off expression value calculated by the Youden's index based on a receiver operating characteristic (ROC) curve analysis for overall survival [12]. The optimal cutoff for *BRCA1* expression was therefore set at the 90th percentile and the optimal cut-off for *BRCA2* expression was the 21st percentile.

Validation of the prognostic impact of BRCA expression in an independent cohort

In the present study, we analyzed HGSOC dataset from The Cancer Genome Atlas (TCGA) project ($n = 581$) originally described in [13]. This publicly available dataset was accessed *via* http://firebrowse.org (courtesy of Broad Institute of MIT and Harvard) and includes clinical characteristics as well as *BRCA1/2* mutation status ($n = 466$) and *BRCA1/2* mRNA expression data (Affymetrix HuEx 1_0 st v2) ($n = 563$).

Non-parametric Mann–Whitney *U* test was applied to correlate *BRCA1/2* mutation status and *BRCA1/2* mRNA expression. Univariate Kaplan-Meier analyses and multivariable Cox survival analyses were used to explore the prognostic impact of *BRCA1/2* expression on survival. For survival analyses, patients were categorized into low and high *BRCA1/2* mRNA expression groups using the established optimal cutoffs [10]. *p* Values less than 0.05 were considered as statistically significant. Statistical analysis was performed using SPSS statistical software (version 20.0.0; SPSS Inc., Chicago, IL, USA).

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Results

The used TCGA dataset included clinical data on 581 patients with HGSOC. *BRCA1/2* mRNA expression data were available for 563 patients, *BRCA1/2* mutation status was recorded in 466 of these patients, 4.1% showed a *BRCA1* mutation and 2.6% had a *BRCA2* mutation. In this cohort *BRCA1* mutation was associated with lower *BRCA1* mRNA expression ($p = 0.016$) and *BRCA2* mutation was associated with lower *BRCA2* mRNA expression ($p = 0.001$) (data not shown).

In order to evaluate the clinical value of *BRCA1/2* mRNA expression we used the earlier established cutoff for discrimination between 'high' and 'low' expression [10]. In the univariate survival analysis low *BRCA2* mRNA expression ($<21^{st}$ percentile) was associated with longer PFS ($p = 0.029$) and longer OS ($p = 0.011$) (Figure 1). Cox-regression survival analysis confirmed the prognostic significance of *BRCA2* mRNA-expression for both PFS ($p = 0.021$) and OS ($p = 0.001$) (Table 1). A subgroup analysis revealed that the survival impact of low *BRCA2* mRNA expression was observed only in patients with *BRCA* wildtype tumors.

The previously described clinical impact of *BRCA1* expression was not confirmed in the herein evaluated cohort, as no statistically significant differences in either PFS or OS were revealed when the cohort was dichotomized at the predefined 90^{th} percentile. Therefore, we searched for an optimal cutoff for *BRCA1* expression specifically adjusted to the TCGA cohort. The best discriminative power was revealed at the 14^{th} percentile but even at this threshold, the observed differences did not reach statistical significance either for PFS or for OS.

Discussion

In the present confirmatory work, we were able to endorse the independent survival impact of *BRCA2* expression in HGSOC tumors for PFS as well as for OS in the large independent TCGA cohort. These results are in line with previous studies on *BRCA* expression in ovarian and breast cancer [14–17].

On the other hand, our preceding findings that low *BRCA1* expression on the transcriptome level was independently associated with favorable OS in HGSOC patients could not be confirmed in this large cohort. However, it is important to notice the limitations of the present dataset, such as incomplete clinical data, but also the rather unrealistic low number of *BRCA* mutated tumors as compared to data from literature as well as to our originally examined cohort [4–6,10,18,19]. Furthermore, the optimal cutoff to discriminate between 'low' and 'high' *BRCA* expression was the same for *BRCA2* in both cohorts, but not for *BRCA1* (90^{th} percentile in the original study vs. 14^{th} percentile in the present study). However, even at the best discriminatory level of the 14^{th} percentile neither for PFS nor for OS *BRCA1* transcript levels proved to be of statistical predictive value regarding disease outcome. This is probably due to multifaceted functional character of the BRCA1 protein, which has multiple functional domains and interacts with multiple proteins. Besides DNA repair BRCA1 is also involved in various crucial cellular processes, such as transcriptional regulation, cell cycle control, apoptosis and others more. Such a broad involvement in various cellular pathways, can easily have opposing effects on disease outcome and may preclude *BRCA1* mRNA

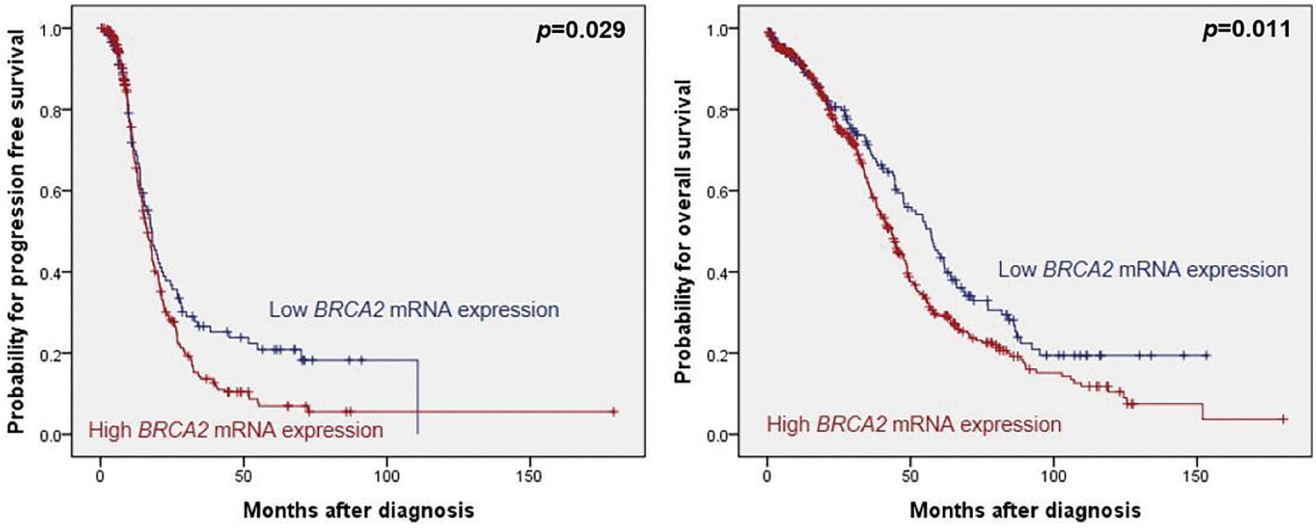

Figure 1. Low *BRCA2* mRNA expression is associated with better survival in HGSOC. *BRCA2* mRNA expression and PFS (a) and OS (b) in 563 HGSOC patients.

Table 1. Multivariate survival analysis in 563 OC patients.

		PFS ($n = 334$)			OS ($n = 463$)		
Variable		RR	(95 CI)	*p* Value	RR	(95 CI)	*p* Value
Age	≤50 years>	1.088	(0.81–1.46)	0.577	1.597	(1.19–2.15)	0.002
FIGO stage	I/II vs. III/IV	2.41	(1.22–4.76)	0.011	3.073	(1.25–7.52)	0.014
Residual disease after surgery	no vs. yes	1.161	(0.96–1.40)	0.121	1.399	(1.16–1.68)	<0.001
BRCA2 mRNA expression	low vs. high	1.409	(1.05–1.89)	0.021	1.592	(1.22–2.08)	0.001

expression as an ideal candidate for a clinically relevant biomarker in OC.

In contrast to *BRCA1*, *BRCA2* is crucial for recruiting an essential HR protein RAD51C to DSB sites, therefore the role of *BRCA2* in the DSB repair by HR is more direct and selective [9]. Nonetheless, *BRCA2* as well as *BRCA1* aberrations in tumor cells lead to HR deficiency (HRD) and the so called '*BRCA*ness' phenotype, which correlates with high platinum-sensitivity and thus predicts better clinical outcome in OC. Interestingly, several reports evidenced that patients with *BRCA2* mutation showed a greater survival advantage compared with patients carrying a *BRCA1* mutation. This was especially highlighted in the analyses done by Norquist *et al.* showing that *BRCA2* but not *BRCA1* mutation carriers exhibited a statistically significant improved PFS and OS when compared with patients without mutations [18]. In this evaluation the difference in PFS between *BRCA2* and *BRCA1* mutated OC was particularly pronounced in the first 3 years. With regard to OS, the improvement for *BRCA2* carriers was in fact due to a sustained long-term better OS paralleled with a distinctive decline in long-term OS for *BRCA1* mutated patients, which is probably due to the occurrence of secondary tumors [18,19]. Based on the herein revealed findings, we suppose that not only mutations in *BRCA2*, but also low *BRCA2* expression on transcriptome level may cause relevant HR pathway alterations.

Platinum-containing DNA crosslinking anticancer drugs are the most decisive pillar in the treatment of advanced OC and responsiveness to these compounds is faithfully mirrored in the patient's survival. As almost all patients from the present cohort received platinum-based chemotherapy, our data on the association of low *BRCA2* mRNA expression and improved PFS and OS may reflect platinum hypersensitivity, which is probably related to malfunction of HR in concerned tumors.

Clinical studies in primary and recurrent OC evidenced that the sensitivity of HGSOC to PARP inhibitor (PARPi) maintenance therapy is particularly related to the response to the actual platinum-based chemotherapy as well as to HR-deficiency [20–22]. Tumors with *BRCA1/2* mutation as well as cancers with *BRCA* unrelated HRD belong to high responders to PARPi treatment, while advantages of PARPi therapy in HR-proficient tumors are of a borderline clinical significance [22]. Our findings temp us to speculate that in *BRCA* wildtype OC, low *BRCA2* transcript levels indicate high sensitivity to platinum-based drugs. This biological feature may define a relevant fraction of HRD tumors, which in turn may also exhibit responsiveness to PARPi therapy although they are possibly not covered by the available HRD tests. Thus, we suggest that a simple determination of the *BRCA2* mRNA level with RT-PCR out of the tumor tissue could represent an additional tool to delignate a further HGSOC subgroup with sensitivity to PARP inhibitors.

Conclusions

Based on the large TCGA cohort we were able to validate the clinical impact of *BRCA2* expression on survival of HGSOC patients. Our findings may reflect higher platinum-sensitivity due to reduced capacity of DNA damage repair *via* HR in tissues with low *BRCA2* expression. We suppose that low *BRCA2* expression may define a relevant fraction of HR deficient tumors, which in turn may also exhibit responsiveness to PARP inhibitor therapy.

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Disclosure statement

The authors declare no conflict of interest.

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