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INVITED REVIEW ARTICLE

Next-generation sequencing-based clinical sequencing: toward precision medicine in solid tumors

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Abstract
Numerous technical and functional advances in next-generation sequencing (NGS) have led to the adoption of this technique in conventional clinical practice. Recently, large-scale genomic research and NGS technological innovation have revealed many more details of somatic and germline mutations in solid tumors. This development is allowing for the classification of tumor type sub-categories based on genetic alterations in solid tumors, and based on this information, new drugs and targeted therapies are being administered to patients. This has largely been facilitated by gene panel testing, which allows for a better understanding of the genetic basis for an individual's response to therapy. NGS-based comprehensive gene panel testing is a clinically useful approach to investigate genomic mechanisms, including therapy-related signaling pathways, microsatellite instability, hypermutated phenotypes, and tumor mutation burden. In this review, we describe the concept of precision medicine in solid tumors using NGS-based comprehensive gene panel testing, as well as the importance of quality control of tissue sample handling in routine NGS-based genomic testing, and we discuss issues for the future adoption of this technique in Japan.

Keywords Next-generation sequencing · Precision medicine · Solid tumors · Genomic panel test · Hypermutation · Tumor mutation burden

Introduction

Next-generation sequencing (NGS), also referred to as massively parallel sequencing, represents an ideal way to detail a substantial volume of genomic detail of cancers. The human genome sequence has exceptionally transformed the perception of the scholars in field of biology, personal diversity, and disease [1]. Genomic profiling technologies like genome-wide NGS are progressively utilized to reveal different facets of genomic heterogeneity in various types of human being disorders, including cancer [2, 3]. The effective use of NGS, primarily via whole-genome sequencing (WGS) and whole-exome sequencing (WES), has generated a surge in understanding of cancer genomic variations, including point mutations, modest insertions or deletions, copy number alterations, and architectural adaptations. By evaluating these variations to coordinated regular samples, scientists have managed to discern two classes of mutations, namely somatic and germline of variants [3].

The Cancer Genome Atlas (TCGA) project was an extensive and synchronized attempt to accelerate our understanding of the molecular basis of numerous cancers by implementing genome-wide analysis. The result was a greater understanding of the relationships between tumor genetics and of the transformable history of cellular functions throughout distinct cancer varieties [4]. From the clinical point of view, there is the promising possibility of utilizing NGS within routine clinical care and patient management. The immediate impact may be a substitution or enhancement of current technology for genetic screening. Together with the advancement of NGS, cancer DNA testing has advanced from phenotype-directed, single-gene testing to extensive panel assessment, whereby various genes are interrogated concurrently.

Despite high expectations for NGS panel assessment, there are several constraints of the technique that need consideration. Along with high penetrance genes, NGS panels

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commonly consist of a combination of moderate and low-penetrance genes, which are devoid of well-established cancer risk estimations and professional medical management recommendations [5, 6]. The very first human cancer genome sequenced by NGS triggered an improved understanding and depiction of several cancers, contributing to the concise explanation of new subtypes, continuing development of biomarkers, the institution of novel remedial objectives, and the realization of TCGA project (TCGA; http://cancergenome.nih.gov) [7, 8]. Genome databases, like the Catalogue of Somatic Mutations in Cancer (COSMIC), provide curated information about somatic mutations from several million tumor trial samples [9, 10]. NGS data from these massive conceptional study initiatives have revealed persistent genomic aberrations throughout numerous tumor types [11, 12]. The knowledge extracted from genome and transcriptome sequencing, which has become integrated into clinical practice, has allowed for the identification of molecular subtypes of breast cancer [13] and targetable genetic variants in lung cancer [14, 15] and has had considerable influence on our understanding of additional cancer types [16].

Many are of the belief that NGS-based genomic sequencing will realize "precision medicine", meaning that every patient is provided with customized treatment depending on the unique genetic alterations in their solid tumors [17]. In this overview, we summarize the concept of precision medicine in solid tumors using NGS-based comprehensive gene panel testing while emphasizing the incredible importance of the standard control of tissue samples in routine NGS-based genomic testing. We also touch on concerns that must be tackled for the future adoption of NGS-based genomic sequencing in clinical practice in Japan.

Precision medicine in solid tumors using NGS-based comprehensive gene panel testing

With the advent of genomic sequencing technology, it is now feasible to ascertain the unique genomic changes associated with the tumor of an individual patient. Considerable time and effort have been dedicated to deciphering the human and cancer genome as part of the first step toward former US President Barack Obama's precision medicine initiative. The Human Genome Project, which spanned over a decade, revealed the complete map of a normal human genome [18]. Approaches for recognition of some genetic changes are set for methodical linking to clinical data. However, it would be essential for the physicians to analyze improvements in the cancer genome to cure the disease [19]. Throughout the sequencing of the human genome, cancer experts discovered virtually all significant oncogenes and tumor suppressors [20]. Precision medicine is anticipated to be adopted particularly for cancer to produce efficient cancer treatment strategies by determining genomic alterations in individual cancers. Many experts have determined that targeted sequencing of the key genes in the cancer genome is among the best ways of recognizing characteristics of the disease and devising therapeutic approaches.

Of roughly 25,000 genes in the human genome, 500 genes are likely linked to cancer, including those that are driver genes of cancer. For this small number of genes, it is easy to carry out deep sequencing, an approach which enhances precision by continuously sequencing the same site. In the United States, services for oncogenic panel testing are already offered by different enterprises and research facilities, including FoundationOne® by Foundation Medicine, Oncomine™ by ThermoFisher Inc., CANCERPLEX® by KEW, and MSK-IMPACT ™ by Memorial Sloan Kettering Cancer Center (Table 1). Many of these genomic tests developed in the United States have been introduced in Japan and are obtainable in the clinical research setting. In addition, original Japanese panels, such as NCC Oncopanel, Todai Oncopanel, and others, have been commercialized for research (Table 1).

Some of the panel tests may have the capability to regulate tumor mutation burden (TMB) (Table 1), which we have described below. Whatever the case, it is very important to verify the precision associated with a panel test prior to it being utilized in clinical practice. Though a lot of the panel tests have already been authorized by the Food and Drug Administration (FDA) in the United States, these panel tests will likely need authorization from the Pharmaceutical drugs and Medical Devices Agency (PMDA) in Japan before being used in clinical practice. Cancer gene panel testing allows us to evaluate genetic mutations that are manageable with molecular targeted drugs and to discover improved therapies for cancers.

Quality control of tissue samples for NGS in clinical practice

Most clinical samples are kept as formalin-fixed, paraffin-embedded (FFPE) tissue, in which the DNA necessary for NGS is regularly fragmented [21]. Many experts have revealed that the caliber of FFPE trial samples differs based on the surgical specimen's preparation and preservation [22, 23]. Consequently, it is essential that the next generation of surgeons who might be collecting samples for NGS learn to properly handle samples for FFPE processing [22]. It is also important to shed light on the amount of DNA that is usually gathered from the minimally sized sample, because generally, only a modest amount of tissues can be collected in clinical settings, for

Table 1 Representative next-generation sequencing-based gene panel tests

Panel test	Number of targeted genes	Enrichment approach (Capture/Amplicon)	Sample type for test	Tumor Mutation Burden	FDA approval	PMDA approval	References
MSK-IMPACT	468 genes	Capture	T/N	Yes	Yes	–	*Nat Med*. 2017; 23:703–713
Todai OncoPanel	464 genes	Capture	T/N	–	–	–	http://todaioncopanel.umin.jp/#sec01
CANCERPLEX	435 genes	Capture	T	Yes	–	–	*Per Med*. 2017; 14:309–325
FoundationOne CDx	324 genes	Capture	T	Yes	Yes	–	https://assets.ctfassets.net/vhribv12lmne/6Rt6csmCPuaguuqmgi2iY8/e3a9b0456ed71a55d2e4480374695d95/FoundationOne_CDx.pdf
OncoPrime	223 genes	Unknown	T	–	–	–	*Cancer Sci*. 2017; 108:1440–1446
PleSSision	160 genes	Unknown	T/N	–	–	–	http://www.hosp.keio.ac.jp/st/cancer/info/20180529_2.pdf
NCC Oncopanel	114 genes	Capture	T/N	–	–	–	https://www.mhlw.go.jp/file/05-Shingikai-10901000-Kenkoukyoku-Soumuka/0000179757.pdf
P5 report	52 genes	Unknown	T	–	–	–	http://www.okayama-u.ac.jp/user/hos/koganzai/P5report/
Oncomine Dx target test	23 genes	Amplicon	T/N	–	Yes	Yes	https://assets.thermofisher.com/TFS-Assets/LSG/brochures/oncomine-dx-target-test-flyer.pdf
Guardant360	73 genes	Capture	Blood (ctDNA)	–	–	–	http://www.guardant360.com/

FDA food and drug administration, *PMDA* Pharmaceuticals and Medical Devices Agency (Japan), *T* tumor tissue only, *T/N* tumor and normal tissue

instance, from a biopsy or following neoadjuvant therapy. Surgically resected and biopsy specimens were fixed in neutral-buffered formalin (NBF) or unbuffered formalin, and FFPE sample blocks were prepared by fixation, dehydration, clearing, and embedding steps, according to the standard protocol, as summarized in Fig. 1. In brief, tissue samples are fixed with NBF that contains PhosSTOP (Roche) to preserve phosphorylated proteins. The fixation time is restricted stringently to ≤ 24 h to avoid over-fixation, which can lead to considerable cross-linking. Five dehydration steps are then carried out in an ethanol series, accompanied by three steps of clearing with xylene and four steps of infusing in paraffin wax before embedding.

Integration of NGS in clinical oncology

The higher molecular resolution achieved by NGS has made it increasingly evident that most tumors harbor complex genomic changes, including large-scale chromosomal rearrangements described as chromothripsis16 or as chromoplexy depending on their origin [24, 25]. Considering intertumoral and intratumoral heterogeneity, it is currently unknown how these various events may influence patient responses to treatments targeting single driver mutations. The continuing development of DNA sequencing technologies now permitted the control of a huge number of tumors

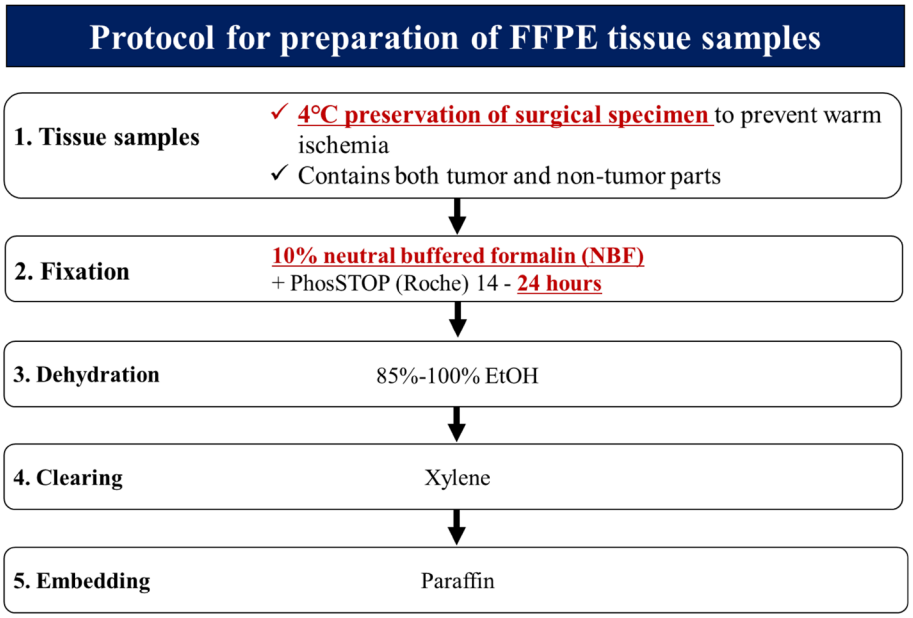

Fig. 1 Protocol for preparation of formalin-fixed, paraffin-embedded tissue samples. Tissue samples were obtained from surgical or biopsy specimens containing tumor and non-tumor tissue. Tissue processing for formalin-fixed, paraffin-embedded (FFPE) block preparation included fixation, dehydration, clearing, and embedding

of several forms for systematic mutation breakthrough, perhaps the most common set of driver mutations exists in every cancer's type, the combination of drivers inside a cancer type in addition to their distribution [26]. Within the beginning, clones and subclones of DNA differ from individual patients. This indicates that comprehending the clonal design of every single sufferer's tumor will likely be vital for improving their treatment method [26]. Le Tourneau et al. revealed no statistically significant variation in progression-free survival regarding the molecularly targeted agent (MTA) and therapy at physician's choice groups in intensely pretreated patients [27]. Based on their conclusions, it is advised that off-label utilization of MTA ought to be discouraged, and enrollment into clinical studies must be motivated to assist to determine predictive bio markers of effectiveness [27].

The scope of cancer sequencing ranges from targeted gene panels to thousands of base calls via whole-exome sequencing (WES) analysis of the 22,000-human protein-coding genes (40–50 million bases) to WGS throughout all 3.3 billion bases of the human genome. NGS had an initial clinical application in germline testing for recognized monogenic and unusual disorders via a targeted panel [28]. The low frequency of some molecular variations inside a sample also introduces the issue of their driver position as well as their actionability, which cannot be settled by NGS on its own because of the spatial and temporal restrictions of the existing technology [29]. In addition to the recognition of variation of unknown significance within the coding regions of the genome, the decryption of WGS is limited in non-coding regions or chromosomal aberrations [30].

Several of the problems in determining the important value of numerous genomic variations could possibly be reconciled by integrative investigation of the transcriptome. Although it might be too soon to accept WGS within a clinical setting, this technique offers a firm foundation for the extensive evaluation of cancer and provides novel insights into cancer biology, resulting in improved diagnosis and therapies.

Microsatellite instability (MSI), hypermutation, and TMB

Lately, the innovative technology of immune checkpoint inhibitors has resulted in a paradigm shift in the area of cancer research and cancer patient care. Immune checkpoint inhibitors have demonstrated remarkable improvements for numerous solid types of cancer [31, 32]. However, immune checkpoint inhibitors have had a substantial impact within a small subset of patients, and existing approaches like immunohistochemistry of PD-L1 are unable to entirely recognize these responders to immunotherapy. Therefore, predictive biomarkers to pinpoint responders are very much desired.

The latest development in genomic analysis employing NGS has allowed for greater understanding of the "mutation burden" in various cancers. Hypermutated cancer cells are believed to express various neo-antigens, which attract cytotoxic (CD8+) T-lymphocytes and activated Th1 cells to the tumor microenvironment [33]. The mutation burden was originally characterized by whole-exome analysis, such as TCGA [34]. Utilizing the NGS-based panel test, we were able to ascertain the TMB [35], which is anticipated to be useful in the area of immuno-oncology and cancer treatment. We have previously established that a panel test with 415 genes is related to WES in generating mutation rates and in

differentiating hypermutated and non-hypermutated tumors [35]. However, the ordinary mutation rate observed through the panel test was more than that recognized by WES, highlighting the reality that the panel content consists of genes that might be repeatedly mutated in cancer.

MSI is triggered by an impaired DNA mismatch repair (MMR) system, which frequently results from germline or somatic mutations or promoter hypermethylation of genes in the DNA MMR system, such as *MLH1, MLH2, MSH6*, and *PMS2* [36]. Lately, NGS-based analysis has been designed to identify MSI better by evaluating several MSI loci, which helps panel tests to elucidate the driver and passenger mutations concurrently with the existence of MSI [37, 38]. We have witnessed that 17 out of 201 (8%) Japanese colorectal cancers were hypermutated tumors, as recognized by a gene panel test [35]. Among them, we found 11 patients with MSI, and two patients with polymerase ε (POLE) mutation, which is an additional cause of hypermutation [35]. We also found 32 out of 207 (15.5%) gastric cancer patients in Japan with hypermutated tumors, which were detected to be an MSI subtype with Epstein–Barr virus (EBV) infection. Through integrative genomic analysis NGS technologies, chromosomal instability (CIN) and genomically stable (GS) subtypes of TCGA were distinguishable [39]. We also identified TMB in in triple-negative breast cancer sufferers and found that 3 out of 51 (5.7%) patients were hypermutated. Components other than MSI, such as substantial APOBEC, may possibly be involved in the hypermutation in breast cancer, which requires additional exploration.

Role of NGS to guide cancer therapy

Many forms of cancer are genetically intricate and are, therefore, much better determined by the activation of signaling pathways than by an outlined set of mutations. The NGS method for developing targeted small-molecule cancer drugs captures excitement in conjunction with minimizing the pre-existing designing load on scientists in cancer therapy. We have witnessed many challenges related to the conventional design of medicines. These obstacles symbolize the intricacy of the anti-cancer drug breakthrough approach, the minimal accuracy level of target recognition, the very high cost of drug synthesis and clinical studies, the limited expertise in the underlying molecular mechanisms, and the lack of authenticated biomarkers for the depiction of tumor type [40]. The achievement of the Human Genome Project led to comparable projects looking at the genome in several cancers [41]. Amplicon sequencing augments target genes via PCR with a set of primers for exons of particular genes prior to NGS analysis [42]. In a few types of cancer, patient risk and prognosis are usually forecasted in line with the mutation profile recognized by NGS. Numerous targeted treatment plans have already been developed for cancer patients who display distinct mutations. On the other hand, deciding on the best NGS method for the proper clinical application can often be difficult, particularly in clinical oncology, where materials for NGS tests are commonly limited and also the turn-around time (TAT) for cancer assessments is restricted to a couple of days. Currently, amplicon-based NGS strategies are the most efficient techniques used in clinical oncology [42].

An individual genome will have numerous digressions from the reference genome, called single nucleotide variants (SNVs), and/or structural alterations like insertions, deletions, or translocations. Somatic mutation analysis, which is carried out for cancers, poses further obstacles [43]. There are robust algorithms available for identifying many clinically relevant alterations that occur as point mutations, short insertions or deletions, or copy number aberrations in clinical samples analyzed by NGS [43].

It is common for hereditary cancers to overlap, so broad phenotypes are apparent objectives for NGS panels. This is in contrast to cautious genetic evaluation, which may recognize distinctive physical or family history attributes, making specific gene testing a tiered testing approach [6]. Panels are readily available and are affordable, providing utility for both patients and professionals. To support professionals in recognizing individuals suitable for genetic counseling referral, the American College of Medical Genetics and Genomics (ACMG) and the National Society of Genetic Counselors (NSGC) have created practice guidelines for cancer predisposition assessment [44]. Specific considerations, such as the age of the patient, the onset of cancer, the main occurrence site, the existing age range and ages at the time of death of close relatives, and the ethnic background of maternal and paternal lineages, need to be documented [6].

The discovery of biomarkers like BRCA1, BRCA2, HER2, PR, and ER has been enormously important in molecular profiling and targeted drug design [45]. The NGS technology incorporates genomics, transcriptomic, and epigenomic mutations in cancer biology, and classification of different types of cancer for earlier medical diagnosis and targeted therapies [46].

Future perspectives of NGS

Since the AJCC 8th edition, multiple gene expression assays, such as OncotypeDx, have been incorporated into the TNM staging system of breast cancers as the prognostic modifier. Within this version, the TNM stage of breast cancer is affected by the outcomes from the molecular diagnosis [47]. In parallel to the advancement of PARP inhibitors for advanced breast cancer sufferers, germline assessment has increased and is necessary for family genetic counseling.

Preventive medicines have appeared to reduce the cancer risk of an individual with germline mutations [48]. Given that the BRCA germline mutations associated with inherited breast and ovarian cancer syndrome are also associated with prostate, pancreatic, and gastric cancer, monitoring for this particular group is an important task and should be handled carefully [49].

NGS-based gene panel tests cost thousands of US dollars per test, depending on the supplier, and WGS is even more expensive. Another significant problem with NGS-based precision medicine is that it still has insufficient therapeutic modalities [50]. It is also critical to develop an interpersonal program that enables genetic testing. For individuals with feasible BRCA1/2 germline mutations, genetic counseling should be carried out and further germline assessments should be analyzed in accordance with the individual's requirements. Aside from *BRCA1/2*, other genes, such as *PALB2, PTEN*, and *TP53*, seemed to be documented to result in genetic carcinogenesis [51]. For each gene, the mutation type and penetrance differed. It is critical that genetic counseling be formulated without delay. There is a paucity of specialists in Japan who are competent to implement this in the field of cancer. Thus, extensive training at the accredited level would also be essential.

Following NGS analysis, the primary obstacle lies in the interpretation and judgment of the data regarding gene alterations and clinical information. This field is steadily expanding in Japan compared with European nations. There are actually numerous published genomic databases; nevertheless, the available data associated with medical therapy results are inadequate. Drug efficacy could possibly be distinctive, primarily based not merely on transformed genes by themselves but also by the types and locations of gene variations. The challenge of mutations of unidentified relevance prevails, not only for *BRCA1/2* mutations as explained previously, but also for other genes for which far more clinical information will be required. Drug effectiveness can even vary in accordance with the organ, where the cancer develops or metastasis ensues. Many experts have revealed that the efficaciousness in HER2-mutant malignancies is associated with tumor type and the mutant allele [52].

Increased database knowledge and access will be essential for the appropriate clinical treatments for patients, taking into account the particular genomic alterations and particular tumor type or organ or origin. Bioinformatics is fundamental for handling massive hereditary and clinical cancer information attained from patients by NGS analysis. Bioinformatics for precision oncology is a new discipline, and for that reason, the need to increase human resources is significant. Increased genetic testing, with the ensuing improved cancer genomic databases and clinical outcomes, will allow development of bioinformatic foundations for improved patient care in the future.

Most NGS-dependent gene panel assessments use tissue samples acquired by surgical procedures or biopsy. Consequently, it is extremely hard for patients who may have only metastasis that cannot be operatively taken off or biopsied to evaluate their tumor by panel assessments. Lately, liquid biopsy, in which cancer-derived DNA in blood is analyzed by NGS, has received attention. There are a number of liquid biopsy technological innovations, based on cell-free DNA (cfDNA) produced by damaged cancer cells within the blood, and circulating tumor cells (CTC) [53]. Monitoring via liquid biopsy is anticipated to allow for earlier recognition of postoperative recurrence. Close monitoring using microRNA is also envisioned to be possible with liquid biopsy [54].

Although these methods are anticipated to simplify the determination of cancerous growth and gene mutations in sufferers with significantly less intrusion, there are still numerous issues that must be resolved, such as the accuracy and reliability of assessment. This is particularly so in breast cancer, since it is regarded as more challenging than carcinomas with numerous driver genes. For instance, in breast cancer, unlike colon cancer and carcinoma of the lung, there is a handful of innate mutations that are typical among the patients, and the overall range of CTC in blood is significantly less compared with prostate cancer. It is anticipated that more and more convenient and useful laboratory tests might be formulated in the foreseeable future as technological innovations advance.

Conclusion

In this overview, we have tried to define the utility of NGS-based gene panel assessment and have reviewed problems of importance to foreseeable future research. Cancer is really a disorder of the genome, and therefore, it is truly reasonable that treatment methods should depend on genomic transformation. Although there are still numerous concerns over the accuracy of most cancer medicine characterization, we were also quickly advancing along the path to improving a cancer patient's prognosis. It is imperative for specialists to understand the application of NGS-based genomic testing to assist patients with cancer.

Compliance with ethical standards

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