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Review

Application of next-generation sequencing in clinical oncology to advance personalized treatment of cancer

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Abstract

With the development and improvement of new sequencing technology, next-generation sequencing (NGS) has been applied increasingly in cancer genomics research over the past decade. More recently, NGS has been adopted in clinical oncology to advance personalized treatment of cancer. NGS is used to identify novel and rare cancer mutations, detect familial cancer mutation carriers, and provide molecular rationale for appropriate targeted therapy. Compared to traditional sequencing, NGS holds many advantages, such as the ability to fully sequence all types of mutations for a large number of genes (hundreds to thousands) in a single test at a relatively low cost. However, significant challenges, particularly with respect to the requirement for simpler assays, more flexible throughput, shorter turnaround time, and most importantly, easier data analysis and interpretation, will have to be overcome to translate NGS to the bedside of cancer patients. Overall, continuous dedication to apply NGS in clinical oncology practice will enable us to be one step closer to personalized medicine.

Key words: Next-generation sequencing (NGS), cancer, personalized treatment

Our understanding of genetics and the genome has been greatly enhanced by the overwhelming revolution in sequencing technology in recent years. Next-generation sequencing (NGS) has been widely implemented for whole-genome sequencing, whole-exome sequencing, transcriptome sequencing, targeted region sequencing, epigenetic sequencing, and other sequencing[1]. With the advent of this technology, there is great potential for NGS application in disease management and treatment, genetic counseling, and risk assessment. From a clinical perspective, the technology can be used for but is not limited to molecular diagnosis of genetic disease[2] and infectious disease [3], prenatal diagnosis [4], carrier detection[5], medical genetics and pharmacogenomics [6], cancer molecular diagnosis[7,8], and prognosis[9,10].

Cancer is a heterogeneous disease that arises from accumulation of DNA mutations. New sequencing technologies will have a significant impact on cancer diagnosis, management, and treatment. Human genomes and thousands of cancer genomes sequenced with NGS provide a road map of the normal human genome and a landscape of mutations in cancer genomes across many cancer types. This allows us to better understand the molecular mechanism of oncogenesis and the rationale for molecule-guided therapies[11]. It will be possible in the near future to sequence both the normal and cancer genomes of every patient. Normal genome sequencing will indicate the patient's genetic background for inherited cancer risk and drug metabolism, thus enabling identification of high-risk patients and chemotherapeutic drugs with the greatest potential effectiveness and fewest potential side effects. Cancer genome sequencing can be used to monitor the disease-specific molecular genotype and guide targeted therapy design or selection.

NGS Technology

Over 30 years ago, Sanger *et al.*[12] developed a DNA sequencing approach based on electrophoresis. However, due to its limitations in throughput and relatively high costs, it was never possible to sequence a

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large number of genes and samples. To overcome this obstacle, new sequencing technologies were created. NGS technologies sequence thousands of DNA molecules in a parallel fashion. It affords high speed and high throughput. It is able to generate both quantitative and qualitative sequence data, equivalent to the data from Human Genome Project, in two weeks. Currently, there are several NGS platforms commercially available: the Illumina Hiseq and Miseq, the Roche 454 GS and Junior version, the personal genome machine Ion torrent, and the Life Technologies SOLiD (Table 1)[13-18]. Some NGS platforms, such as Miseq and Ion torrent, are more favorable for clinical use because of their more flexible throughput and shorter turnaround time. In this review, we focus on the application of NGS to cancer prevention, diagnosis, and treatment.

Whole-genome sequencing involves resequencing the entire genome and mapping the sequence back to the human genome to identify mutations. The main advantage of whole-genome sequencing is full coverage of the entire genome, including promoters and regulatory regions. Therefore, whole-genome sequencing is mostly used to identify novel and rare mutations. In whole-exome sequencing, all exons of all known genes are sequenced at a relatively deeper depth. Compared to whole-genome sequencing, the major advantage of exome sequencing is that the cost has been reduced significantly. Whole-exome sequencing has been used to identify genes associated with cancer [19], diabetes [20], immunologic disorders[21], and other conditions. Transcriptome sequencing involves sequencing cDNA fragments generated by reverse transcription of RNA. Researchers can determine an RNA expression and splicing profile based on results from transcriptome sequencing. Epigenetic analysis is an emerging NGS application to characterize epigenetics in cancer. The potential prognostic and diagnostic application of methylation and protein-DNA binding profiles have been shown[22].

Application of NGS in Clinical Oncology

Identification of novel cancer mutations using NGS

NGS technologies have enabled efficient and accurate detection of novel and rare somatic mutations. NGS has been successfully employed to identify novel mutations in a variety of cancers, including bladder cancer[23], renal cell carcinoma[24], small-cell lung cancer[25], prostate cancer[26], acute myelogenous leukemia[27,28], and chronic lymphocytic leukemia [29]. Whole-genome sequencing with NGS was used in patients with a rare form of acute promyelocytic leukemia and successfully identified a novel *PML-RARA* genetic recombination that was undetectable with standard cytogenetic techniques[30]. In our department, Gui *et al.*[23] sequenced the exomes of 9 transitional cell carcinoma tumors to find somatic mutations, then screened in tumor samples from 88 individuals with transitional cell carcinoma at different stages and grades. They found 55 notable mutations related to transitional cell carcinoma, 49 of which were first found in bladder cancer. Furthermore, they sequenced the whole exomes of 10 clear cell renal cell carcinomas and screened thousands of genes in an additional 88 samples, ultimately discovering 12 new mutated genes[31]. Both studies were published in *Nature Genetics* in the same year.

In another study, Keller *et al.*[32] used specific target selection and NGS to identify novel SNPs in genes already associated with glioblastoma. Over 6000 SNPs, including more than 1300 SNPs located in targeted genes, were identified. These results provide evidence that targeted resequencing is well suited to detect SNPs that may relate with disease risk. The approach may be especially applicable in personalized medicine to assess the relevant genotype of a patient at a reasonable cost[32].

By using whole-genome (or whole-exome) sequencing, numerous novel genetic aberrations and associated potential therapeutic targets have been found in many cancers. These findings have provided key insights into mechanisms of tumorigenesis. Many of these studies on new genetic aberrations were summarized by Tran *et al.*[33].

NGS in hereditary cancer syndrome genetic testing

About 5%−10% of cancers are hereditary. Genetic testing has been used for hereditary cancer patients for more than ten years in the US and Europe[34]. Today, the most widely used method for genetic testing is Sanger-based sequencing, which is considered the gold standard for detecting mutations. Nevertheless, because genes related to hereditary cancers are very large and there is no particular mutation hot spot, this traditional method for genetic testing of hereditary cancer has been proven to be time consuming, high cost, and low throughput[35].

The development of NGS provided many opportunities for genetic testing. Walsh *et al.* [35] used target region capture and NGS to detect 21 genes associated with hereditary breast and ovarian cancer. This combined method allowed detection of several kinds of variations, including single-nucleotide substitutions, small insertions and deletions, and large genomic duplications and deletions[35]. In the US and Europe, commercial testing for breast cancer 1 (*BRCA1*) and *BRCA2* are based on polymerase chain reaction (PCR) amplification of individual exons and Sanger sequencing

Table 1. Comparisons of next-generation sequencing platforms

Sequencing platform	Technology	Applications	Run time	Read length	Output	Advantage	Disadvantage
Roche 454 GS FLX+	Emulsion PCR pyrosequencing	Targeted region sequencing, SNP discovery	10 hours	~400 bases	700 Mb	High throughput, long reads, short turnaround time	High cost, homopolymer errors
Roche 454 GS Junior	Emulsion PCR pyrosequencing	Same as GS FLX+	10 hours	~400 bases	35 Mb	Small size, long reads, low price, short run time	Small scale, low output
Illumina HiSeq 1000/2000	Bridging amplification, reversible terminator dye and imaging system	Whole-genome sequencing, targeted resequencing, *de novo* sequencing, amplicon sequencing, SNP discovery	8.5–11 days	~100 bases	300–600 Gb	Ultra high output, widely used	High cost, low multiplex capability, short read assembly
Illumina HiSeq 1500/2500	Bridging amplification, reversible terminator dye and imaging system	Same as Illumina HiSeq 1000/2000	High output: ~11 days Rapid run: ~27 hours	~100 bases	600 Gb	Two modes ready for large and small scales	High cost, short reads
Illumina MiSeq	Same as Hiseq	Amplicon sequencing, clone checking, ChIP-Seq, and small-genome sequencing	4–39 hours	36–250 bases	540 Mb –8.5 Gb	Personal sequencer, more rapid, fully automated, more suitable for clinics	Unproven
Life Technologies SOLiD	Emulsion PCR, ligation sequencing	Whole-genome/ exome sequencing, SNP detection	2–7 days	35–75 bases	77–155 Gb	More accurate, high throughput	Short read assembly, long run time
Life Technologies Ion Torrent	Hydrogen release detection, semiconductor sequencing	Targeted sequencing, amplicon sequencing, small-genome sequencing	~2 hours	35–200 bases	1 Gb	Short run time, low cost, more suitable for clinical application	Homopolymer errors, semi-automated
Complete Genomics	DNA nanoball PCR, ligation sequencing	Whole-genome sequencing	12 days	70 bases	20–60 Gb	Complete genome analysis	Only offer whole-genome sequencing
Helicos	Single molecule dye terminator	Whole-genome sequencing	8 days	35 bases	21–35 Gb	Unbiased read	High error rates
Pacific Biosicence PacBioRS	Single molecule sequencing using fluorescent dNTP	Genomic DNA, PCR products, infectious agent sequencing	<1 day	3–10 kb	1.2 Gb	Longest read length, short run time	Highest error rate

of the products. For large exonic deletions and duplications, multiplex ligation-dependent probe amplification (MLPA) has been added for supplementary testing. However, MLPA can only be used to test known variations[36].

NGS provides a good solution for detecting rare variations. Because it allows testing of multiple genes at once, NGS greatly improves the variation detection rate. Many patients with hereditary cancer have tested negative for genetic variations, but with NGS, it is easier to find causative mutations. In a study of 300 high-risk breast cancer families, Walsh *et al.*[37] found previously undetected mutations in 52 probands. And the reduced sequencing costs and turnaround time made the approach even more practical in clinics[37]. Ozcelik *et al.*[38] introduced a method that used long-range PCR plus NGS to detect *BRCA1* and *BRCA2* and demonstrated that it was useful for *BRCA* testing. For a small sample size, the method is combined with the Miseq or Ion torrent platform. Moreover, this approach may be more flexible and economic than a capture strategy[38]. A similar method has also been reported previously by Hernan *et al.*[39] and De Leeneer *et al.*[40].

The use of NGS in genetic testing for hereditary

cancer syndromes will be the first and closest step for its transition into clinical practice. It is more exciting that whole-genome or whole-exome sequencing of malignant tumors has been used in several clinical trials for personalized therapy.

NGS for personalized cancer treatment

Apart from identifying genetic and novel somatic mutations, another use of NGS is to improve rationally designed individualized medicine. To date, many studies have applied NGS for personalized treatment of cancer. For example, NGS has been used in the treatment of pancreatic cancer[41]. It has been also used in the detection of epidermal growth factor receptor (*EGFR*) deletions in non–small cell lung cancer, which showed important pathogenetic and clinical implications for patients with non–small cell lung cancer[8]. In addition, it has been used in the detection of *PML-RARA* fusion gene in acute promyelocytic leukemia, which led to a change of a patient's therapeutic schedule[31]. By using Affymetrix SNP arrays, Illumina mRNA expression arrays and sequencing method, Holbrook *et al.*[42] observed the *KRAS*, *ERBB2*, *EGFR*, *NET*, *PIK3CA*, *FGFR2*, and *AURKA* gene mutations in gastric cancer, and their data suggested targeted therapies for gastric carcinoma that would benefit 11 of the 50 patients in their study. Roychowdhury *et al.*[43] reported the first study of whole-genome and whole-exome plus transcriptome sequencing in personalized treatment. In the study, they tested the integrated strategy in several patients, and the resultant sequencing data were discussed by the Sequencing Tumor Board, a panel of clinical and genome sequencing experts, to determine the appropriate treatment. Next they used the strategy in actual patients and sequenced tumor and normal DNA. Two patients, one with colorectal cancer and the other with melanoma, who were previously treated unsuccessfully, were enrolled into the pilot study. The integrative sequencing and analysis were completed within 4 weeks after biopsy. In the patient with colorectal cancer, variations in *CDK8* and *NRAS* were found and considered the potential targets to match to a clinical trial in the future. For the patient with melanoma, a structural rearrangement in *CDKN2C* and *HRAS* was detected, prompting the Sequencing Tumor Board to suggest combined treatment with PI3K and MEK inhibitors for this patient[43].

Although most of the findings in the pilot trial were not clinically significant, the study demonstrated that NGS is a practical clinical application in terms of cost and time. Other investigations with NGS have also been reported. In an inspiring study, genetics researchers at Washington University did whole-genome and transcriptome sequencing for a researcher in their team, who had adult acute lymphoblastic leukemia[44]. The cancer relapsed twice in 10 years from the time of first diagnosis. Then, his colleagues found a clue about the disease through RNA sequencing. Their results showed that a normal gene, *FLT3*, was wildly active in the leukemia cells. Luckily, however, the drug sunitinib, which is approved to treat advanced renal cancer, inhibits *FLT3*. With sunitinib treatment, his blood counts appeared more normal. This is a very successful case of translating NGS into clinical practice[44]. At present, only DNA and RNA sequencing are used to view genetic aberrations. Moving forward, the epigenetic genome and small RNA will be sequenced to provide complementary information and enable cross-validation.

As illustrated in Figure 1, it is possible that one day, patients will have their normal and tumor genomes sequenced before doctors design personalized treatment regimens. With the improvement of NGS technology, it is possible to have a comprehensive molecular profile for a patient available within a clinically acceptable timeframe and cost. Furthermore, as these studies demonstrate, the application of NGS in clinical oncology is progressing. The first step, exploration, is underway, but further clinical investigation and validation trials are required to implement this approach in personalized treatment for cancer.

Detection of circulating cancer DNA by NGS

Rare mutations in circulating DNA have long been used to detect somatic mutation for cancer diagnosis and management. There are many available methods to detect these mutations, such as peptide nucleic acid and primer extension and bead-based digital PCR in emulsions ("BEAMing"). Nonetheless, it is difficult to identify rare mutations in tumor suppressor genes like *TP53*, which is highly mutated throughout the gene. NGS may be the cost-effective method to detect and measure the allele frequency of *TP53* and other tumor gene mutations in the plasma. Forshew *et al.*[45] developed a tagged-amplicon deep sequencing (TAm-Seq) method that used NGS and designed primers to amplify approximately 6000 bases that covered the selected regions of cancer-related genes, including *EGFR*, *TP53*, and *KRAS*. By using plasma samples, they showed that the method could identify mutations in *TP53* at allelic frequencies of 2% to 65%, thereby demonstrating that it is feasible to sequence large regions of circulating DNA by NGS[45].

Advantages of NGS

More comprehensive analysis of variation type

Sanger sequencing is the gold standard for single-

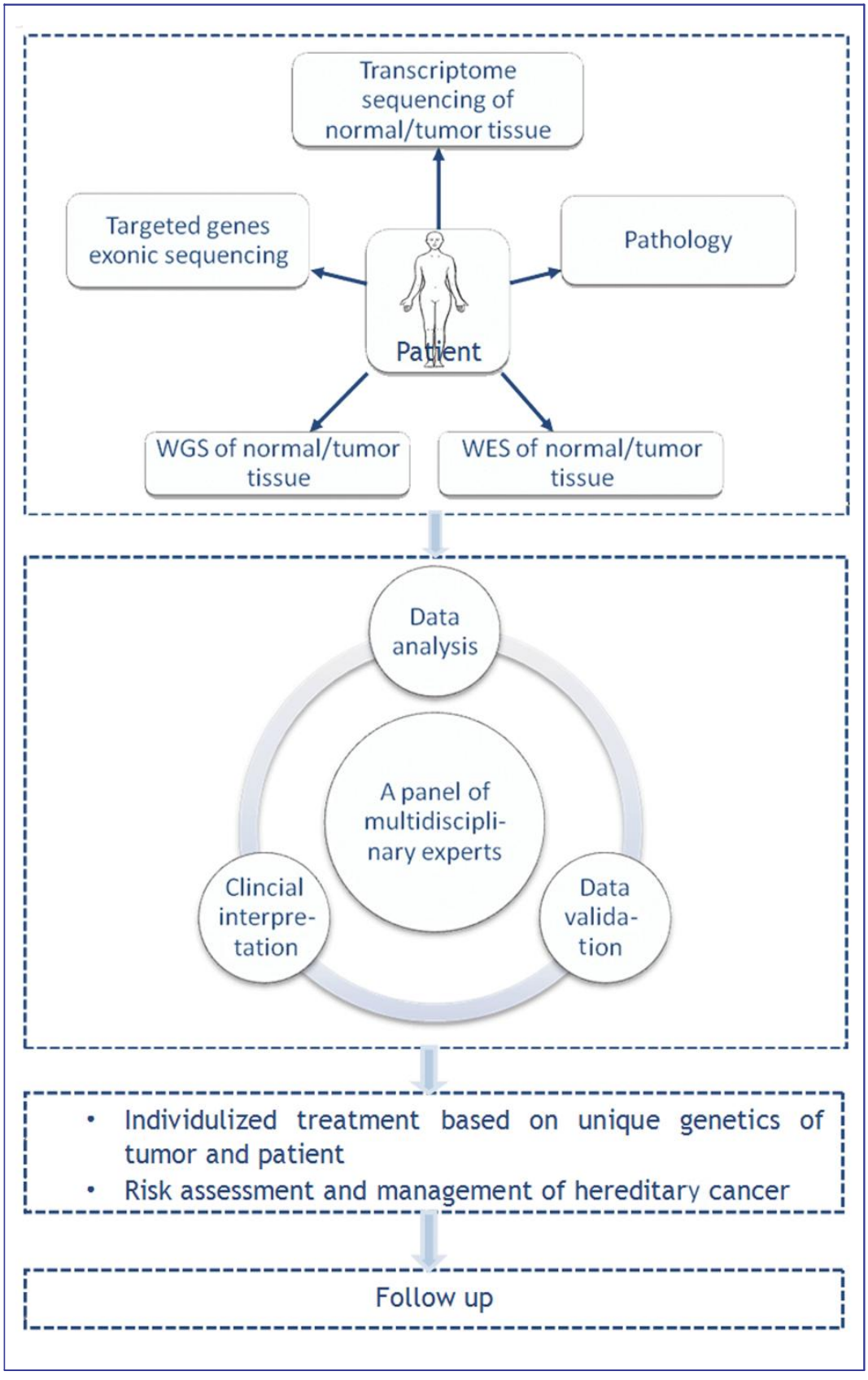

Figure 1. **The anticipated work flow of individualized cancer treatment based on the unique molecular prolife of a patient.** For a given patient, the normal genome and tumor genome is sequenced by using next-generation sequencing. The genetic information is analyzed, validated, and clinically interpreted by a panel of multidisciplinary experts. A personalized treatment regimen is designed based on the unique genetics of the tumor and the patient's normal genome. In addition, the patient's family may benefit from knowing the risk of hereditary cancer and taking appropriate intervention. WGS, whole genome sequencing; WES, whole exome sequencing.

nucleotide variants and small insertions/deletions[46], but it is not effective for gross insertions/deletions and large rearrangement. NGS can fully detect all mutation types of target genes and even chromosomal abnormalities[47]. Walsh *et al.*[48] reported that they developed a genomic assay to detect all mutations in 21 genes associated with breast and ovarian cancer. Not only were all the mutations found, there were no false-positive declarations of deleterious mutation[48].

Less DNA is required

It is not always possible to get a large quantity of DNA for genetic tests, especially from small amounts of tumor tissue. Traditional Sanger sequencing for *BRCA1* and *BRCA2* requires approximately 3 μg of DNA, whereas 500 ng is enough for chip-captured NGS sequencing.

Challenges of NGS

Data analysis and computing infrastructure

Hundreds of gigabytes of data will be generated from NGS. It is a difficult and complicated task for bioinformatics staff to filter redundant and huge amounts of data. Some open source tools are available, though only for simple and routine cases. Specialized programs must be used for most NGS projects. In addition, storing, processing, and analyzing NGS data must be done on a high-performance computer. It is impractical for small diagnostic laboratories and clinics to satisfy these essential requirements and to afford the associated costs. NGS platforms such as the Miseq and the Ion torrent have been purchased in some clinics due to their automated data analysis and storage pipelines; however, low throughput restricts their applications.

Interpretation of variation data

Interpretation and clinical translation of data collected on genetic variants remains a bottleneck for routine adoption of NGS in clinical settings. Because few variants contribute to disease pathogenesis, it is difficult to accurately define clinically significant variants or to effectively assess disease risk based on current research[49,50]. For personalized cancer treatment, filtering out tumor-promoting mutations from passenger mutations is also a challenge, especially considering that the roles of both may change as the tumor develops[51].

Ethical issues

There are some legal and ethical concerns for DNA sequencing. It is easy to share a patient's genetic testing result with that patient and among doctors and family members. However, this disclosure may lead to discrimination based on genetic information and social disorder, especially in job-hunting and applications for health insurance. With the promulgation of some laws, such as the Genetic Information Nondiscrimination Act (GINA) (US, 2008), genetic testing is gradually becoming accepted by the public. However, more particular patent law should be adapted to push the NGS application from research to routine clinical use.

Conclusion

NGS has been used in cancer genomics study and transitioned to be applied in clinical practice. Several studies have demonstrated the potential of NGS application in cancer personalized treatment. Compared to traditional sequencing, NGS holds many advantages, such as high throughput and low cost. Moreover, personal sequencers, such as Miseq and Ion torrent, have vastly improved in speed, which is helping to accelerate NGS to routine clinical practice. However, significant challenges, particularly with respect to the data analysis and interpretation and ethical issues, will have to be overcome to translate NGS to the bedside of cancer patients. Overall, continuous dedication to apply NGS in clinical oncology practice will enable us to be one step closer to personalized medicine.

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