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REVIEW PAPER

Next-Generation Sequencing in Cancer

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Abstract

Objective In this article, we provide a gestalt idea about NGS technologies and their applications in cancer research and molecular diagnosis.

Background Next-generation sequencing (NGS) advancements like DNA sequencing and RNA sequencing allow uncovering of genomic, transcriptomic, and epigenomic scenes of individual malignant growths. An assortment of genomic abnormalities can be screened at the same time, for example common and uncommon variations, auxiliary variations like insertions and deletions, copy-number variation, and fusion transcripts.

Conclusion NGS innovations together with bioinformatics investigation, which extend our insight, are progressively used to analyze multiple genes in a cost-effective way and have been applied in examining clinical cancer samples and offering NGS-based molecular diagnosis.

Application NGS is progressively significant as a device for the diagnosis of cancers.

Keywords NGS · Next generation sequencing · Cancer · Cancer research · Molecular diagnosis

Abbreviations

NGS	Next generation sequencing
DNA	Deoxy ribonucleic acid
RNA	Ribonucleic acid
WES	Whole exome sequencing
WGS	Whole genome sequencing
NCDs	Non-communicable diseases
WHO	World Health Organization
HNCs	Head and neck cancers
SCC	Squamous cell carcinoma
GWAS	Genome-wide association study
SBS	Sequencing by synthesis
SMRT	Single-molecule real-time
CNV	Copy number variations
SNPs	Single-nucleotide polymorphisms
TCGA	The cancer genome atlas
WGBS	Whole genome bisulphite sequencing
RRBS	Reduced representation bisulfite sequencing
PC	Prostrate cancer
BC	Breast cancer
HNSCC	Head and neck squamous cell carcinoma

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Introduction

Historically, NGS technology helps in investigating mutations in those genes that have known or suspected associations with a particular disease or phenotype. For example, targeted gene sequencing panels have helped clinical scientists establish diagnoses, guide treatments, and produce disease risk assessments for individuals and families. More recently, NGS technology has been used to conduct comprehensive surveys of genetic material.

Besides employing targeted sequencing panels, clinical scientists may use whole-exome sequencing (WES) and whole-genome sequencing (WGS).

The Sanger DNA sequencing technique was developed in 1997, later automated with some modifications, and formed sequencing gold standard until the late 2000's [1]. This NGS sequencer was based on pyrosequencing technology and was commercialized in the year 2004 Roche 454® (Roche Diagnostics, Almere, The Netherlands). Initially, NGS was so expensive and was difficult to use for studies and then decreased continuously, with a massive decline during the last 8 years. Some advantages of NGS sequencers are as per need the wide sequencing of large genomic regions or small regions for many samples can be achieved. Recently, NGS replaced conventional Sanger sequencing for several clinical and non-clinical applications.

Noncommunicable diseases (NCDs) are now responsible for the majority of global deaths [2] and cancer is expected to rank as the leading cause of death and the single most important barrier to increasing life expectancy in every country of the world in the twenty-first century. In 2005 according to the World Health Organization (WHO), cancer is the first or second leading etiology of death before age 70 years.

Head and neck cancers (HNCs) are the ninth most common malignancy in the world, with unacceptably high mortality rates in the developing countries [3]. More than 90% of these cancers are squamous cell carcinomas (SCCs) and variants thereof, originating from the epithelium of the mucosal lining of the upper aerodigestive tract [3, 4]. Significantly, 10–30% of patients with cancer of the lip and oral cavity subsequently develop second primary neoplasms of the upper aerodigestive tract [5, 6].

Cancer transitions are most striking in emerging economies, where an increasing magnitude of the disease is paralleled by a changing profile of common cancer types. A recurring observation is an ongoing displacement of infection-related and poverty-related cancers by those cancers that already are highly frequent in the most developed countries (e.g., in Europe, North America, and high-income countries in Asia and Oceania). These cancers are often ascribed to a so-called westernization of lifestyle [7–9], yet the differing cancer profiles in individual countries and between regions signify that marked geographic diversity still exists, with the persistence of local risk factors in populations at quite different phases of social and economic transition. This is illustrated by the prominent differences in rates of infection-associated cancers, including the cervix, stomach, and liver, observed in countries at opposite ends of the human development spectrum [8].

Genome-wide association study (GWAS) is a screening methodology to recognize the area of pathogenically pertinent varieties and identifies numerous normal variations with little impacts for malignancies and other complex ailments [10, 11]. Nonetheless, GWAS utilizing tagSNPs are underpowered for identifying relationships with uncommon variations, albeit some uncommon variations and haplotypes were distinguished; while uncommon and possibly pernicious variations may not be recognized by GWAS [12].

Next-generation sequencing (NGS) is progressively used to identify sequence variations and has given plenteous genetic markers including common and uncommon variations and has been applied in the examination of cancer cases including NGS-based molecular diagnosis [13–17].

Evolving molecular techniques are now able to target biomarkers with high accuracy in all kinds of body fluids (i.e., liquid biopsy). This enables minimally invasive profiling of tumor-specific transcriptomic signatures or (epi) genetic targets. This allows a more personalized approach to cancer management by improving disease prognostication and post-treatment disease monitoring while avoiding burdensome and invasive tissue biopsy procedures.

Cancer Research

Two major types of NGS technology have been proposed like short- and long-read sequencing. Short-read sequencing conducted as per Illumina® protocols and machines and is described as "sequencing by synthesis" (SBS) of reads lesser than 300 bp [18]. The ion semiconductor method (Ion Torrent®) is another cheap short-read sequencer [19]. The long-read sequencing is performed mainly by PacBio® or Roche®, which is a costly "single-molecule real-time" (SMRT) technology of reads longer than 2.5 Kb [20, 21]. The Oxford Nanopore Technologies® MinION, using single-stranded pore technology, actually allows to sequence very long molecules (> 10 kb) [22] and at a relatively low cost but with a relatively higher error rate compared with other sequencers.

DNA sequencing comprises whole genome sequencing (WGS), whole-exome sequencing (WES), and targeted sequencing [15–17]. WGS permits sequencing of the whole malignancy genomes, which can be utilized to recognize a wide range of substantial/germline including normal and uncommon variations, nucleotide substitutions, insertions and deletions, copy number variations (CNVs), chromosomal modifications, and examination of the non-coding regions [11, 23–25].

WES targets the coding regions (exons) of a genome, to discover rare or common variants associated with a disorder or phenotype; therefore, WES can be used to determine the sequence of the regulatory regions, such as promoters and non-protein-coding regions, defining the functional parts of the genome [16, 24]; however, WES is not ideal for

the identification of CNVs and other structural alterations in the genome [24]. WES make use of targeted sequencing for a specific disease could be more accurate and accessible in terms of time and cost for clinical applications for more laboratories [11, 16, 24]. WES promises to accelerate the discovery of genetic causes and contributors to disease in both the research and clinical settings [26]. Besides, precision cancer medicine, the use of genetic/genomic profiles of patient tumors at the point-of-care to inform treatment decisions, is rapidly changing treatment strategies across cancer types. Treatment efficacy can be increased and toxicity can be reduced with the proper knowledge of NGS and therefore decrease the overall cost of cancer treatment for both individual family and society.

RNA sequencing (RNA-Seq) is to group the all-out RNA of the cell to acquire data concerning mutations/single-nucleotide polymorphisms (SNPs), levels of gene expressions, gene fusions, genomic adjustments, allele-specific expression, posttranscriptional alterations, microRNAs, small and long noncoding RNAs [13, 23, 24].

The advancement of NGS advances has made projects like The Cancer Genome Atlas (TCGA) [27] and the International Cancer Genome Consortium [28] plausible, and gave multi-stage information to a large number of tumors from different cancer types and subtypes and integrative examinations of genomic, transcriptomic and epigenomic information and increment our comprehension of cancer biology [27, 28].

Non-coding RNA species such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) may play significant jobs in a variety of cellular processes and have been demonstrated to be generally dysregulated in cancer [29]. miRNAs can control the expression of target genes and act as tumor suppressors or oncogenes and exert widespread gene- and pathway-level effects [30]. Long non-coding RNAs (lncRNAs) may assume a significant job in oncogenesis and cancer pathology [30].

Among the epigenetic alterations, DNA methylation is very much reported and well-considered in cancer. Whole-genome bisulphite sequencing (WGBS) or reduced representation bisulfite sequencing (RRBS) has been utilized to distinguish methylated cytosines, particularly in the CpG islands, which are interspersed in the promoter and other administrative areas of a gene. Such epigenetic regulation interceded through DNA methylation balances the expression of specific genes [23, 24].

Cancer Diagnosis

Different approaches can be used according to the needs and the questions to be addressed. The initial input material can be genomic DNA (DNA-seq), messenger or non-coding RNA (RNA-seq), or any nucleic/ribonucleic material obtained after specific procedures.

Even though NGS is broadly utilized for cancer research, of late NGS technology has been applied in NGS-based cancer molecular diagnosis [15, 31]. NGS helps in early diagnosis which in turn allows effective treatments by simultaneous sequencing of a large number of target genes and provides rich early diagnostic markers to develop NGS-based molecular diagnosis [14, 16, 17, 32].

Targeted genetic tests are currently used as diagnostic and prognostic apparatuses in clinical oncology, and progressively broad genomic tests appear to probably come into customary use in the near future [33, 34]; while WES as of now finds the most use in clinical diagnostics since it covers over 95% of the exons, which contains 85% of disease-causing mutations [35]. WGS can be used to compare tumor progression, treatment efficacy, the systems related to resistance development; in the meantime, these can be the hotspot for the future, as new inventions in clinically significant adjustments are made. WGS provides a large volume of data in return it remains an expensive technique [16, 24]. For instance, targeted cancer panels are beneficial because of their minimal effort and moderately straightforward interpretability, and many exist both for cancers [36].

Ongoing advances in NGS innovation have improved the comprehension of Prostrate Cancer (PC) biology and clinical inconsistency. With the help of NGS technology DNA-Seq, RNA-Seq, chromatin immunoprecipitation-Seq, and methyl-Seq tests have recognized some new repetitive adjustments in PC like TMPRSS2-ERG translocation, ATM, SPOP, and CHD1 changes and chromoplexy and better clarified the significant pathways influencing prostate tumorigenesis, which are the AR signaling, PI3K/PTEN/AKT, RB1, TP53 misfortune/transformations, and RTK–Ras–MAPK pathways [16, 24, 37]. Be that as it may, at present, the investigation of genetic alterations in PC is not recommended for routine analytic purposes; because of its exceptionally heterogeneous nature of genetics and phenotypes, PC keeps on representing an enormous challenge in terms of diagnosis and prognosis [16, 24].

Past examinations have indicated that most of the Breast Cancer cases are brought about by BRCA1 and BRCA2 changes. Genetic tests utilizing BRCA1 and BRCA2 changes have been prescribed; be that as it may, different genes like ATM, CHEK2, PALB2, and TP53 have been appeared to give high Breast cancer risk [16, 17]. For instance, Lin et al. built up a numerous quality sequencing board utilizing the NGS, which included 68 genes responsible for causing mutations for BRCA1 and BRCA2 qualities, ATM, TP53 those having a high risk for cancer development [32]. The multiple genes sequencing utilizing the NGS is a successful strategy to increment the

recognized pace of high-risk cases [32]. Utilizing DNA methylation and miRNA profiling, an ongoing study reports that DNA methylation adds to the deregulation of 12 cancer-causing miRNAs and BC metastasis [38]. Some studies reveal a solid relationship between hypermethylation of MIR-127 and MIR-125b-1 and Breast Cancer (BC) metastasis and proposed that MIR-127 and MIR-125b-1 hypermethylation can be potential biomarkers of Breast Cancer metastasis. Additionally, an ongoing report utilizing NGS innovation joined with protein articulation distinguishes PI3K pathway aberrations are among the most widely recognized in malignant growths like Breast Cancer and PC by analyzing more than 19,784 cancer samples from a great many clinicians in 60 countries [39].

Literature reveals Cbioportal in 2014, processed and preanalyzed data from 3 independent whole-exome NGS studies, by reporting 412 cases of HNSCC [40–42]. In 15,293 genes, somatic non-synonymous mutations were detected. In which only 2.3% of these genes were altered by a mutation in $> 3\%$ of the tumors. In HNSCC, when the non-synonymous mutations were filtered using the MutSigv2.0 algorithm, only 0.3% of all affected genes were identified as significantly mutated genes.

Conclusion

NGS innovation can be utilized to recognize single nucleotide variations (SNVs), multi-nucleotide variations (MNVs), auxiliary varieties (SVs), CNV, quality transcripts, epigenetic varieties, and has prompted enhancements in disease order frameworks and sub-atomic determination [14–16, 23]. Besides, the TCGA, ICGC, and different gatherings have directed examinations with various malignancy types to incorporate genomic, transcriptomic, and epigenomic information from a few cancer types and have helped us refine characterization frameworks just as our general comprehension of cancer biology [16, 23, 27, 28]. The successful uses of NGS in routine molecular diagnostics depend on the high discovery rate of new markers, expanded lucidity concerning the approval and execution of NGS tests, and innovative upgrades [14]. Later on, to more readily comprehend the genetic etiology of cancers, to improve effective molecular diagnosis and to apply for genome information in precision medicine, and disease facility care. It will be valuable to consolidate the consequences of GWAS, gene–gene, and gene-environment interactions, with the ongoing fast advances in NGS technologies including entire exome sequencing, transcriptome sequencing, and entire genome sequencing.

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