BRCA1 and BRCA2 mRNA-expression prove to be of clinical impact in ovarian cancer

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BACKGROUND: Mutations in BRCA1 and BRCA2 are associated with better survival in ovarian cancer (OC) patients due to a better response to platinum-based chemotherapy. However, the impact of the BRCA1/2 mRNA-expression is not well characterized in OC.

PATIENTS AND METHODS: We investigated BRCA1/2 mRNA-expression in 12 non-neoplastic fallopian tubes and 201 epithelial OCs in relation to their clinical characteristics.

RESULTS: We found higher BRCA1/2 mRNA-expression in OCs compared to controls (P = 0.011, P < 0.001, respectively). BRCA1 mutated OCs exhibited lower BRCA1 (P = 0.014) but higher BRCA2 mRNA-expression (P = 0.001). Low BRCA1-expression was associated with favorable overall survival (OS) (P = 0.012) and low BRCA2-expression with better progression-free survival (PFS) and OS (P = 0.004, P = 0.001, respectively). A subgroup-analysis showed that this effect was confined only to the BRCA1-wildtype cancers. Cox-regression confirmed the prognostic significance of BRCA1-expression for OS (P = 0.028). Independence of the prognostic value of BRCA2-expression for PFS (P = 0.045) and OS (P = 0.015) was restricted to high-grade serous OCs. Fully platinum-sensitivity was characterized by lower BRCA1/2 mRNA-expression in BRCA1-wildtype cancers in comparison to platinum-refractory OC.

CONCLUSION: Our findings may reflect higher platinum-sensitivity due to reduced capacity of DNA damage repair in tissues with low BRCA1/2-expression. In this context, especially in BRCA-wildtype cancers both parameters could also be potential predictors for PARP-sensitivity.

British Journal of Cancer (2018) 119:683–692; https://doi.org/10.1038/s41416-018-0217-4

INTRODUCTION

BRCA1 and BRCA2 are tumor suppressor genes that are involved in cell growth inhibition, apoptosis, regulation of gene transcription and DNA damage repair through homologous recombination.1 Thus, germ line mutations in BRCA genes are considered to be associated with cancer susceptibility, especially with an increased risk of developing ovarian and breast cancer.2,3 Epithelial ovarian cancer (OC) is one of the leading causes of cancer death in women in the western world3–5 Approximately 5–10% of all epithelial OC are hereditary and at least two-third of them are due to BRCA1/2 mutations.6,7

The current opinion is that OC patients who carry a pathogenic BRCA mutation show better survival rates possibly due to a better response to platinum-based chemotherapy and inhibitors of poly(ADP)-ribose polymerase (PARP).6,9 However, there are also conflicting data, showing worse survival in hereditary OC cases or no significant difference in survival rates between patients with BRCA associated and sporadic epithelial OC.10–12 Such discrepancies could be due to different duration of follow-up, different histological type or a death caused by secondary malignancies.

Besides BRCA mutation-status, the expression of these genes could contribute to the tumor pathogenesis and therapeutical response. Currently there is lack of data on BRCA1/2 expression and their clinical significance in epithelial OC patients. The implication of BRCA-expression in BRCA-wildtype epithelial OC is also poorly studied but could be clinically relevant. We wondered whether the expression of BRCA1/2 on the transcriptome level could be a reliable predictor for platinum response and thus for the clinical outcome in OC patients.

In our study, we evaluated BRCA1/2-mRNA-expression in frozen tissues of 201 epithelial OC patients. We analyzed progression-free survival (PFS), overall survival (OS), the association between the BRCA-expression and mutation-status and methylation-status as well as FIGO stage and achievement of a complete resection during debulking surgery.

PATIENTS AND METHODS

Patients and samples

Ovarian tissue samples from 201 patients with OC obtained at primary debulking (patients were 24–90 years old; median age at

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Received: 26 April 2018 Revised: 12 July 2018 Accepted: 16 July 2018
Published online: 15 August 2018

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diagnosis was 62 years) and non-neoplastic tubal tissues from 12 patients obtained by elective salpingo-oophorectomy for benign conditions (patients were 30–73 years old, median age: 50 years) were collected and processed at the Department of Obstetrics and Gynecology of the Medical University of Innsbruck between 1989 and 2015 as described recently. Systemic treatment of OC patients consisted of six adjuvant cycles of platinum-based chemotherapy. We used a categorization which defines "platinum-refractory" as disease progressing during therapy or within one month after the last dose, "platinum-resistant" as disease progressing within 6 months, "partially platinum-sensitive" as disease progressing between 6 and 12 months, and "platinum-sensitive" as disease progressing with an interval of more than 12 months. Written informed consent was obtained from all patients before enrolment. The study was reviewed and approved by the Ethics committee of the Medical University of Innsbruck (reference number: AN2015-0038 346/4.17) and conducted in accordance with the Declaration of Helsinki. The median observation period of all patients was 1.6 years (0.03–73 years old, median age: 50 years) concerning the median overall survival. Clinicopathological characteristics are shown in Table 1.

RNA isolation and reverse transcription
Total cellular RNA extraction from and reverse transcription were performed as previously described. Primers and probes for BRCA2 [GenBank: NM_000059.3] were determined with the assistance of the computer program Primer Express (Life Technologies, Carlsbad, CA, USA). BRCA2 forward primer: 5′-GAA AAT CAA GAA AAA TCC TTA AAG GCT-3′; BRCA2 reverse-primer: 5′-GTA ATC GGC TCT AAA GAA ACA TGA TG-3′; BRCA2 TaqMan probe: 5′-FAM-AGC ACT CCA GAT GGC ACA ATA AAA GAT-3′-BHQ1. Primer sequences were selected from literature, also primers and probes for BRCA1 were purchased from Applied Biosystems (Foster City, CA, USA). DNA-methylation analysis an additional MethyLight reaction was selected from literature, also primers and probes for BRCA2 were selected from literature. Cases were scored as positive if a percentage of methylated reference (PMR) value of ≥4.0% was obtained, according to studies published in the literature.

Statistical analysis
To compare clinicopathological characteristics and BRCA1/2 mRNA-expression or BRCA1/2 mutation-status, the non-parametric Mann-Whitney U test or Kruskal-Wallis test or Chi-squared test were applied. The correlations between BRCA1/2 mRNA-expression were assessed by Spearman-rank correlation analyses. Progression-free survival (PFS) was defined as the time from diagnosis of the primary tumor to the histopathological confirmation of recurrence or metastases and overall survival (OS) as the time from diagnosis of the primary to tumor to death from any cause or to the last clinical inspection. Univariate Kaplan-Meier analyses and multivariable Cox survival analyses were used to explore the association of BRCA1/2 expression or with PFS and OS. 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 curve analysis for overall survival. 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).

RESULTS
We analyzed BRCA1/2 mRNA-expression levels in 201 OC tissues and 12 non-neoplastic fallopian tubes. We found 1.6-fold higher BRCA1 and 5.0-fold higher BRCA2 mRNA-expression levels in OC samples in comparison to control tissues (P = 0.011, P < 0.001, Fig. 1a, b).

Molecular and clinicopathological characteristics
Associations of BRCA1/2 mRNA-expression with clinicopathological characteristics are shown in Table 2. We found that BRCA2 mRNA-expression was associated with poor tumor differentiation as it increases with tumor grade (P < 0.001; Table 1). Higher BRCA2 mRNA-expression was observed in patients with any residual disease (P = 0.032; Table 1) in comparison to patients with no residual disease. The highest BRCA1/2 mRNA-expression levels were identified in endometrioid OCs in comparison to the other histologic subtypes (P = 0.025, and P = 0.005, respectively; Table 1). Ninety-one percent of the patients included in this study had type II tumors (N = 183) which showed higher, intratumoral BRCA1/2 mRNA-expression compared to type I tumors (P = 0.034 and P = 0.001, respectively; Table 1).

OC tissues with BRCA1-mutations showed lower BRCA1 mRNA-expression (P = 0.014) but higher BRCA2 mRNA-expression (P = 0.001) (Table 1; Fig. 1c, d) in comparison to tissues without BRCA1 mutations. No association between BRCA2 mutation-status and BRCA1/2 mRNA-expression was identified (Table 1). Among 201 OC patients 36 patients (18%) presented BRCA1-mutations, 11 patients (6%) BRCA2-mutations. Interestingly, there was no correlation between BRCA1 mutation-status and any clinicopathological characteristics. In the herein investigated cohort, no differences in the expression of BRCA-1/2-mRNA could be revealed for the various subtypes of mutations detected in the BRCA1/2 genes (data not shown).

As expected, we found an inverse association between BRCA1 DNA-methylation-status and BRCA1 mRNA-expression (P < 0.001;
Table 1. Association of BRCA1 and BRCA2 mRNA-expression and mutations with clinicopathological characteristics in 201 ovarian cancer patients

| Variable                  | Number (%) | mRNA expression values (arbitrary units) | Somatic mutations | DNA methylation status |
|---------------------------|------------|------------------------------------------|-------------------|------------------------|
|                           |            | BRCA1 Median (IQR) | P value | BRCA2 Median (IQR) | P value | Non-mutated (%) | Mutated (%) | P value | BRCA1 | Non-mutated (%) | Mutated (%) | P value | Unmethylated (%) | Methylated (%) | P value |
| Age                       |            |                           |          |                    |          |                |             |        |         |                |             |        |                |              |        |
| ≤62.3 years               | 101 (50%)  | 0.81 (0.53–1.30)        | 0.491    | 3.72 (2.14–6.15)   | 0.875    | 73 (73%)       | 27 (27%)    | 0.001  | 95 (94%)| 5 (5%)      | 0.743      | 88 (88%)| 12 (12%)       | 0.504       |
| >62.3 years               | 100 (50%)  | 0.86 (0.55–1.37)        |          | 3.65 (2.47–6.01)   |          | 90 (90%)       | 9 (9%)      |        | 93 (93%)| 6 (6%)      | 0.943      | 90 (91%)| 9 (9%)        |
| FIGO stage                |            |                           |          |                    |          |                |             |        |         |                |             |        |                |              |        |
| I/II                      | 50 (25%)   | 0.91 (0.57–1.35)        | 0.361    | 3.22 (2.08–4.87)   | 0.089    | 42 (84%)       | 7 (14%)     | 0.426  | 48 (96%)| 1 (2%)      | 0.219      | 48 (96%)| 2 (4%)        | 0.081       |
| III/IV                    | 151 (75%)  | 0.80 (0.53–1.30)        |          | 4.02 (2.67–6.17)   |          | 121 (80%)      | 29 (19%)    |        | 140 (93%)| 10 (7%)     | 0.871      | 130 (87%)| 19 (13%)      |
| Tumor grade               |            |                           |          |                    |          |                |             |        |         |                |             |        |                |              |        |
| 1                         | 14 (7%)    | 0.52 (0.43–1.01)        | 0.099    | 2.07 (1.19–2.92)   | <0.001   | 12 (86%)       | 2 (14%)     | 0.789  | 14 (100%)| 0 (0%)     | 0.624      | 13 (93%)| 1 (7%)        | 0.004       |
| 2                         | 90 (45%)   | 0.85 (0.56–1.26)        |          | 3.55 (2.22–5.35)   |          | 71 (80%)       | 18 (20%)    |        | 84 (94%)| 5 (6%)      | 0.871      | 87 (97%)| 3 (3%)        |
| 3                         | 95 (47%)   | 0.82 (0.58–1.55)        |          | 4.51 (3.00–7.30)   |          | 78 (83%)       | 16 (17%)    |        | 88 (94%)| 6 (6%)      | 0.943      | 76 (82%)| 17 (18%)      |
| n.a.                      | 2 (1%)     | –                         |          | –                   |          | –              |             |        | –             | –             | –       | –              |              | –       |
| Residual disease          |            |                           |          |                    |          |                |             |        |         |                |             |        |                |              |        |
| Macroscopically tumor-free| 100 (50%)  | 0.87 (0.58–1.34)        | 0.261    | 3.36 (2.04–5.16)   | 0.032    | 82 (82%)       | 17 (17%)    | 0.588  | 92 (92%)| 7 (7%)      | 0.399      | 94 (95%)| 5 (5%)        | 0.013       |
| Any tumor residual        | 95 (47%)   | 0.78 (0.51–1.33)        |          | 3.99 (2.82–6.23)   |          | 75 (79%)       | 19 (20%)    |        | 90 (95%)| 4 (4%)      | 0.743      | 79 (84%)| 15 (16%)      |
| n.a.                      | 6 (3%)     | –                         |          | –                   |          | –              |             |        | –             | –             | –       | –              |              | –       |
| Histology                 |            |                           |          |                    |          |                |             |        |         |                |             |        |                |              |        |
| HGSOC                     | 129 (64%)  | 0.79 (0.55–1.31)        |          | 4.13 (2.59–6.16)   | 0.025    | 102 (79%)      | 25 (19%)    | 0.848  | 117 (91%)| 10 (8%)     | 0.314      | 114 (89%)| 14 (11%)      | 0.993       |
| LGSOC                     | 12 (6%)    | 0.48 (0.38–0.67)        |          | 2.05 (1.16–2.87)   |          | 10 (83%)       | 2 (17%)     |        | 12 (100%)| 0 (0%)     | 0.621      | 11 (92%)| 1 (8%)        |
| Endometrioid              | 45 (22%)   | 1.05 (0.68–1.48)        |          | 4.44 (2.91–6.40)   |          | 37 (82%)       | 8 (18%)     |        | 44 (98%)| 1 (2%)      | 0.340      | 40 (89%)| 5 (11%)       |
| Clear cell                | 11 (5%)    | 0.97 (0.74–1.44)        |          | 2.88 (2.00–4.56)   |          | 10 (91%)       | 1 (9%)      |        | 11 (100%)| 0 (0%)      | 0.290      | 9 (90%) | 1 (10%)       |
| Unknown                   | 4 (2%)     | –                         |          | –                   |          | –              |             |        | –             | –             | –       | –              |              | –       |
| Ovarian cancer type       |            |                           |          |                    |          |                |             |        |         |                |             |        |                |              |        |
| Type I                    | 14 (7%)    | 0.52 (0.43–1.00)        |          | 2.07 (1.19–2.92)   | 0.034    | 12 (86%)       | 2 (14%)     | 0.676  | 14 (100%)| 0 (0%)     | 0.342      | 13 (93%)| 1 (7%)        | 0.650       |
| Type II                   | 183 (91%)  | 0.85 (0.57–1.36)        |          | 4.09 (2.63–6.16)   | 0.001    | 147 (80%)      | 34 (19%)    |        | 170 (93%)| 11 (6%)     | 0.743      | 161 (89%)| 20 (11%)      |
| Variable          | Number (%) | mRNA expression values (arbitrary units) | Somatic mutations | DNA methylation status |
|-------------------|------------|------------------------------------------|-------------------|------------------------|
|                   |            | BRCA1 Median (IQR)                         | BRCA2 Median (IQR) | BRCA1 Non-mutated (%) | BRCA2 Non-mutated (%) | BRCA1 Mutated (%) | BRCA2 Mutated (%) | Unmethylated (%) | Methylated (%) | P value |
| Unknown           | 4 (2%)     | 0.90 (0.57–1.40)                          | –                  | –                      | –                      | –                  | –                  | –                  | –                  | –                  |
| BRCA1 mutation    |            |                                          | –                  | –                      | –                      | –                  | –                  | –                  | –                  | –                  |
| Wild type         | 163 (81%)  | 0.90 (0.57–1.40)                          | 3.48 (2.23–5.48)   | 0.014                  | 0.001                  | 141 (87%)          | 21 (13%)          | 0.024              |
| Mutate            | 36 (18%)   | 0.66 (0.42–0.89)                          | 5.92 (3.27–8.38)   | –                      | –                      | 35 (100%)          | 0 (0%)            | –                  | –                  | –                  |
| n.a.              | 2 (1%)     | –                                         | –                  | –                      | –                      | –                  | –                  | –                  | –                  | –                  |
| BRCA2 mutation    |            |                                          | –                  | –                      | –                      | –                  | –                  | –                  | –                  | –                  |
| Wild type         | 188 (94%)  | 0.82 (0.53–1.29)                          | 3.84 (2.46–6.16)   | 0.073                  | 0.346                  | 166 (89%)          | 20 (11%)          | 0.862              |
| Mutate            | 11 (6%)    | 1.59 (0.59–2.39)                          | 3.40 (1.97–4.18)   | –                      | –                      | 10 (91%)           | 1 (9%)            | –                  | –                  | –                  |
| n.a.              | 2 (1%)     | –                                         | –                  | –                      | –                      | –                  | –                  | –                  | –                  | –                  |
| BRCA1/2 mutation  |            |                                          | –                  | –                      | –                      | –                  | –                  | –                  | –                  | –                  |
| Wild type         | 152 (76%)  | 0.88 (0.55–1.33)                          | 3.48 (2.26–5.51)   | 0.178                  | 0.015                  | 131 (87%)          | 20 (13%)          | 0.033              |
| Mutate            | 47 (24%)   | 0.68 (0.44–1.35)                          | 5.14 (2.80–7.55)   | –                      | –                      | 45 (98%)           | 1 (2%)            | –                  | –                  | –                  |
| n.a.              | 2 (1%)     | –                                         | –                  | –                      | –                      | –                  | –                  | –                  | –                  | –                  |
| BRCA1 DNA methylation |       | Unmethylated 178 (89%) | 0.88 (0.58–1.35)   | <0.001                 | 0.001                  | –                  | –                  | –                  | –                  | –                  |
| Methylated        | 21 (11%)   | 0.20 (0.14–0.46)                          | 5.52 (3.99–8.21)   | –                      | –                      | –                  | –                  | –                  | –                  | –                  |
| BRCA2 DNA methylation |       | Unmethylated 168 (100%) | 0.78 (0.53–1.28)   | 3.68 (2.36–6.01)       | –                      | –                  | –                  | –                  | –                  | –                  |
| Methylated        | 0 (0%)     | –                                         | –                  | –                      | –                      | –                  | –                  | –                  | –                  | –                  |

Bold values indicate $P < 0.05$
Table 2. Univariate survival analysis in 201 ovarian cancer patients

| Variable                  | Progression-free survival | Overall survival |
|---------------------------|---------------------------|------------------|
|                           | Median, years (95% CI)    | P value          |
|                           |                           | Median, years (95% CI) | P value |
| A                         |                           |                  |         |
| Age ≤62.3 years           | 2.05 (1.47–2.63)          | 0.805            | 8.20 (5.63–10.78) | **0.006** |
| Age >62.3 years           | 1.81 (1.13–2.50)          |                  | 3.35 (2.68–4.02)  |         |
| FIGO stage                |                           |                  |         |
| I/II                      | n.r.                      | **<0.001**       | n.r.     |         |
| III/IV                    | 1.48 (1.10–1.86)          | 3.62 (3.06–4.18) |         |
| Tumor grade               |                           |                  |         |
| 1/2                       | 2.05 (1.23–2.87)          | 6.24 (2.82–9.67) | 0.057   |
| 3                         | 1.97 (1.16–2.78)          | 3.62 (3.03–4.21) |         |
| Residual disease          |                           |                  |         |
| Macroscopically tumor-free| n.r.                      | **<0.001**       | 13.03 (n.r.) | **<0.001** |
| Any tumor residual        | 1.25 (1.06–1.44)          | 2.68 (1.83–3.53) |         |
| Histology                 |                           |                  |         |
| HGSOC                     | 1.77 (1.35–2.18)          | **0.027**        | 3.62 (3.13–4.12) | **0.006** |
| LGSOC                     | n.r.                      |                  | n.r.     |         |
| Endometrioid              | 5.98 (n.r.)               | 11.06 (n.r.)     |         |
| Clear cell                | 1.81 (1.10–2.53)          | 2.72 (n.r.)      |         |
| Ovarian cancer type       |                           |                  |         |
| Type I                    | n.r.                      | 0.068            | n.r.     | 0.022   |
| Type II                   | 1.91 (1.52–2.29)          | 3.82 (2.11–5.52) |         |
| BRCA1 DNA methylation     |                           |                  |         |
| No                        | 2.00 (1.41–2.59)          | 4.54 (2.55–6.53) | 0.521   |
| Yes                       | 1.95 (0.46–3.45)          | 4.89 (2.25–7.53) |         |
| BRCA1 mRNA expression     |                           |                  |         |
| Low                       | 2.02 (1.38–2.65)          | 5.74 (3.63–7.85) | **0.012** |
| High                      | 0.87 (0.00–1.82)          | 1.66 (0.00–5.00) |         |
| Subgroup analysis         |                           |                  |         |
| BRCA1 non-mutated         |                           |                  |         |
| Low                       | 2.06 (1.03–3.10)          | 0.169            | 4.89 (2.88–6.90) | **0.023** |
| High                      | 0.87 (0.00–1.82)          |                  | 1.67 (0.00–5.00) |         |
| BRCA1 mutated             |                           |                  |         |
| Low                       | 2.00 (1.60–2.39)          |                  | 8.20 (4.52–11.88) | –         |
| High                      | –                        | –                | –        | –        |
| BRCA2 mRNA expression     |                           |                  |         |
| Low                       | n.r.                      | **0.004**        | n.r.     | **0.001** |
| High                      | 1.81 (1.41–2.22)          | 3.70 (2.78–4.62) |         |
| B                         |                           |                  |         |
| Age ≤62.3 years           | 1.98 (1.34–2.62)          | 0.590            | 6.86 (4.23–9.50) | **0.012** |
| Age >62.3 years           | 1.77 (1.27–2.26)          |                  | 3.32 (2.63–4.01) |         |
| FIGO stage                |                           |                  |         |
| I/II                      | n.r.                      | **<0.001**       | n.r.     | **<0.001** |
| III/IV                    | 1.47 (1.10–1.84)          | 3.43 (3.01–3.85) |         |
| Tumor grade               |                           |                  |         |
| 2                         | 1.90 (1.40–2.41)          | 5.74 (2.79–8.68) | 0.177   |
| 3                         | 1.95 (1.20–2.71)          | 3.55 (3.01–4.08) |         |
| Residual disease          |                           |                  |         |
| Macroscopically tumor-free| 5.98 (n.r.)               | **<0.001**       | 13.03 (n.r.) | **<0.001** |
| Any tumor residual        | 1.25 (1.06–1.44)          | 2.55 (1.58–3.51) |         |
| Histology                 |                           |                  |         |
| HGSOC                     | 1.77 (1.35–2.18)          | 3.62 (3.13–4.12) | **0.029** |
| HGEOC                     | 5.11 (1.10–2.53)          | 8.94 (5.85–12.02) |         |
| HGCCOC                    | 1.81 (1.10–2.53)          | 2.72 (n.r.)      |         |
| BRCA1 DNA methylation     |                           |                  |         |
| No                        | 1.91 (1.35–2.47)          | 3.92 (2.09–5.76) | 0.531   |
| Yes                       | 1.95 (0.51–3.40)          | 3.71 (1.65–5.77) |         |
| BRCA1 mRNA expression     |                           |                  |         |
| Low                       | 1.98 (1.34–2.62)          | 4.89 (2.74–7.04) | **0.004** |
| High                      | 0.87 (0.02–1.72)          | 1.65 (0.68–2.63) |         |
| Subgroup analysis         |                           |                  |         |
| BRCA1 non-mutated         |                           |                  |         |
| Low                       | 1.95 (1.07–2.84)          | 0.094            | 3.94 (1.94–5.94) | **0.011** |
| High                      | 0.87 (0.02–1.72)          | 1.65 (0.68–2.63) |         |
| BRCA1 mutated             |                           |                  |         |
| Low                       | 2.00 (1.80–2.19)          |                  | 8.20 (3.28–13.12) | –         |
Table 2 continued

| Variable                  | Progression-free survival | Overall survival |
|---------------------------|---------------------------|-----------------|
|                           | Median, years (95% CI)    |                |
|                           | P value                   | Median, years (95% CI) | P value |
| **BRCA2 mRNA expression**|                          |                 |
| Low                       | n.r.                      | 0.006           | n.r.     | 0.002 |
| High                      | 1.81 (1.40–2.23)          | 3.62 (2.97–4.27) |
| **Subgroup analysis**     |                          |                 |
| **BRCA1 non-mutated**     |                          |                 |
| Low                       | n.r.                      | 0.022           | n.r.     | 0.005 |
| High                      | 1.65 (1.19–2.11)          | 3.43 (2.96–3.90) |
| **BRCA1 mutated**         |                          |                 |
| Low                       | 7.49 (n.r.)               | 12.58 (n.r.)    | 0.461 |
| High                      | 1.98 (1.62–2.34)          | 6.03 (0.28–11.78) |
| **C**                     |                          |                 |
| Age ≤62.3 years           | 1.81 (1.20–2.42)          | 5.74 (2.89–8.58) | 0.035 |
| >62.3 years               | 1.68 (1.07–2.29)          | 3.32 (2.74–3.89) |
| FIGO stage                |                          |                 |
| I/II                      | n.r.                      | <0.001          | 7.78 (1.38–14.18) | 0.049 |
| III/IV                    | 1.47 (1.09–1.84)          | 3.55 (3.15–3.94) |
| Tumor grade               |                          |                 |
| 1/2                       | 1.65 (1.10–2.20)          | 3.82 (3.15–6.29) | 0.257 |
| 3                         | 1.95 (1.49–2.42)          | 3.55 (3.10–3.99) |
| Residual disease          |                          |                 |
| Macroscopically tumor-free| 3.57 (0.00–7.23)          | <0.001          | 8.17 (2.26–14.08) | <0.001 |
| Any tumor residual        |                          |                 |
| **BRCA1 DNA methylation** |                          |                 |
| No                        | 1.77 (1.33–2.21)          | 3.62 (3.07–4.17) | 0.750 |
| Yes                       | 1.95 (0.92–2.99)          | 3.71 (2.02–5.40) |
| **BRCA1 mRNA expression** |                          |                 |
| Low                       | 1.84 (1.51–2.17)          | 3.71 (2.73–4.69) | 0.027 |
| High                      | 0.87 (0.14–1.60)          | 1.65 (0.42–2.88) |
| **Subgroup analysis**     |                          |                 |
| **BRCA1 non-mutated**     |                          |                 |
| Low                       | 1.68 (1.20–2.17)          | 3.62 (3.07–4.18) | 0.051 |
| High                      | 0.87 (0.14–1.60)          | 1.65 (0.42–2.88) |
| **BRCA1 mutated**         |                          |                 |
| Low                       | 1.98 (1.70–2.26)          | –               | 6.03 (1.35–10.71) |
| High                      | –                         | –               | – |
| **BRCA2 mRNA expression** |                          |                 |
| Low                       | n.r.                      | 0.006           | n.r.     | 0.005 |
| High                      | 1.67 (1.31–2.03)          | 3.39 (3.01–3.77) |
| **Subgroup analysis**     |                          |                 |
| **BRCA1 non-mutated**     |                          |                 |
| Low                       | n.r.                      | 0.016           | n.r.     | 0.001 |
| High                      | 1.46 (1.06–1.87)          | 3.39 (2.87–3.91) |
| **BRCA1 mutated**         |                          |                 |
| Low                       | 7.49 (n.r.)               | 12.58 (n.r.)    | 0.571 |
| High                      | 1.98 (1.69–2.27)          | 4.08 (0.00–9.78) |

The significance level (P) was determined by log-rank test HGCCCOC high grade clear cell ovarian cancer, HGEOC high grade endometrioid ovarian cancer, HGSOCSO high grade serous ovarian cancer, LGSOCL low grade serous ovarian cancer, n.r. not reached A: Progression free and overall survival in 201 ovarian cancer patients B: Subgroup analysis: progression free and overall survival in 183 high grade OC patients C: Subgroup analysis: progression free and overall survival in 129 high grade serous OC patients. The optimal cutoff points for BRCA1/2 mRNA expression were calculated by the Youden index for overall survival (BRCA1 expression: low/ high; <90th percentile; BRCA2 expression: low/ high; >21st percentile). Bold values indicates P < 0.05.

Table 1; Fig. 1e). Interestingly we observed a direct association between BRCA1 DNA-methylation and BRCA2 mRNA-expression (P = 0.001; Table 1; Fig. 1f). Eighteen percent of undifferentiated tumors (tumor grade 3, N = 17) and 16% of tumors from patients with any residual disease (N = 15) were positive for BRCA1 DNA-methylation (P = 0.004, and P = 0.013; respectively; Table 1). Epigenetic silencing of BRCA1 was mutually exclusive with BRCA1 mutations (Table 1). No BRCA2 DNA-methylation was detected in the analyzed OC tissue samples.

Survival analysis of BRCA1 and BRCA2 mRNA-expression and DNA-methylation-status

In order to investigate the prognostic value of BRCA1/2 mRNA-expression levels we identified the optimal threshold for “high” and “low” expression using Youden’s index. Univariate survival analysis in the entire cohort showed that a lower BRCA1 mRNA-expression (<90th percentile) was associated with a favorable OS (P = 0.012; Table 2A; Fig. 2a). This was also observed in the subgroups of high grade OC (P = 0.004; Table 2B) and high grade serous OC (P = 0.027; Table 2C). A detailed analysis revealed that these prognostic effects were only observed in patients with BRCA1 non-mutated (wildtype) tumors in all patients (P = 0.023; Table 2A; Fig. 2b) and in high grade OC patients (P = 0.011; Table 2B). Lower BRCA2-expression levels (<21st percentile) were associated with favorable PFS and OS in the entire cohort (P = 0.004; P = 0.001; Table 2A; Fig. 3a, b), in high grade OC (P = 0.006; P = 0.002; Table 2B) and in high grade serous OC (P = 0.006; P = 0.001; Table 2C). A detailed analysis showed again the prognostic relevance of low BRCA2 mRNA-expression only in patients with BRCA1 non-mutated tumors. This was true for the entire patient cohort (PFS: P = 0.012; OS: P = 0.002; Table 2A; Fig. 3c, d), high grade OC (PFS: P = 0.022; OS: P = 0.005; Table 2B) and high grade
serous OC (PFS: $P = 0.016$; OS: $P = 0.001$; Table 2C). No impact of $BRCA1$ gene promoter methylation-status on progression-free survival and overall-survival rates was found (Table 2).

Cox-regression survival analysis confirmed the prognostic significance of $BRCA1$ mRNA-expression for OS in the whole cohort (HR$_{death}$ 2.0 (1.1–3.7), $P = 0.028$; Table 3A) but not in high grade serous OC (Table 3B). However, independency of the prognostic value of $BRCA2$ mRNA-expression was approved in patients with high grade serous OC, representing 64% of the entire cohort, as well for PFS (HR$_{progression}$ 2.4 (1.0–5.7), $P = 0.045$) as for OS (HR$_{death}$ 2.9 (1.2–6.8), $P = 0.015$); (Table 3B).

To answer the question whether the identified favorable survival in tumors with low $BRCA1/2$-mRNA expression may be interpreted by platinum-sensitivity we compared the expression levels in $BRCA1$-wildtype tumors from platinum-refractory and fully platinum-sensitive patients. We found statistically significant lower $BRCA1$- and $BRCA2$ mRNA-expression levels in platinum-sensitive tumors ($P = 0.004$ and $P = 0.045$; Supplemental Fig. 1).

**DISCUSSION**

$BRCA1/2$ belong to genes that play key roles in the homologous recombination repair, which represents the main mechanism to repair DNA double-strand breaks. While $BRCA1$ is multifunctional, $BRCA2$ functions almost exclusively in homologous recombination by recruiting an essential homologous recombination protein RAD51C to double-strand break sites. Our investigations revealed higher $BRCA1/2$-expression on the transcriptome level in OC tissues in comparison with non-neoplastic fallopian tube tissue. The cause of this finding may be the higher proliferation

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**Fig. 1** $BRCA1$ and $BRCA2$ mRNA-expression in ovarian tissues. **a** $BRCA1$ mRNA-expression in 12 non-neoplastic fallopian tubes and 192 OC tissues, **b** $BRCA2$ mRNA-expression in 11 non-neoplastic fallopian tubes and 168 OC tissues. **c** $BRCA1$ and **d** $BRCA2$ mRNA-expression according the $BRCA1$ mutation-status. **e** $BRCA1$ and **f** $BRCA2$ mRNA-expression according the $BRCA1$ DNA-methylation-status.
rate in malignant tissues which together with genetic instability may increase the need for more DNA damage repair. In accordance higher BRCA1/2-expression was also found in high-grade (Type II) tumors. This notion is supported by Gudas et al. who suggest that the upregulation of BRCA1-expression by steroid hormones is caused indirectly by increasing proliferation of breast cancer cells.21

Multivariate Cox-regression analysis showed a favorable OS for low BRCA1-expression in the whole cohort of included patients. For BRCA2-expression in the subgroup of high grade serous OC an independent, prognostic value in terms of PFS and OS was confirmed. These findings could be explained by a reduced capacity of DNA damage repair via homologous recombination in cancers with low BRCA1/2 expression, enhancing the therapeutic effects of DNA-crosslinking agents such as platinum compounds. In fact, low BRCA1/2 mRNA-expression in BRCA1-wildtype cancers was associated with fully platinum-sensitive disease and high expression was evidenced in platinum-refractory disease. These BRCA mRNA-expression data are in line with the plethora of data showing that OC patients carrying a BRCA1 or 2 germline mutation exhibit high responsiveness to platinum-based chemotherapy consecutively associated with an improved clinical outcome. In recurrent OC, similar beneficial therapeutic effects in BRCA mutation carriers have been reported for PARP-inhibitors and for trabectedin a drug that is crosslinking the DNA in the minor groove.

Until to date there are only very few studies on BRCA-expression in OC and these are only dealing with the expression of BRCA1 but not with that of BRCA2. In a retrospective analysis of OC specimens obtained from patients included in the GOG-172 study comparing intraperitoneal (IP) with intravenous (i.v.) platinum/taxane chemotherapy, Lesnock et al. assessed BRCA1-expression on the protein level with regard to clinical outcome and responsiveness to chemotherapy considering especially the efficacy of the high loco-regional platinum doses reached by IP administration. The authors revealed that patients with cancers exhibiting BRCA1-immunostaining in less than 10% of the tumor cells was the only subgroup exhibiting a significant benefit in OS from a platinum-based IP chemotherapy.22 In addition, Carser et al. found also a strong response improvement to classical i.v. platinum-based chemotherapy in tumors with absent or low BRCA1-expression in immunohistochemistry. This effect was translated into a favorable PFS and OS in affected patients and the predictive value of BRCA1-immunostaining was confirmed in the multivariate analysis.23 These considerations were indirectly confirmed by Swisher et al., showing that in primary BRCA1-mutated OCs, recurrent platinum-resistant tumors exhibited secondary genetic changes within the BRCA1 gene, which interestingly were accompanied by restored expression of BRCA1-protein.24 Furthermore, Quinn et al. reported from an in vitro and in vivo approach that inhibition of BRCA1-expression via siRNA knock-down leads to increased sensitivity to platinum therapy but impaired responsiveness to anti-microtubule agents such as taxanes. In a small series of patients, they corroborated their in vitro data by showing a significant improved OS in patients with tumors exhibiting low levels of BRCA1-mRNA.25 Also in this study BRCA2-expression has not been accessed.

In breast cancers exhibiting low BRCA2 mRNA-levels, a significantly higher 5-year disease free survival rate was shown.26 Interestingly, our study emerged that in BRCA1-mutated OC the expression of BRCA1-transcripts was lower, but in contrary those of BRCA2 were significantly higher as compared with BRCA1-wildtype cancers. Furthermore, also down regulation of BRCA1-transcripts by methylation of the BRCA1-promotor was associated with increased BRCA2 mRNA levels. In contrast in cancers carrying a BRCA2-mutation, no up- or down-regulation of the BRCA1/2 mRNA was found. However, the latter findings should be interpreted with caution due to the low number of BRCA2-mutated cancers within our cohort. The reason of the “compensatory” upregulation of BRCA2-mRNA in low BRCA1-expressing cancers remains speculative because there is no exact knowledge on how the BRCA protein expression is regulated either in normal or in malignant tissues. High BRCA-expression could determine a distinct phenotype with a high constitutive expression or could reflect a transitory upregulation triggered by various situations (e.g., proliferative or genomic stress). Thus, it is theoretically possible that the functional loss of multifunctional BRCA1 is leading to genetically instable cancers requiring higher BRCA2 recruitment for repeated double-strand break repair.

In BRCA1 we found DNA-methylation in 11% of all tumors, which is in accordance with previously published data.27 We could not identify a prognostic relevance of BRCA1 DNA-methylation for PFS and OS consistent with recent studies.28,29 Our data show that low BRCA1/2 mRNA-expression confers platinum-hypersensitivity to OCs. As clinical studies in recurrent OC recently evidenced that the sensitivity of high grade serous OC to PARP-inhibitor maintenance therapy is particularly related to
Fig. 3 Kaplan Meier survival analysis and BRCA2 mRNA-expression in OC patients according the 21st percentile as cut-off value. a Progression-free survival and b overall survival in 168 OC patients. c Progression-free survival and d overall survival in 136 patients with BRCA1-wildtype tumors.

Table 3. Multivariable analysis in ovarian cancer patients

| Variable                          | Progression-free survival | Overall survival |
|----------------------------------|---------------------------|-----------------|
|                                  | HR of progression (95% CI)| P value         | HR of death (95% CI) | P value |
| A                                |                           |                 |                           |         |
| Age                              |                            |                 |                           |         |
| Low vs. high (<or>median age)    | –                         | –               | 1.9 (1.3–2.9)           | 0.001   |
| FIGO stage I/III vs. III/IV      | 2.6 (1.2–5.5)             | 0.013           | 1.1 (0.6–2.1)           | 0.692   |
| Residual disease after surgery   | 2.7 (1.6–4.6)             | <0.001          | 3.5 (2.1–6.0)           | <0.001  |
| Histology HGSOC vs. Others       | 0.9 (0.6–1.5)             | 0.675           | 0.6 (0.4–1.1)           | 0.087   |
| Ovarian cancer type               |                            |                 |                           |         |
| Type I vs. Type II               | –                         | –               | 0.9 (0.3–2.7)           | 0.829   |
| BRCA1 mRNA expression            | Low vs. high (<or>optimal cut-off) | 1.7 (0.8–3.5) | 0.028           |
| BRCA2 mRNA expression            | Low vs. high (<or>optimal cut-off) | 1.9 (1.0–3.7) | 0.058           |
| B                                |                            |                 |                           |         |
| Age                              | Low vs. high (<or>median age) | –               | 2.1 (1.3–3.4)           | 0.002   |
| FIGO stage I/III vs. III/IV      | 2.3 (0.8–6.3)             | 0.119           | 1.1 (0.5–2.4)           | 0.812   |
| Residual disease after surgery   | 2.4 (1.3–4.7)             | 0.008           | 3.2 (1.7–6.0)           | <0.001  |
| BRCA1 mRNA expression            | Low vs. high (<or>optimal cut-off) | –               | 1.7 (0.8–3.5) | 0.151   |
| BRCA2 mRNA expression            | Low vs. high (or>optimal cut-off) | 2.4 (1.0–5.7) | 0.045           |

The significance level was determined by Cox regression analysis HR hazard ratio. Bold values indicates P < 0.05.
the response to the actual platinum-based chemotherapy. Our data are tempting to speculate that BRCA1/2 mRNA levels may be reliable biomarkers to also predict responsiveness of cancers to PARP-inhibitors. The same may be true for other drugs whose effectiveness is related to platinum-sensitivity such as trabectedin.

ACKNOWLEDGEMENTS
We thank Inge Gaugg, Martina Fleischer and Annemarie Wiedemair for excellent technical assistance. This work was supported by the Verein zur Krebsforschung in der Frauenheilkunde (no grant number is applicable), the Österreichische Krebshilfe - Krebsgesellschaft Tirol (15010/2015) and AstraZeneca (NCR-15-11443).

AUTHOR CONTRIBUTIONS
H.F., A.G.Z., and C.M. developed the study concept, H.F., A.G.Z., C.M., and I.T. designed the project and edited the manuscript, I.T., V.W., C.D., G.S., S.W., S.S., and S.F.L. were involved in data acquisition, I.T., V.W., C.D., G.S., S.W., S.S., S.F.L., and H.F. performed quality control of data and algorithms, I.T., V.W., C.D., G.S., S.W., S.S., S.F.L., C.M., H.F., and A.G.Z. analyzed and interpreted data and reviewed the final manuscript, I.T. and H.F. performed statistical analyses, I.T., H.F., and A.G.Z. prepared the manuscript.

ADDITIONAL INFORMATION
Supplementary information is available for this paper at https://doi.org/10.1038/s41416-018-0217-4.

COMPETING INTERESTS
The authors declare no competing interests.

DATA AVAILABILITY
The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

ETHICAL APPROVAL
The study was reviewed and approved by the Ethics committee of the Medical University of Innsbruck (reference number: AN2015-0038 346/4.17) and the Medical University of Innsbruck (reference number: AN2015-0038 346/4.17) and conducted in accordance with the Declaration of Helsinki.

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