ADVANCES IN DRUG DEVELOPMENT

Current Developments in Oncology Drug Research

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Clinical Applications of Next-Generation Sequencing

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H&O What is next-generation sequencing (NGS)?

MK NGS (also known as second-generation sequencing) is a massive parallel sequencing technology that can be used to search the whole genome sequence of an individual in a short period of time. NGS technologies have resulted in mapping of the human genome in many people, and have thereby aided in providing the blueprint to understanding how genetic changes lead to disease. In the past, the size and complexity of the human genome was a major obstacle for the sequencing of human cancer genomes. The development of NGS technologies has revolutionized our ability to analyze cancer genomes, as massive parallel sequencing leads to the simultaneous generation of millions of short DNA sequences (50–100 nucleotides). Those sequences are then mapped back to the human reference genome, ultimately creating a picture of the cancer genome. With regard to cancer studies, sequencing both the tumor and the normal tissue from patients is crucial. For each human genome, there are 3–4 million inherited sequence variants. As such, inherited polymorphisms are the majority of sequence variants identified in a cancer genome, not acquired mutations. Thus, one must compare a tumor genome with its paired normal genome in order to appropriately identify acquired sequence variants. Currently, the cost of sequencing 1 human genome is approximately $5,000; this cost is expected to drop down to $1,000 in the near future. As the cost of sequencing continues to decline and the number of molecular assays continues to rise, it is anticipated that the point will be reached where whole-genome sequencing becomes one of, if not the most, cost-effective diagnostic tool for cancer patients.

H&O How is NGS different from traditional DNA sequencing?

MK To comprehensively analyze a whole genome, NGS performs sequence analysis using massive short reads of a genome simultaneously. Compared to the traditional DNA sequencing method (Sanger sequencing), NGS can detect structural variations other than single nucleotide variations. NGS is capable of producing from thousands to millions of sequences at once, compared to the 96 sequences processed by the conventional capillary-based Sanger method. However, due to the short read length, NGS cannot accurately read highly similar sequences in a human genome, which is one of its limitations at present.

H&O In what settings has NGS already been successfully employed?

MK In the research setting, NGS has been widely implemented for de novo genome sequencing, DNA resequencing, transcriptome sequencing, and epigenomics. NGS has also succeeded in the comprehensive analysis of hematologic tumor genomes and the detection of their mutations. Now, NGS can detect all types of mutations—including single nucleotide variants (SNVs), small insertions/deletions (indels), copy number variants (CNVs), and complicated structural changes—in a broad range of tumor types, by comparing the genomic sequences between tumors and normal tissues. However, because most of the sequencing analyses for cancer genomes were conducted by exome sequencing or RNA sequencing, detectable mutations

Table 1. Summary of Next-Generation Genomic-Sequencing Assays

Sequencing Target	Approach	Comment
Whole genome	Unselected genomic DNA is fragmented and sequenced	Comprehensive but expensive; detects point mutations, indels, translocations, deletions, and amplifications
Exome	Exonic DNA is enriched by capture and then sequenced	Only identifies mutations in exons, misses many structural variants
Transcriptome	RNA is reversed transcribed to generate cDNA and then sequenced	RNA expression and splicing are assessed; only identifies mutations in expressed genes
Methylome	gDNA is treated with bisulfite to mark methylated cytosines before sequencing*	Assay being optimized; provides genome-wide information about gDNA methylation
Histone-associated genome	ChIP using antibodies against modified histones followed by sequencing	Used to assess chromatin modifications, in particular genome-wide occupancy by mofidied histones

gDNA indicates genomic DNA.
Data from Welch JS, Link DC. Genomics of AML: clinical applications of next-generation sequencing. *ASH Education Book*. 2011;2011:30-5.

are mainly focused on the single nucleotide variations or fusion genes. Table 1 illustrates a summary of next-generation genomic-sequencing assays.

H&O What are some challenges for implementing NGS in the clinical setting, and what progress has been made thus far?

MK Clinical sequencing, or the implementation of NGS in the clinical setting, remains a big challenge. NGS can explore all variations in the human genome, but most of these genomic changes are almost uncharacterized, and their biologic or clinical implications are unknown. In order to achieve a deeper interpretation of the diversity of the human genome and cancer genomes, we must analyze more associations of genotypes or mutations with diverse phenotypes, such as drug response and side effects, in larger sets of clinical samples. Another concern is that the amount of data produced by NGS may quickly overwhelm the existing data management and storage infrastructures of clinical laboratories. A single sequencing run can generate up to 15 terabytes of information and, as such, clinical laboratories will need to implement efficient methods of tracking and backing up data. Lastly, because NGS is not yet widely used for clinical diagnostic purposes, quality control standards are still being developed to address acceptable precision, accuracy, contamination, and sequence coverage parameters. Until such standardization is reached, the integrity and accuracy of both raw data and interpretation of variants identified by NGS cannot be ensured.

H&O What are some potential benefits of utilizing NGS in the management of cancer?

MK There are several potential advantages for using NGS in the management of cancer. Data generated from the complete molecular profiling of the cancer genome can be used for the accurate molecular diagnosis and classification of cancer types. Such data will also help predict individual prognosis and likely treatment response, which is currently based on probabilistic measures derived from the general population. NGS can provide a faster, less invasive, more clinically useful tool for diagnostics, treatment monitoring, and personalized detection of recurrence. NGS will also provide useful information to develop new drug targets.

NGS can detect somatic mutations in a minor cell population of cancer tissues with far greater sensitivity than Sanger sequencing. Given that cancer is a heterogeneous disease, only a fraction of the cells will contain the many clinically relevant mutations. NGS employs high base coverage, resulting in a higher sensitivity of minor alleles or mutations appearing in tumor cells. This will help us further understand and characterize each of these tumors at the clinical level.

H&O What role do you think NGS will play in the future for cancer care and individualized treatment?

MK Proof-of-concept studies have already successfully employed NGS technologies for the management of cancer patients. In the future, NGS will play a central role in determining treatment approaches for individual patients. Clinicians will select the appropriate treatment based on

the sequencing data of each tumor obtained by NGS. Moreover, NGS may detect the recurrence of cancer and early-stage cancer, if we can sequence circulating tumor cells or cancer biomarkers in the blood or other biofluids.

NGS technologies may also play a role in pharmacogenomics, or how one's genes influence their response to drugs. By sequencing the "normal" and disease genomes in cancer patients, it may be possible to identify genetic variants known to effect the efficacy and toxicity of certain agents, which will help guide the choice and dosing of the treatment regimens used. NGS may also identify genetic variants that contribute to cancer susceptibility, which may also affect a patient's treatment and prognosis.

Suggested Readings

Schweiger MR, Kerick M, Timmermann B, Isau M. The power of NGS technologies to delineate the genome organization in cancer: from mutations to structural variations and epigenetic alterations. *Cancer Metastasis Rev*. 2011;30:199-210.

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