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Proper genomic profiling of (*BRCA1*-mutated) basal-like breast carcinomas requires prior removal of tumor infiltrating lymphocytes

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ABSTRACT

Introduction: BRCA1-mutated breast carcinomas may have distinct biological features, suggesting the involvement of specific oncogenic pathways in tumor development. The identification of genomic aberrations characteristic for BRCA1-mutated breast carcinomas

Abbreviations: aCGH, micro-array based competitive genomic hybridization; BAF, B-allele frequency; BLC, basal-like carcinomas; BRCAX, non-BRCA1/2 mutated familial breast carcinomas; CIN, chromosomal instability; CK, cytokeratin; CNA, copy number aberration; ER, estrogen receptor; FE, Fischer exact; FFPE, formalin-fixed, paraffin-embedded; HER2, human epidermal growth factor receptor 2; HR, homologous recombination; H&E, hematoxylin and eosin; LOH, loss of heterozygosity; mBAF, mirrored B-allele frequency; MSP, methylation specific PCR; PR, progesterone receptor; SNP, single nucleotide polymorphism; TIL, tumor infiltrating lymphocyte; WGS, whole genome sequencing.

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could lead to a better understanding of *BRCA1*-associated oncogenic events and could prove valuable in clinical testing for *BRCA1*-involvement in patients.

Methods: For this purpose, genomic and gene expression profiles of basal-like *BRCA1*-mutated breast tumors (n = 27) were compared with basal-like familial BRCAX (non-*BRCA1/2/CHEK2**1100delC) tumors (n = 14) in a familial cohort of 120 breast carcinomas.

Results: Genome wide copy number profiles of the *BRCA1*-mutated breast carcinomas in our data appeared heterogeneous. Gene expression analyses identified varying amounts of tumor infiltrating lymphocytes (TILs) as a major cause for this heterogeneity. Indeed, selecting tumors with relative low amounts of TILs, resulted in the identification of three known but also five previously unrecognized *BRCA1*-associated copy number aberrations. Moreover, these aberrations occurred with high frequencies in the *BRCA1*-mutated tumor samples. Using these regions it was possible to discriminate *BRCA1*-mutated from BRCAX breast carcinomas, and they were validated in two independent cohorts. To further substantiate our findings, we used flow cytometry to isolate cancer cells from formalin-fixed, paraffin-embedded, *BRCA1*-mutated triple negative breast carcinomas with estimated TIL percentages of 40% and higher. Genomic profiles of sorted and unsorted fractions were compared by shallow whole genome sequencing and confirm our findings.

Conclusion: This study shows that genomic profiling of in particular basal-like, and thus *BRCA1*-mutated, breast carcinomas is severely affected by the presence of high numbers of TILs. Previous reports on genomic profiling of *BRCA1*-mutated breast carcinomas have largely neglected this. Therefore, our findings have direct consequences on the interpretation of published genomic data. Also, these findings could prove valuable in light of currently used genomic tools for assessing *BRCA1*-involvement in breast cancer patients and pathogenicity assessment of *BRCA1* variants of unknown significance. The *BRCA1*-associated genomic aberrations identified in this study provide possible leads to a better understanding of *BRCA1*-associated oncogenesis.

1. Introduction

Breast tumors of *BRCA1* germ line mutation carriers have been shown to be primarily of the basal-like subtype (75–90%) (Sorlie et al., 2003; Jonsson et al., 2010). These basal-like breast carcinomas (BLCs) represent about 10–20% of all breast carcinomas (Foulkes et al., 2003; Sorlie et al., 2003) and are generally high grade, triple negative ((ER-), (PR-), and (HER2/ERBB2-) and express high molecular weight cytokeratins (CK5/6, 14 and 17) (Lakhani et al., 2002; Foulkes et al., 2003; Honrado et al., 2005; Rubinstein, 2008).

High levels of genomic instability are frequently observed in *BRCA1*-mutated carcinomas and BLCs in general, both sporadic and hereditary (Bergamaschi et al., 2006; Jonsson et al., 2010; Smid et al., 2010). Although frequent, these genomic aberrations mostly represent low copy number gains and losses of large DNA segments rather than (focal) high level amplifications (Bergamaschi et al., 2006; Stefansson et al., 2009). This observation fits well with the described role of *BRCA1* in DNA damage response and DNA repair through homologous recombination (HR) (Roy et al., 2012). Hyper-methylation of the *BRCA1* promoter region and reduced *BRCA1* mRNA expression levels have been observed in sporadic BLCs and could act as an alternative mechanism of *BRCA1* inactivation (Wei et al., 2005; Birgisdottir et al., 2006; Turner and Reis-Filho, 2006). However, the involvement of a dysfunctional *BRCA1* gene in sporadic BLCs has also been questioned due to lack of somatic mutations in the *BRCA1* gene (Futreal et al., 1994; Gonzalez-Angulo et al., 2011). Nevertheless, the observed similarities between *BRCA1*-mutated and non-mutated BLCs have led to the suggestion that targeting a dysfunctional *BRCA1*-pathway, exploiting in particular a defective HR, might be effective in all BLCs (Turner et al., 2004). However, platinum based chemotherapy and treatments with PARP inhibitors have shown inconsistent results in BLCs (Silver et al., 2010; Lips et al., 2011a). For this reason, the role of a dysfunctional *BRCA1* gene or pathway as a general characteristic of BLCs remains uncertain.

To allow discrimination between *BRCA1*-mutated and unselected sporadic/hereditary breast tumors, micro-array based comparative genomic hybridization studies (aCGH) have been used to identify characteristic genomic aberrations in *BRCA1*-mutated breast tumors (Tirkkonen et al., 1997; Wessels et al., 2002; van Beers et al., 2005; Jonsson et al., 2005; Joosse et al., 2009). Recent studies comparing BLCs with and without *BRCA1* mutations have reported few or even no differential regions of genomic aberrations (Stefansson et al., 2009; Jonsson et al., 2010; Waddell et al., 2010). A well known difficulty in DNA-copy-number determination by aCGH and SNP array analyses is variable tumor purity due to normal DNA contamination. In literature, a number of approaches have been proposed to try to mitigate this effect (Yau et al., 2010). However, such methods have not been applied in aforementioned genomic profiling efforts of *BRCA1*-mutated breast carcinomas.

The use of *BRCA1* specific classifiers would facilitate the identification of women and their family members with