Editorial

BRCA1 and BRCA2: two genes, multiple clinical applications

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The discovery of BRCA1 and BRCA2 hereditary breast-ovarian cancer genes was driven by a well-defined practical purpose: it was expected, that the identification of genetic causes of these diseases will allow to ascertain yet healthy women at-risk, and therefore to reduce cancer mortality through implementing relevant diagnostic and preventive programs. BRCA-associated syndrome is apparently the most common Mendelian genetic disease in humans. Studies on geographic spread of BRCA1/2 mutations revealed a number of founder alleles, thus permitting non-expensive and rapid identification of thousands of mutation carriers in genetically homogenous human communities (1). On the other hand, the majority of patients with suspicion for hereditary breast or ovarian cancer require comprehensive BRCA1/2 testing; exhaustive BRCA1/2 analysis remains complicated even for the time being, as it includes the detection of both small mutations and gross rearrangements across the entire coding regions of these genes (2). BRCA1 and BRCA2 gene alterations appear to be associated with somewhat distinct disease phenotypes, with a plethora of genetic and non-genetic factors modifying their penetrance (3,4). Diagnostic attitudes towards BRCA1/2 mutation carriers continue to evolve, given that some of the BRCA-driven cancers are notoriously difficult to detect at yet manageable stages (5,6).

BRCA1 and BRCA2 tests were initially thought to play a purely diagnostic role. A series of studies revealed, that tumors arising in BRCA1/2 germ-line mutation carriers are characterized by somatic loss of the remaining allele of the involved gene and therefore deficient for DNA repair by homologous recombination. This renders tumor sensitivity to a number of well-known cheap cytotoxic drugs, such as cisplatin and mitomycin C. In addition, a novel class of drugs, i.e., poly (ADP-ribose) polymerase inhibitors (PARPi), was intentionally developed to target BRCA-deficient cells. Given that BRCA1/2 status may critically influence the choice of antitumor drugs, it is getting evident that in ideal situation BRCA1/2 germ-line testing requires to be completed before the treatment planning. Furthermore, the analysis of blood DNA may no longer be sufficient: some studies demonstrate that sporadic tumors may also exert BRCA1/2 deficiency, and somatic BRCA1/2 gene defects correlate with tumor response to BRCA-specific drugs (7,8).

In addition to offering novel options for cancer therapy, BRCA1/2 research also led to the discovery of an amazingly elegant mechanism of tumor escape. Some tumors with acquired resistance to platinum or PARPi demonstrate a second mutation in the affected gene, which is located in the vicinity of the germ-line mutation and restores the open reading frame (ORF) for the mutated allele. This is a clearly non-random event, considering that restoration of BRCA1/2 function was repeatedly documented in treatment-resistant tumors from BRCA1/2 germ-line mutation carriers and that the appearance of multiple ORF-restoring clones was observed in a subset of cases (9-12).

In theory, monitoring of the emergence of BRCA1/2 ORF-restoring mutations may have practical implication by guiding the choice between the continuation of BRCA-targeting therapy and the switch to other therapeutic modalities. The re-biopsy of the treatment-resistant tumor lumps does not look as an optimal approach: depending on location of the cancer lesions, it can be a highly traumatic procedure; furthermore, multiple treatment-resistant metastases are likely to have distinct roots of evolution of treatment-resistant clones, therefore the analysis of a single piece of tumor tissue may not provide a cumulative picture for the entire tumor burden.

A so-called liquid biopsy is a viable approach for the monitoring of tumor resistance. It may rely either on the analysis of circulating tumor cells (CTCs), which are collected on specific beads and then used for molecular analysis, or, alternatively, subject to the examination circulating nucleic acids. Liquid biopsy was shown to be able to detect somatic mutations and RNA splice variants, which are associated with resistance to inhibitors of signaling pathways (13). Also, the potential capability of liquid biopsy to detect secondary mutations in BRCA1/2 genes has been shown in several recent studies. Christie *et al*. (14) investigated BRCA1/2 gene status in circulating plasma DNA obtained from BRCA1/2 mutation carriers with ovarian cancer. The authors aimed to detect clones with ORF-restoring genetic events, therefore they developed individual PCR assays for every woman in order to amplify gene fragments surrounding the patient-specific germ-line mutation. To ensure the detection of minor mutated clones, multiple reads of the analyzed nucleotide fragments were performed by the next generation sequencing (NGS). The results appear to be promising. There were no false-positives, i.e., none of 30 included patients showed the secondary BRCA1/2 mutation in circulating DNA in the absence of the one in tumor tissue. The sensitivity of the method was moderate: while the analysis of cancer lumps revealed 5 instances of BRCA1/2 reversion, only 3 women carried the same alteration in plasma DNA. As expected, the emergence of secondary BRCA1/2 mutations correlated with the cessation of the effect of BRCA-specific therapy. The utility of circulating free DNA for detection of secondary BRCA1/2 mutations was also demonstrated in the recently published studies of Quigley *et al*. (12) and Goodall *et al*. (15).

Several issues related to the detection of BRCA1/2 reversion by liquid biopsy deserve discussion. BRCA1 and BRCA2, being distinct genes, render similar increase of the risk of breast and ovarian cancer and are associated with a similar patterns of tumor sensitivity to platinum drugs and PARPi, therefore they are often mentioned interchangeably in the medical literature. However, it is important to recognize that the majority of the data for secondary mutations were obtained for BRCA2, and only a few instances of these events were described for BRCA1 (9,14). There are several alternative mechanisms for BRCA1-driven tumors to acquire platinum and PARPi resistance, and the detection of these events by liquid biopsy may look more challenging. Swisher *et al*. (16) and Norquist *et al*. (17) described back mutations in BRCA1 gene, which restored BRCA1 sequence back to the norm; these mutations cannot be detected in circulating DNA due to the excess of the wild-type allele (14), however, to our knowledge, the findings of Swisher *et al*. (16) and Norquist *et al*. (17) have not been replicated yet in subsequent studies. Alternative splicing and the use of alternative sites for initiation of translation may result in the skipping of BRCA1 frame-shift mutation and restoration of the activity of DNA repair (18-20). Sokolenko *et al*. (21) recently demonstrated that the somatic deletion of the wild-type allele may not necessarily be the first event in the pathogenesis of BRCA1-related ovarian cancer and that primary tumors obtained from BRCA1 mutation carriers contain residual cancer cells with preserved BRCA1 function. These cells undergo rapid expansion upon the pressure of platinum therapy and repopulate tumor mass within a very short period of time. Overall, there is an enormous plasticity of BRCA1/2-driven cancers, and secondary mutations are responsible only for a share of therapy-resistant phenotypes.

The actual clinical utility of liquid biopsy for the detection of secondary BRCA1/2 mutations is also not self-explanatory. Even if the potentially treatment-resistant cell clones already exist in the body of the patient, it is unclear from the plasma DNA test to what extent they dominate across all detectable tumor lumps and therefore determine the systemic non-response to the therapy. Studies on EGFR T790M mutation rendering resistance to EGFR tyrosine kinase inhibitors demonstrate, that monitoring of this allele correlates well with the emergence of treatment resistance while analyzing multiple patients as a group, but have less obvious value when considering treatment decisions in individual cases (22). In fact, in the study of Sueoka-Aragane *et al*. (22) some patients continued to be stable despite the emergence of EGFR T790M allele in the blood, while others progressed some weeks before the appearance of this resistance-associated mutation in circulating DNA.

Furthermore, while the gain of EGFR T790M mutation is a clearly actionable event as it calls for the switch to the T790M-targeting drugs, e.g., osimertinib, there is currently little to offer to ovarian or breast cancer patients progressing on BRCA1/2-specific therapy. Noteworthy, Christie *et al*. (14) described an ovarian cancer patient, who responded to gemcitabine and bevacizumab after acquiring the platinum-resistant mutation.

Overall, the limitations of liquid biopsy are well familiar to the clinical oncologists. They mirror well-known dilemmas on the use serum markers for guiding treatment decisions in the absence of disease progression by RECIST (23). Irrespectively of the method of detection of platinum/PARPi resistance, one may conclude that the concept of the treatment of BRCA1/2-driven tumors deserves further discussion. The best available therapeutic options, i.e., platinum derivatives and PARP inhibitors, appear to have highly overlapping if not identical mechanisms of tumor escape and their conventional use seems unlikely to deliver the dramatic improvement of long-term outcomes. Some innovative strategies may have relevance to BRCA1/2-associated cancers. High-dose chemotherapy was shown to be particularly effective for BRCA1/2 mutation carriers, probably because of robust elimination of cell clones resistant to standard therapy regimens (24). Some evidences suggest that rapid elimination of treatment-sensitive cell population, as observed in case of use of some potent anticancer drugs, is not neutral for the rest of the tumor mass but has a strong stimulatory effect on the expansion of residual multidrug-resistant neoplastic cells; these theories call for modification of dosing and scheduling of existing therapies in order to achieve a more balanced mode of the tumor control (25). The molecular aspects of pathogenesis, treatment response and drug resistance are significantly better defined for BRCA1/2-related neoplasms than for other malignancies, therefore BRCA1/2-associated cancers continue to serve as an excellent model for breakthrough studies on fundamental principles of cancer therapy.

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