BRCA sequencing of tumors: understanding its implications in the oncology community

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Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: None; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Abstract: In the current era of personalized medicine, much more information can be gleaned through genetic testing and tumor sequencing. Unfortunately, this comes at a price of obtaining results that may beget more uncertainties. Sequencing for mutations on tumor samples is increasingly performed, more commonly to guide treatment for oncology patients, and occasionally as a proxy for germline testing when the ideal index patient to initiate genetic testing in a family at risk for hereditary cancer syndrome is no longer alive. Next-generation sequencing (NGS) involving tens to hundreds of genes as a testing platform is being used more routinely in the clinic now. However, one should keep in mind that the larger number of genes included in an NGS panel will yield a correspondingly higher probability of finding an incidental germline pathogenic mutation, which will have both clinical and ethical implications for patients and their families. The probability of identifying a tumor pathogenic *BRCA1/2* variant is about 3–4%, with the majority (~80%) being germline in nature; thus, patients should be counselled accordingly prior to having their tumor samples sequenced. On the flip side, caution should be exercised when tumor sequencing is intended to be a surrogate for germline testing. This is because false negative rate is high at ~30%, making it an inadequate tool to sufficiently dismiss the presence of a germline *BRCA1/2* mutation, especially in a setting where there is already a high clinical suspicion for a hereditary condition.

Keywords: *BRCA1/2*; tumor sequencing; germline mutation

Submitted Sep 05, 2019. Accepted for publication Jul 28, 2020.
doi: 10.21037/cco-19-198
View this article at: http://dx.doi.org/10.21037/cco-19-198

Introduction

The breast cancer susceptibility genes *BRCA1/2* are tumor suppressor genes, that when mutated, increases an individual's risk of developing not just breast cancer, but also ovarian, pancreatic, and prostate cancer (1-4). This occurs as the *BRCA1/2* genes encode for proteins that play crucial roles in the maintenance of genomic integrity by ensuring accurate and precise repair of damaged DNA, as well as controlling cell cycle checkpoints (5,6). Women who harbor pathogenic *BRCA1/2* mutations are at a 45–65% risk of developing breast cancer and a 11–39% risk of ovarian cancer by the age of 70 (7), compared to the corresponding general population cumulative risk of 5.03 % and 0.72% respectively (8). The past 25 years have seen an increase in the number of genetic tests being offered, owing to advances in testing technology and increase in access to testing (9). With lowered test costs and high-throughput sequencing technologies, many more laboratories have been able to offer tests that cover more genes, beyond the conventional *BRCA1/2* genes, to diagnose hereditary breast cancer syndrome. Indeed, the rates of testing for hereditary breast cancer including *BRCA1/2* have been increasing over the years (10,11). This has led to a need to

address the implications of acquiring genetic information; be it intentionally sought or incidentally discovered. In this review article, we seek to provide a brief history of DNA sequencing, highlight the differences between sequencing DNA from blood and tumor samples, with particular emphasis on the *BRCA1/2* genes, as well as understand the clinical implications of tumor *BRCA1/2* testing.

The process of DNA sequencing and its utility

DNA sequencing was first described in the late 1970s by Fred Sanger and his colleagues (12) and is better known as the Sanger sequencing method. Sanger sequencing involves mixing non-extendable, fluorescently labeled dideoxy nucleotides together with standard nucleotides that may be randomly incorporated by DNA polymerase to generate fragments of varying lengths of nucleotides which are copies of the original template of DNA. These fragments are then separated by high-resolution capillary electrophoresis, and the color tag linked to the last incorporated dideoxy nucleotide on each fragment is then used to interpret the original sequence of DNA (13). It was the Sanger sequencing method which was employed to elucidate the first complete sequence of the human genome (known as the Human Genome Project) (14). However, the traditional Sanger sequencing method is limited by its throughput as well as high cost. In fact, it was estimated that the first human genome sequencing cost an estimated 0.5–1 billion US dollars (15). Since then, much effort has been poured into initiatives to improve DNA sequencing, in a bid to increase throughput whilst reducing cost. The National Human Genome Research Institute created a 70-million-dollar DNA sequencing technology initiative with the aim of achieving a 1,000-dollar human genome within 10 years (16). One such technology that was created is known as next-generation sequencing (NGS). While the concept behind Sanger sequencing and NGS is similar, the critical difference lies in the sequencing volume. The traditional Sanger sequencing method sequences a single DNA fragment at any one time, while NGS, also known as massive parallel sequencing, sequences millions of fragments simultaneously per run. This capability has expanded the breadth of genotyping available in the clinic. We can now utilize different technologies to sequence genetic information of varying lengths (17). Low throughput methods such as Sanger sequencing are suitably used to sequence single variants such as the *BRAF* V600E mutation, part of a gene such as epidermal growth factor receptor (EGFR) mutations in exons 18–21 (18), or an entire gene for example full *BRCA1/2* sequencing. At the other end of the spectrum, high-throughput genotyping such as NGS method has the capability of sequencing tens to hundreds of genes simultaneously, the whole exome, or even the whole genome (*Figure 1*).

After a genomic sequence is determined, the reads are aligned to a published reference genome and compared. Any site with a differing DNA from the reference is considered a sequence variant (19). Variants are classified as "pathogenic", "likely pathogenic", "of uncertain significance", "likely benign" or "benign" based on the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology guidelines (20). By increasing the length of genome that is sequenced, the chance of detecting informative and/or actionable mutations is increased; however, the disadvantages of testing a large panel of genes include higher rates of variants of uncertain significance (VUS) (21) which are uninformative and not actionable, as well as a higher likelihood of incidental and/or unexpected findings in less familiar genes that may have limited or no management guidelines.

Germline *vs.* somatic testing (*Table 1*)

Germline mutations are heritable and present in every cell of a person's body. These mutations are inherited from a person's parent(s), and can be passed on to his or her offspring. Typical starting materials used to diagnose a germline mutation are blood (peripheral blood mononuclear cells), buccal swab, or saliva. Heritable germline mutations exist from birth to death and do not change with time, thus testing only needs to be done once. Heritable germline information can potentially lead to ethical, social, and familial implications, and hence pre-test genetic counseling is required. In oncology, the traditional indications for germline testing are to diagnose hereditary cancer syndrome, such as *BRCA1/2* hereditary breast-ovarian cancer syndrome, to identify individuals who will benefit from early screening and prevention strategies (22,23). More recently, germline *BRCA1/2* testing may lead to therapeutic indications, as germline *BRCA1/2* mutation carriers with metastatic HER2 negative breast, advanced epithelial ovarian, or metastatic pancreatic cancer may benefit from treatment with poly-ADP-ribose polymerase (PARP) inhibitors (3,24-26). In contrast to germline mutations, somatic mutations are acquired genetic changes that occur in a diseased organ, for example, in tumor

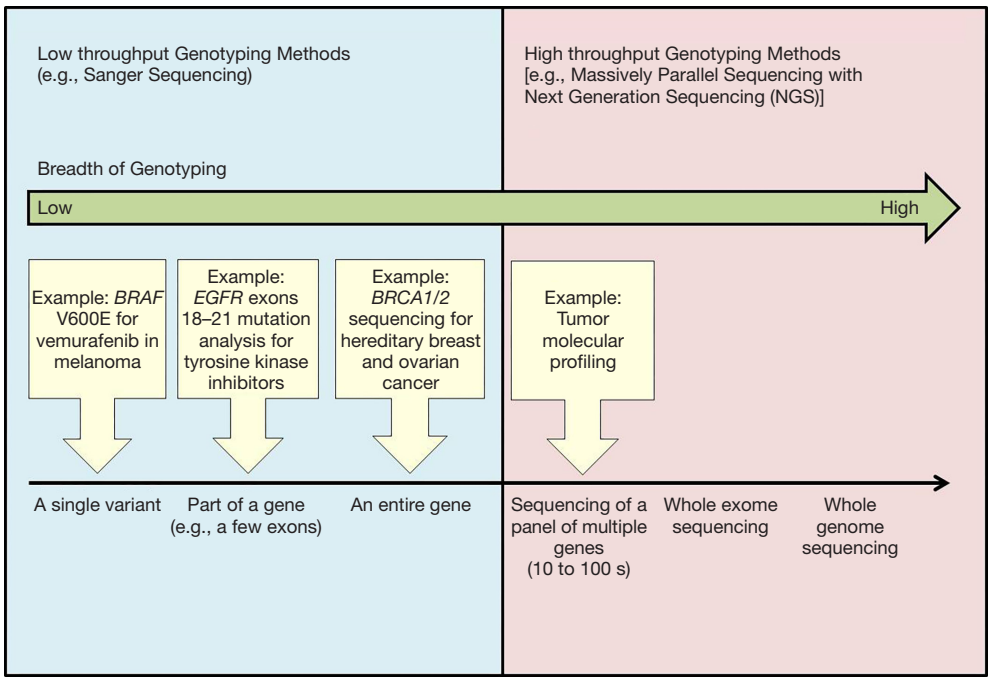

Figure 1 Clinical examples of the various types of genotyping methods.

Table 1 Comparisons between germline and somatic testing

Differences	Germline	Somatic
Features	Heritable	Non-heritable
	Results do not change with time	Results may change with time
	Usually tested only once	May require serial sampling
Indications	Diagnostic: for early screening/prevention in suspected hereditary cancer syndrome, e.g., *BRCA*, Lynch syndrome	Therapeutic: *EGFR*, *KRAS*, *BRAF* mutations, ALK translocation
	Therapeutic: poly-ADP-ribose polymerase (PARP) inhibitors in *BRCA* mutation carriers with advanced ovarian or breast cancer	
Pre-test genetic counselling	Recommended due to potential ethical and social implications with identification of heritable mutation	Not typically required as identified mutation is usually not heritable
Starting materials	Blood (peripheral blood mononuclear cells), buccal swab, saliva	Tumor (biopsy, surgical specimen), pleural fluid, ascites
	Tumor (not typically used, but does contain germline DNA)	Blood (processed to yield circulating tumor cells or cell-free DNA)

cells in a patient with cancer. Somatic testing in cancer is typically done on tumor biopsy or surgical specimens, or any other biological materials that contain malignant cells, e.g., malignant pleural effusion or ascitic fluid. As somatic mutations are not heritable, pre-test genetic counselling is generally not required. The traditional indication to test for somatic mutations in oncology is for therapeutics, e.g., identification of *EGFR* mutations in non-small cell lung cancer (NSCLC) to select patients for treatment with EGFR tyrosine kinase inhibitors (TKIs) (18,27,28). Somatic

mutations may evolve with time and treatment exposure, thus repeated testing may be required, for example, testing for the emergence of *EGFR* T790M mutation in *EGFR*-mutant NSCLC patients who have developed resistance to first- or second-generation EGFR-TKIs (29). While genetic testing of blood is usually associated with germline information and testing of tumor with somatic information, this relationship does not always hold true. For example, although tumor is not commonly used as a source of germline DNA, it originates from normal tissue and thus contains germline DNA. Therefore, tumor genetic testing can potentially yield incidental germline findings. Similarly, if blood is processed to yield circulating tumor cells or cell-free DNA, then testing such materials will provide somatic rather than germline information.

Testing for mutations in the *BRCA1/2* gene in the tumor

Germline testing for the *BRCA1/2* gene is usually done on a blood sample. However, there are instances whereby *BRCA1/2* gene sequencing is performed on a tumor sample. One scenario that is occurring increasingly commonly is when the tumor of a patient with refractory cancer is profiled using NGS in search of an actionable mutation to guide treatment. A panel of tens to several hundred genes is typically tested and often includes the *BRCA1/2* genes. Pathogenic *BRCA1/2* gene mutation may be identified in the tumor, and although some of these mutations are somatic mutations, others may represent incidental germline findings. Another example is when there is a deliberate search for a tumor *BRCA1/2* mutation to guide treatment, for example in the setting of epithelial ovarian cancer, where there is data to support the use of PARP inhibitors in patients whose tumors harbor *BRCA1/2* mutation (3,24), regardless of whether they are germline or somatic in nature (30). Lastly and rarely, tumor may be used as a surrogate for germline testing in the context of a high-risk family with no living affected index patient for direct germline testing.

Are *BRCA1/2* gene mutations identified on tumor sequencing somatic or germline in origin?

One challenge when a pathogenic *BRCA1/2* mutation is identified in tumor is to ascertain its origin, i.e., germline versus somatic. The most conclusive way to determine if a mutation identified in a tumor is germline or somatic in nature is to test an accompanying germline sample (e.g., blood or buccal swab). However, in practice, most laboratories offering tumor NGS testing do not routinely request for a germline sample from the patient, for several reasons. These include ethical concerns, as testing germline samples yields direct information on heritable mutations and will require prior genetic counselling, and practical considerations, such as the need for more bioinformatics analysis with a germline sample, thus increasing testing cost and possibly turnaround time (31). Most tumor NGS reports are silent on whether an identified pathogenic mutation is germline or somatic in origin. However, certain features may help to ascertain if a tumor variant could actually be germline in nature, including a concordant clinical and family history and mutant allele frequency (MAF). When a pathogenic *BRCA1/2* mutation is identified in tumor, the clinician should review the patient's clinical presentation to determine if it is consistent with a heritable mutation. Information such as the cancer type, family history and young age at diagnosis are important clues that could point towards the fact that the identified mutation may be germline in nature. Another clue is the MAF of the pathogenic variant of interest. Since most hereditary cancers are inherited in an autosomal dominant fashion (50% wild type and 50% mutant), the MAF of pathogenic germline variants is usually close to 50%, whereas MAF of pathogenic somatic variants tends to be much more variable. The gene in which the tumor pathogenic mutation is identified may provide further insights on its origin. For example, Meric-Bernstam *et al.* showed that the majority (77.8%) of tumor pathogenic *BRCA1/2* variant was germline in nature, compared to only 2.88% of tumor pathogenic TP53 variants (32). Other clues that could suggest a tumor pathogenic mutation to be germline in nature include detection of the same mutation in different primary tumor specimens from the same patient, and the mutation having been previously reported as a heritable founder mutation (31).

What is the likelihood of finding an incidental germline pathogenic mutation on tumor NGS testing?

In a study from MD Anderson, 1,000 cancer patients underwent tumor NGS testing with a panel of 202 genes; all patients provided a corresponding germline sample (blood or buccal swab), allowing investigators to ascertain

if an identified tumor pathogenic mutation is germline or somatic in nature. A focused analysis on 19 cancer predisposing genes found that ~5% of pathogenic mutations identified in the tumor in these 19 genes were actually germline in nature (31). Schrader *et al.* further reported that the likelihood of picking up incidental germline pathogenic mutations in tumor increases with the number of genes tested, from 6.4% in a 26-gene panel to 12.6% using a 93-gene panel and further to 15.7% when a 187-gene panel is tested (33). These mutations may be linked to increased risk of preventable diseases for which clear management guidelines are available (32), while others may cause non-preventable diseases or have less certain clinical implications. These findings have potential medico-legal implications, and there has been significant debate on whether all or some of these incidental germline findings should be disclosed to the patient. Importantly, disclosing and managing all such results demand significant medical expertise and health resources, which are not available at most institutions. Furthermore, disclosure of a finding that can lead to a non-preventable disease can also be a source of distress and may not be welcome by the patient. Physicians ordering these tests should thus be mindful of these issues.

How should we handle incidental *BRCA1/2* pathogenic germline variants identified in tumor?

The ACMG recommends a list of 59 genes including 25 cancer predisposing genes in which results should be returned to patients if incidental germline mutations are identified; regardless of the original indication for the clinical sequencing (34). This includes the *BRCA1/2* genes (34). Several studies have reported the likelihood of detecting an incidental germline pathogenic *BRCA1/2* mutation on tumor NGS testing to range from 2.1% to 3.3% (32,33,35). Interestingly, the clinical diagnosis was not suspected in 24% to 46% of the cases. Clinicians should be aware of this when ordering tumor NGS testing for their patients; it will be appropriate to provide brief pre-test counseling to inform patients of the possibility of detecting incidental heritable mutations. If a tumor *BRCA1/2* pathogenic mutation is identified, full genetic counseling should be provided with a view to confirm the mutation with clinical testing using a direct germline sample such as blood or buccal swab. Full genetic counselling typically comprises pre-and post-test processes. Pre-test genetic counselling includes taking a thorough family history for risk assessment followed by counselling on the characteristics of the suspected hereditary cancer syndrome, including mode of inheritance, lifetime cancer risks, screening and preventive options for proven mutation carriers, as well as highlighting potential ethical, social, and legal implications of genetic information. During post-test counselling, the implications of the test results are explained and follow-up plans including screening and prevention as well as cascade testing of family members of proven mutation carriers are formulated (22).

Is tumor *BRCA1/2* gene testing reliable enough to diagnose germline mutations?

Since tumor NGS testing may uncover incidental germline *BRCA1/2* mutations, an important clinical question arises: how reliable is tumor *BRCA1/2* testing in diagnosing or excluding germline mutations? We studied 60 patients who had undergone clinical *BRCA1/2* germline testing using blood samples, including 22 patients who were diagnosed with pathogenic germline *BRCA1/2* mutations and 38 patients without (36). Paraffin-embedded tumors from these patients were retrieved for tumor *BRCA1/2* testing via NGS. The laboratory was blinded to the germline test results and was asked to commit if an identified tumor pathogenic *BRCA1/2* is germline in origin, using MAF and other variant classification algorithms. In the 38 patients with no germline pathogenic *BRCA1/2* mutation, tumor tests were 100% concordant with no false positive results. However, in patients who carry germline pathogenic *BRCA1/2* mutation (n=22), only 70% of these germline mutations were conclusively diagnosed on tumor testing, while 30% of germline mutations were missed. Among the false negative cases, 40% was due to technical error, i.e., the mutation was not detected in the tumor, while 60% was due to interpretative error, i.e., mutation was detected in the tumor but the classification algorithm used by the laboratory erroneously classified the variant as non-pathogenic or somatic. These results highlight that a tumor NGS test that is negative for *BRCA1/2* mutation does not conclusively exclude germline pathogenic *BRCA1/2* mutation, thus germline testing should still be considered if the patient fulfills clinical criteria for germline *BRCA1/2* testing. On the other hand, tumor *BRCA1/2* testing can detect some pathogenic germline mutations and false positive rates appear low, with high concordance rate of more than 90% between tumor *BRCA1/2* mutations assessed to be likely germline in nature versus blood testing

Figure 2 A summary of the clinical scenarios in which tumor *BRCA1/2* may be identified and their implications.

(37-39). Thus, in the context of a high risk family with no living affected who can be tested as the index patient, archival tumor specimen from a deceased cancer-affected family member can potentially be used as a surrogate for germline; the detection of a pathogenic germline mutation in this context could potentially facilitate cascade testing in family members, although this is best done in the context of a specialized cancer genetics clinic.

Putting it all together (*Figure 2*)

When sequencing a tumor using NGS testing, the probability of finding an incidental pathogenic *BRCA1/2* mutation is ~2–4%, of which ~80% is germline in origin. Consequently, clinicians should consider pre-test counselling to warn patients of such a possibility (32). Subsequently, when a pathogenic *BRCA1/2* gene mutation is indeed reported in the tumor, the patient should be counseled that this may represent an incidental germline finding. It is recommended that these patients be referred for formal genetic counselling followed by confirmatory germline testing with a blood sample or buccal swab. On the other hand, due to a relatively high false negative rate, failure to identify pathogenic *BRCA1/2* mutation on tumor NGS testing does not definitively exclude germline *BRCA1/2* mutations. Therefore, if an individual fulfills conventional genetic testing criteria, germline testing will still be indicated even if the tumor testing returns negative. Finally, using tumor as a surrogate to test for germline mutations in a deceased individual should only be used as a last resort, best done in the context of a cancer genetics clinic, and results interpreted with caution.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editor (Shaheenah Dawood) for the series "Targeting the DNA Damaging Pathway: PARPi and Beyond" published in *Chinese Clinical Oncology*. The article was sent for external peer review organized by the Guest Editor and the editorial office.

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.

org/10.21037/cco-19-198). The series "Targeting the DNA Damaging Pathway: PARPi and Beyond" was commissioned by the editorial office without any funding or sponsorship. Dr. SCL reports grants and other from Pfizer, other from Novartis, other from Astra Zeneca, grants and other from Taiho, grants and other from Eisai, other from Roche, grants and other from ACT Genomics, grants and other from ASLAN Pharmaceuticals, other from MSD, other from Amgen, other from Eli Lilly, outside the submitted work. RSJW has no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Wong RSJ, Lee SC. *BRCA* sequencing of tumors: understanding its implications in the oncology community. Chin Clin Oncol 2020;9(5):66. doi: 10.21037/cco-19-198