Associating BRCA1 hypermethylation with clinicopathological and molecular variables in triple-negative breast cancers

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**BACKGROUND**

The aim of this study was to investigate the correlation between BRCA1 methylation status and clinicopathological and molecular variables and outcome in patients with triple-negative breast cancer (TNBC) (Fig. 1).

**MATERIAL AND METHODS**

145 patients enrolled 2015-2018 in the Sweden Cancerome Network - Breast (SCAN-B) study (ClinicalTrials.gov ID NCT02306906) [3] were included. 109 had chemotherapy (>90% with sequential epirubicin + cyclophosphamide and a taxane, EC–T) according to national guidelines either as neoadjuvant (32%, NAC) or adjuvant (68%, ACT) therapy. Germline screening was performed in 54 patients with pathogenic BRCA1/2 variants in 11 cases. Microsatellite instability (MSI) and BRCA1 promoter methylation (BRCA1met) using pyrosequencing were investigated in all cases, including analyses of pre-treatment biopsies and surgical resections for NAC patients with residual disease (RD). Clinicopathological and molecular variables were obtained through clinical review and complementary RNA-seq data for all patients and tumor specimens (Table 1).

**RESULTS**

MSI and BRCA1met were observed in 2% and 17% of patients, respectively. BRCA1met was correlated with younger age and the PAM50 Basal phenotype (92%) and was frequent (40%) in women without pathogenic BRCA1/2 germline variants. No association was observed between BRCA1met and distant recurrence-free interval (DRFI) in adjuvant EC–T treated patients (log-rank p=0.77) (Fig. 2A-C) or FEC treated patients vs. EC–T treated patients (Fig. 2D), nor was there an association between BRCA1met and pathological complete response (pCR) in NAC patients (p=0.69) (Table 2). In-depth analyses of BRCA1met NAC cases revealed differences in methylation level and BRCA1 mRNA expression in pre-treatment versus surgically resected tissue in patients with RD (Fig. 3) that will be further analyzed using WGS to map the genomic traits of these tumors (Fig. 4). No gene fusions were detected.

**CONCLUSIONS**

BRCA1met is associated with young age in TNBC but hold no predictive or prognostic value in this patient cohort given EC–T treatment. We observe a difference in BRCA1met patterns in NAC patients with RD that may potentially be related to treatment resistance.

**TABLE 1. COHORT CHARACTERISTICS.** Intrasubject associations of clinical and molecular variables across the patient cohorts 2010-2015 and 2015-2018.

**TABLE 2. ASSOCIATION BETWEEN BRCA1 HYPERMETHYLATION AND DRFI.** Association between BRCA1 hypermethylation and distant recurrence-free interval (DRFI).

**FIGURE 1. CIRCOS PLOT DEMONSTRATING THE RESULTS FROM WGS.** To better characterize the genomic landscape of patients receiving NAC with RD as treatment response, we have analyzed pre- and post-treatment specimens using WGS. This circos plot demonstration the genomic traits of a pre-treatment specimen from patient PD35986a enrolled in the SCAN-B study during the years 2010-2015. (See Supplementary material for the complete WGS data). The genomic landscape is then compared to the genomic landscape of the same patient PD35986a after a period of NAC treatment (2015-2018) in Figure 2. 

**FIGURE 2. KAPLAN-MEIER OVERALL SURVIVAL ESTIMATES.** A) Overall survival in patients with neoadjuvant (NAC) and adjuvant treatment (ACT) with EC–T or FEC. Fisher's exact test showed no associations between two therapy groups (P=0.69). B) Comparison of survival analysis among patients with residual disease treated with neoadjuvant (NAC) and adjuvant with EC–T or FEC. No significant differences were observed in survival probability between these groups (P=0.25). C) Survival analysis comparing two different patient cohorts. Patients included in the current study (2015-2018) and the previously published SCAN-B cohort (2010-2015). No association was observed between type of treatment and survival probability (P=0.53). D) Survival analysis of patients treated with ACT comparing two different patient cohorts. Patients included in the current study (2015-2018) and the previously published SCAN-B cohort (2010-2015). No association was observed between type of treatment and survival probability (P=0.57).