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Mini Review

The Clinical Versatility of Next-Generation Sequencing in Colorectal Cancer

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Abstract

Next-Generation Sequencing is an evolving technology employed in the field of cancer biology. This mini review is intended as a brief overview of NGS for the clinical utility in colorectal cancer. The pathogenesis and treatment of colorectal cancer will continue to evolve as NGS is applied to more patient samples, correlating tumor biology and outcomes.

Keywords: Next-Generation sequencing, Colorectal cancer

Abbreviations: CRC: Colorectal Cancer; NGS: Next-Generation Sequencing; DNA: Deoxyribonucleic Acid; SNP: Single Nucleotide Polymorphism; MMR: Mismatch Repair; MSI: Microsatellite Instability; PCR: Polymerase Chain Reaction; LS: Lynch Syndrome; cfDNA: Cell Free DNA; ctDNA: Cell Tumor DNA

Introduction

Colorectal Cancer (CRC) is the third most common cancer worldwide resulting in over a half million deaths annually [1]. Shortly after the advent of Next-Generation Sequencing (NGS) in the mid 2000's, it was utilized to examine tumor biology with applications in cancer diagnosis and detection as well. There are several different NGS platforms that utilize disparate deoxyribonucleic acid (DNA) preparation and sequencing methods. All NGS techniques involve massive amounts of parallel DNA templates with resulting sequencing outputs in a digital format [2]. These large files of sequencing data undergo additional filtering and processing to ultimately give a user their desired information [3]. Additionally, all NGS platforms have associated error rates, differences in costs, advantages and disadvantages [4]. The previous gold standard to evaluate DNA was with Sanger sequencing, which requires a 15-25% allele frequency to detect a mutation [5]. NGS can detect allele frequencies that occur at a frequency of 2-10% with additional DNA preparation steps to reduce error rate detection below 10-6 [2,5]. While NGS and its clinical application in the study of cancer are continually evolving, this mini review will describe current utilizations in multiple areas of CRC.

Whole Genome Sequencing for CRC

A prominent application of NGS when it was first introduced was for drastically reducing the cost and time to sequence the entire genome of a species. Thousands of species' genomes have been fully sequenced, identifying common variants. Additionally, whole genome sequencing for many human diseases can uncover unique characteristics like genomic rearrangements and gene fusions that have been discovered in CRC [6]. Other investigators have utilized whole genome sequencing of plasma to identify biomarkers that may reveal response to anti-EGFR therapies [7]. There is some evidence that the gut microbiome may play a role in CRC development or progression [8]. These bacteria species have been identified with NGS through whole genome shotgun sequencing of 16S rRNA in feces samples [9]. Such large-scale approaches to sequencing yield lower read coverage, limiting the

sensitivity and specificity of reads. In turn, this limits the clinical utility of NGS, leading many to prefer targeted sequencing [10].

Targeted Sequencing for CRC

Targeted sequencing allows the similar number of base pairs as whole genome sequencing to be focused on specific exons of interest [10]. While this decreases the number of genes analyzed, the "depth" of reads increases, detecting less common mutations and giving greater reliability to sequencing outputs. Several dozen genes have been implicated in CRC with microsatellite instability (MSI) and single nucleotide polymorphisms (SNP) being increasingly investigated with NGS to determine clinical relevancy.

Single nucleotide polymorphisms (SNP) are defined as point mutations at a specific position in the genome. In colorectal cancer, common genes at which SNPs occur are TP53 (56.7%), KRAS (48.1%), and PIK3CA (9.3%), among others [11]. Identifying these mutations from patient tumor samples is used to drive decisions on neoadjuvant or adjuvant therapy in clinical practice. For example, anti-EGFR antibodies, cetuximab and panitumumab, have greatly improved CRC patient outcomes [12]. The utility of investigating multiple genes and associated patient outcomes has been demonstrated with discovering ineffective anti-EGFR therapy in patients with concomitant BRAF or PIK3CA mutations are present [11]. Additional mutations such as KRAS and NRAS have found patients to be non-responders to anti-EGFR therapy. Multiple other studies involving several hundred CRC patients have reported similar tumor mutations with concomitant patient outcomes [13]. Using NGS enables sub categorization of CRC and is helping to identify responders from non-responders to treatment, shaping future targeted therapies that will be offered to patients.

Microsatellite instability is the spontaneous acquisition or loss of nucleotides from regions of repeated nucleotides due to impaired DNA mismatch repair [14-16]. MSI has been extensively studied in colorectal cancer since first reported in 1993 and is a marker of favorable prognosis because therapies can be developed for the immunogenic nature of these tumor types [17-19]. There are several methods for detecting MSI such as mSINGS and MSIseq using targeted sequencing data with similar results to standard polymerase chain reaction (PCR) based detection [16,20,21]. The advantage of using NGS to detect MSI is scalability, cost-effectiveness, and reproducibility of quantifiable data for statistical purposes.

Cell Free DNA in CRC

NGS has also been applied to the concept of a "liquid biopsy" that is not performed from a tumor sample. While a blood sample is commonly referred to as a liquid biopsy, there are a variety of other bodily fluids, such as saliva, urine, and feces, from which cell free DNA can be obtained. Cell free DNA (cfDNA) refers to any DNA present in plasma while cell tumor DNA (ctDNA) is DNA that only comes from a tumor [22].

In a multi-institutional study of 1,397 patients with CRC, using cfDNA from blood samples detected single-nucleotide variants (SNV) that were similar to tumor sample sequencing [23]. Identifying liquid biopsy DNA that is similar to tumor sample DNA portends a potential significant role in screening for CRC.

Genetic Screening for CRC

Approximately 5% of CRC patients have a hereditary CRC syndrome such as Lynch syndrome (LS) or familial adenomatous polyposis (FAP) [24]. Patients with hereditary CRC syndromes present earlier in life than sporadic CRC, making diagnosis important so that other family members can undergo age appropriate screening. Lynch syndrome is the most common heredity CRC syndrome due to autosomal dominant mutations in MMR genes [25]. Traditionally, detection of LS has been performed with several steps including immunohistochemical staining for MMR proteins or MSI [26]. A study assessing 419 patients with LS found that using NGS for tumor sequencing is simpler and analytically superior to current screening methods [26. Similar results of NGS accuracy have been reported in other populations and with other heredity CRC syndromes [24].

Conclusion

While the role of NGS continues to evolve, the ability to identify a litany of targeted genes at one time, from a variety of DNA sources, is a powerful tool for both researchers and clinicians. Continued research using NGS techniques will likely help to identify cancers sooner, become a part of screening and/or surveillance guidelines, and help the push toward personalized medicine.

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