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How Can Next-Generation Sequencing (Genomics) Help Us in Treating Colorectal Cancer?

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Abstract

Next generation sequencing methods have exponentially increased the amount of genomic information available to scientists and clinicians. This review will explain the evolution of tumor gene sequencing and identify its potential to accelerate therapeutic progress by using colorectal cancer to illustrate the benefits of this type of analysis. A milestone in sequencing occurred when The Cancer Genome Atlas investigators characterized the genomes of 276 colorectal cancer samples, with the resulting information expected to provide future clinical applications and help to guide the treatment of colorectal cancer. Data regarding colorectal cancer mutational frequencies, prognostic and predictive biomarker usefulness, and signaling pathway alterations are emerging from various next generation sequencing platforms. Next generation sequencing methods are also enhancing our understanding of the causes and consequences of both the chromosomal instability and microsatellite instability pathways, as well as expanding our knowledge of the origins of familial colorectal cancer. Limitations to next generation sequencing methods include the need for storage and analysis of massive quantities of data, as well as assurance that the data is of the highest possible quality. However, this genomic technology carries with it the potential to revolutionize our treatment of colorectal cancer patients through better understanding of the underlying disease biology and subsequent development and application of therapeutic approaches targeting the genetic abnormalities specific to individual malignancies.

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Conflict of Interest

Kristen K. Ciombor, Sigurdís Haraldsdóttir, and Richard M. Goldberg declare that they have no conflict of interest.

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Keywords

Next-generation sequencing; gene expression profiling; microsatellite instability; chromosomal instability; *RAS*

Introduction

Colorectal cancer (CRC) is both the third most common and third most deadly malignancy in the United States, as well as a leading cause of cancer-related death in the world [1]. In the United States alone, it is estimated that 136,830 people will be diagnosed with this disease and 50,310 people will die of it in 2014 [1]. While the colorectal cancer death rate is incrementally decreasing through early disease detection and treatment, a substantial burden remains for patients diagnosed with advanced or incurable disease. Development of new colorectal cancer treatments have improved the therapeutic landscape in this disease over the past fifteen years, but better tailored, more effective therapies are urgently needed. Fundamentally, the biological underpinnings of colorectal cancer development and progression need to be more clearly understood in order to exploit these therapies most effectively. With the advent and development of powerful genomic technologies, much is being discovered regarding the genetic drivers of disease, data that can be employed to optimize the likelihood of predicting an individual tumor's response to cancer therapies.

Next Generation Sequencing (NGS) in Colorectal Cancer

Colorectal cancer has long served as a model to understand the molecular events that lead to cancer formation. It was investigated thoroughly back in the 1980–1990s by Sanger sequencing, when the adenoma to carcinoma pathway was established [2]. Further investigations subsequently identified two critical pathways, the chromosomal instability pathway and the microsatellite instability pathway. Colorectal cancer was also one of the first tumors to undergo whole genome sequencing, completed in 2012 by the investigators that constructed The Cancer Genome Atlas (TCGA), identifying the abnormalities responsible for a cancer's development [3]. The molecular basis of colorectal cancer has been previously reviewed [4].

In comparison to conventional “first-generation” Sanger sequencing, which was first introduced in 1977, next generation sequencing technology has recently revolutionized the way human genomes are analyzed. NGS can accomplish massively parallel sequencing of DNA templates at a cost and speed far superior to older technologies, and it can identify multiple different derangements of the genome, including subtle nucleotide sequence alterations, copy number variants and translocations, among others. NGS methods have made it feasible to sequence a whole tumor genome (or exome) in order to establish possible driver mutations, including activating oncogene mutations and inactivating tumor suppressor gene mutations.

The NGS process consists of various steps such as library preparation/enrichment, sequencing, base calling, sequence alignment and variant calling [5, 6]. Details of particular NGS platforms used and comparisons of their individual throughput, run times and other

defining characteristics are beyond the scope of this article but are nicely reviewed in the literature [5, 7–9]. Clinically relevant NGS applications currently include whole genome sequencing, exome sequencing, transcriptome profiling, and epigenomic characterization, among others (Table 1), all of which have been explored in colorectal cancer [10]. In colorectal cancer, genomic profiling has most recently identified at least three different genetic signatures likely representing different patterns and heterogeneity within the spectrum of a disease with a relatively monotonous histologic appearance. This review article will focus on how NGS has and will continue to shed further light on the pathogenesis of CRC and the therapeutic potential of drug development for this tumor.

The Cancer Genome Atlas and the Characterization of Colorectal Cancer

As previously mentioned, next generation sequencing of colorectal carcinoma tumor samples was performed by The Cancer Genome Atlas Network in 2012 [3], and these analyses provided an interesting example of genomic discovery that could potentially lead to paradigm shifts in the future treatment of CRC. A total of 276 human colorectal carcinoma samples were genomically profiled by analyzing exome sequence, DNA copy number, promoter methylation, messenger RNA and microRNA expression, and approximately one third of these samples also underwent whole genome sequencing. In this project, exome capture DNA sequencing was utilized to define the mutational spectrum of CRC, and 16% of the cases in question were found to be hypermutated, defined as >12 mutations per 10^6 bases. As expected, the majority of these hypermutated tumors (77%) had high levels of microsatellite instability (MSI-H). The recurrently mutated genes among the hypermutated cancers included *APC*, *TGFBR2*, *MSH3*, *MSH6*, *ACVR2A*, *TCF7L2*, *BRAF*, and *SLC9A9*, among others, whereas the non-hypermutated cancers demonstrated more mutations in *APC*, *TP53*, *KRAS*, *PIK3CA*, *FBXW7*, *SMAD4*, *NRAS*, *CTNNB1*, *SMAD2*, *FAM123B* and *SOX9*, among others. The differences in gene mutation profile and mutational frequency between the hypermutated and non-hypermutated tumors seem to suggest that these cancers are driven by varying genetic events, such that an enhanced understanding of these differences could lead to more effective therapeutic pathways in the future.

Utilizing an integrated analysis of mutations, copy number and mRNA expression changes in a total of 195 colorectal tumors, TCGA investigators also explored pathway regulation in CRC. Particular pathways of interest included WNT, MAPK, PI3K, TGF- β , and p53, with recurrent mutations of *FAM123B*, *ARID1A*, and *SOX9*, among others, seen in the tumors. From this integrative analysis of genomic data, the authors hypothesized that future therapeutic approaches to CRC could include WNT signaling inhibitors and small-molecule β -catenin inhibitors. Other potentially exploitable targets for which drugs could be designed in this disease could include IGF2, IGFR, ERBB2/3, MEK, AKT and mTOR. While this project was a solid first step in understanding the genomic complexity of colorectal cancer using next generation technology, further genomic analysis is still warranted, especially given the vast molecular heterogeneity of the disease and disparate clinical outcomes. Data on clinical outcomes was not available for the patients whose tumors were analyzed in the TCGA, limiting the value of the data to some degree.

Mutations in MAPK and PI3K Signaling Pathways in Colorectal Cancer

Several approaches have been used to sequentially investigate genomic alterations in tumors such as CRC. One of these approaches utilizes sequencing of specific genes of interest, such as tyrosine kinases. Bardelli *et al.* published the first systematic mutation analysis approach in any human cancer in 2003, where genes coding for 138 tyrosine kinases were sequenced [11]. After identifying mutations in 14 of the 138 genes, they looked for abnormalities in these genes in 147 colorectal cancers. A total of 46 mutations were found in the 14 genes, suggesting that 30% of colorectal cancers contain at least one mutation in a tyrosine kinase gene. A similar approach was used to investigate the tyrosine phosphatase genes which regulate phosphorylation of tyrosine residues. A total of 83 somatic mutations were found in 6 protein tyrosine phosphatase genes affecting 26% of these CRCs and most were found to be inactivating mutations [12]. Parsons *et al.* investigated mutations in 340 serine/threonine kinases and found mutations in eight genes, including 3 members of the PI3K pathway [13]. Around 40% of the tested tumors had mutations in one of eight PI3K pathway genes, suggesting that this pathway could represent a potential therapeutic target in a significant subset of patients with CRC.

Lipson *et al.* also performed NGS on 40 formalin-fixed paraffin-embedded colorectal tumor specimens targeting 145 cancer-related genes [14]. In this study, genomic alterations were found in 59% of samples, including a novel gene fusion between *ALK* and *C2orf44*. *TP53* and *APC* were the most frequently mutated (80% and 67.5%) but other potentially targetable mutations were seen including those in the MAPK pathway (*KRAS*, *BRAF*), DNA repair pathways (*ATM*, *BRCA2*, *MSH6*), and PI3K pathway (*PIK3CA*). More recent studies have focused on a whole exome or whole genome sequencing approach of tumors. Bass *et al.* performed whole genome sequencing on nine individuals with CRC, both primary tumors and matched adjacent normal tissue [15]. The tumors had on average 75 somatic rearrangements including translocations between pairs of chromosomes. Furthermore, RNA sequencing identified novel R-spondin fusions which may have a role in Wnt signaling as they were mutually exclusive with *APC* mutations, when 70 pairs of human colon tumors were tested with NGS [16]. These results, along with the TCGA data, provide a basis for current and future rational drug development in CRC.

The Chromosomal Instability Pathway in Colorectal Cancer

Chromosomal instability was identified early on in colorectal tumors and seems to underlie the pathogenesis in about 80–85% of tumors. This is evident by losses and gains of entire chromosomes or large interchromosomal regions, a mechanism that can lead to the deletion of a wild-type allele of a tumor suppressor gene. In recent years, some discoveries have been made to establish why this pattern develops. Genes that regulate sister chromatid cohesions seem to be involved and down regulation or disruption of certain genes such as *MRE11A* and *CDC4* can predispose to CIN tumors [17]. Further investigations using NGS methods are needed to better understand this important pathway, however.

The Microsatellite Instability Pathway in Colorectal Cancer

Also of great importance to the pathogenesis of CRC, microsatellite instability (MSI) in colorectal tumors was first identified in 1993 [18]. MSI was linked to deficiency in the mismatch repair system (dMMR) [19], and later MSI tumors were found to be caused by germline mutations in the mismatch repair genes, *MLH1*, *MSH2*, *MSH6* or *PMS2* (Lynch syndrome, found in 2–3% of CRC cases) [20] or sporadic inactivation of *MLH1* by hypermethylation (found in 12–15% of CRC cases) [21]. As the mismatch repair genes maintain genomic integrity by repairing errors in base pairing that occur during DNA replication, the dysfunction of the system leads to an accumulation of mutations, leading to hypermutated tumors. Mutations are inherited on one allele and with the acquisition of a second hit tumors can develop. While inherited biallelic MMR mutations are known and cause childhood cancers (also known as the constitutional MMR-deficiency), they are rare. In about 2–3% of all CRC cases, dMMR is found but neither *MLH1* hypermethylation nor germline mutations in MMR genes are present. Recent reports indicate that the majority of these tumors have developed biallelic MMR mutations. Furthermore, *TACSTD1*, a gene encoding for EPCAM upstream of *MSH2*, has been shown to be mutated in the germline in 20–25% of unexplained cases in which *MSH2* is absent on immunohistochemistry. EPCAM mutations lead to a readthrough and inactivating hypermethylation of *MSH2* [22]. *BRAF* mutations are found in up to 50–70% of *MLH1* hypermethylated tumors while LS-associated tumors rarely have mutations in *BRAF*. This fact is utilized when universal screening is done for LS, where a tumor *BRAF* mutation in the setting of *MLH1* deficiency is considered proof of *MLH1* hypermethylation in the tumor, excluding the presence of Lynch Syndrome [23].

As mentioned above, in TCGA, tumors were divided into hypermutated tumors if they had a mutation rate >12 per 10^6 or non-hypermutated with a mutation rate <8.24 per 10^6 [3]. Among the 30 hypermutated tumors, 77% of cases were MSI and 63% were *MLH1* hypermethylated (17 of 19 also had CIMP high). The remaining tumors, including 6 with the highest mutation rate, had *POLE* aberrations or somatic mutations in the mismatch repair genes. The different mutational patterns between the hypermutated tumors (most of which are MSI) and tumors that develop via the chromosomal instability pathway support the fact that they develop via two distinct pathways. Hypermutated tumors had mutations in *TGFBR2* along with *BRAF* mutations and others while the non-hypermutated tumors had significantly higher likelihood of mutations in *TP53* and *APC*. This was further explored by Donehower *et al.* who found frequent *BRAF* mutations and infrequent *KRAS* and *APC* truncating mutations in *MLH1*-silenced hypermutated tumors [24], while the opposite was true for non-*MLH1* silenced hypermutated tumors from TCGA. They also showed that while *MLH1* silenced tumors had higher mononucleotide frameshifts in genes containing mononucleotide repeats (such as *TGFBR2*, *PTEN*, *RAD50*), the overall mutation rates were lower when compared to non-*MLH1* silenced hypermutated tumors, suggesting that there are subgroups of hypermutated tumors that have distinct mutational profiles [24].

The identification of MSI tumors via first generation and NGS methods has had a clinical impact as these tumors have a better prognosis in early disease stages [25, 26] and are rarely diagnosed as stage IV [27]. Preclinical and clinical studies have shown these tumors to be resistant to 5-fluorouracil (5-FU) [28–30], while they are sensitive to irinotecan [31]. Given

the good prognosis, the National Comprehensive Cancer Network (NCCN) guidelines do not recommend adjuvant 5-FU in stage II MSI tumors [32], and we would also caution against the use of 5-FU as a single agent in the adjuvant therapy of stage III patients. More recent preclinical work has shown this 5-FU chemoresistance in *MSH3* deficient cell lines but it is independent of *MLH1* status, suggesting that it might be mediated by *MSH3* deficiency [33].

Another recognized phenotype, the CpG island Methylator Phenotype (CIMP), overlaps with the MSI pathway. In about 15% of CRCs, aberrant methylation occurs within promoter-regions of CpG dinucleotides leading to epigenetic silencing of gene expression and a CIMP-high (CIMP1) phenotype [34]. Nearly all of these tumors are *MLH1* hypermethylated and have a high rate of *BRAF* mutations [35]. Low-level CIMP is commonly associated with microsatellite stability with a limited group of methylated genes but mutations are frequently seen in *KRAS*. CIMP-negative cases, on the other hand, have frequent chromosomal instability and TP53 mutations. While the cause of methylation in cancer is unclear, it has been associated with older age and proximal colon location [36] and has recently been shown to be associated with a high load of *Fusobacterium* in colorectal carcinoma [37].

Sequencing in Familial Colorectal Cancer

Next generation sequencing of tumors from patients suspected to have an inherited cancer syndrome (when no mutations are found in genes associated with CRC such as *APC*, *MMR*, and *MUTYH*) has yielded some novel mutated genes. Mutations in the proofreading domains of the DNA polymerase genes *POLE* and *POLD1* were recently shown to occur as germline mutations in some cases of patients with early colorectal cancer with multiple adenomas [38]. Mutations in the *POLE* gene were associated with hypermutated tumors (with more frequent mutations than MMR deficient tumors) in TCGA [3], which underscores the importance of their proofreading role during DNA replication. Linkage analysis and sequencing of a single large Jewish family with hereditary mixed polyposis syndrome (where individuals develop multiple polyps of mixed morphologies, including serrated lesions, Peutz-Jeghers polyps and adenomas) revealed a duplication in the *GREM1* gene causing increased GREM1 mRNA levels, likely causing a reduction in BMP signaling to crypt cells [39].

Gene Expression Profiling in Colorectal Cancer

Gene expression profiling studies have also been undertaken in colorectal cancer, with two large studies recently reported. A commercial gene expression profiling test, ColoPrint[®] (Agendia), was shown to improve the prognostic accuracy of pathologic factors and MSI in stage II/III CRC [40]. The test analyzes the expression of 18 genes and classifies tumors as having low versus high recurrence risk. As a predictive value has not been shown in terms of benefit from adjuvant chemotherapy, it is not recommended for use by NCCN [32]. In a follow-up study looking at patient outcomes, this same group looked at the 3 distinct molecular subtypes characterized by the test. The A type commonly had MMR deficiency and had the best prognosis; the C-type had an epithelial-to-mesenchymal transition profile

and had the worst outcomes; and the B-type tumors had more of an epithelial phenotype and had a low overall mutation frequency. Only the B type appears to benefit from adjuvant chemotherapy [41]. Interestingly, not all patients with MSI tumors had a good prognosis. The C-type MSI patients had a poor prognosis, while the A-type MSI patients had a very good prognosis. Another group looked at stage I-IV CRC samples and segregated them into two prognostic subtypes, where the EMT subtype was highly prognostic for colon cancer recurrence [42].

Budinska *et al.* also identified 5 gene expression subgroups from the PETACC-3 cohort with 1113 patients and validated their findings in an independent set of 720 patients [43]. The 5 subtypes, called surface crypt-like, lower crypt-like, CIMP-H-like, mesenchymal and mixed, had differences in prognosis and morphological features; MSI, *BRAF* mutation status, site, mucinous histology and *p53* expression were significantly associated with the various subtypes. The results of this study underscore the heterogeneity of colorectal cancer and the complementary value of gene expression profiling to other NGS methods.

Expansion of Mutational Analysis and Effect on Clinical Outcomes for Metastatic CRC Patients

In addition to providing valuable biologic and prognostic information for CRC, next generation sequencing also has a role in predictive biomarker discovery. One example of this predictive capability is the expansion of *RAS* mutational analysis afforded by NGS methods, as discussed below.

The determination that mutations in *KRAS* predicted resistance to anti-epidermal growth factor receptor (EGFR) targeted therapies such as cetuximab and panitumumab was one of the first practical applications of genomic analysis in patients with CRC. However, a substantial minority of patients whose tumors had wild type *KRAS* do not respond to these agents. Further genomic analysis indicates that other *RAS* variants may be equally important in the decision to treat with anti-EGFR therapies. In an exploratory analysis based on The Panitumumab Randomized Trial in Combination with Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy (PRIME) by Douillard *et al.*, tumors previously found to be *KRAS* exon 2 (codons 12 and 13) wild-type were analyzed by traditional Sanger sequencing for mutations in *KRAS* exon 3 (codon 61), *KRAS* exon 4 (codons 117 and 146), *NRAS* exon 2 (codons 12 and 13), *NRAS* exon 3 (codon 61), *NRAS* exon 4 (codons 117 and 146), and *BRAF* exon 15 (codon 600), and clinical outcomes with fluorouracil, leucovorin and oxaliplatin (FOLFOX4) with or without panitumumab were analyzed by mutational status [44]. A total of 1060 patients underwent *RAS* testing, and 108 tumors originally categorized as not having *KRAS* exon 2 mutations were found to have other *RAS* mutations on secondary analysis. When excluding patients with any *RAS* mutation, progression-free survival (PFS) was improved with panitumumab-FOLFOX4 as compared to FOLFOX4 alone (10.1 vs. 7.9 months, $p = 0.004$) [44]. For patients with *RAS* wild-type disease, treatment with panitumumab-FOLFOX4 versus FOLFOX4 alone also led to a significant improvement in overall survival (OS) (26.0 vs 20.2 months, $p = 0.04$), which was not seen in the *KRAS* exon 2 wild-type population treated with panitumumab-FOLFOX4 versus FOLFOX4 alone (23.9 vs. 19.7 months, HR 0.88, 95% CI 0.73–1.06, $p = 0.17$) [44, 45].

Additionally, in the expanded subgroup of patients with mutant *RAS* tumors, PFS (HR 1.31, 95% CI 1.01–1.60, $p = 0.008$) and OS (HR 1.25, 95% CI 1.02–1.55, $p = 0.03$) were shorter in the panitumumab-FOLFOX4 group than in the FOLFOX4 alone group [44], suggesting possible harm to those patients with mutant *RAS* tumors treated with anti-EGFR therapy. Furthermore, in this study 7 patients were found to have mutations in codon 59 of either *KRAS* or *NRAS*; while a small sample size, exclusion of these patients from the overall analysis slightly improved both PFS and OS, indicating that perhaps other *RAS* alleles are negative predictive biomarkers for anti-EGFR therapy as well.

As indicated by this study, knowledge of expanded *RAS* status is critically important in avoiding ineffective therapies for patients with metastatic CRC (mCRC). Other *RAS* alleles may be predictive of negative response as well, but which to test for is still to be determined. While the above study utilized Sanger sequencing for its mutational data acquisition, next generation sequencing platforms that comprehensively test for mutational biomarkers of response stand to more efficiently provide essential genomic information for the selection of appropriate therapeutics in mCRC.

One example of this method is that of NGS mutational analysis of the Cetuximab After Progression in *KRAS* Wild Type Colorectal Cancer Patients-Gruppo Oncologico dell'Italia Meridionale (CAPRI-GOIM) study in which 340 mCRC patients with *KRAS* exon 2 wild type tumors were treated with first line FOLFIRI plus cetuximab [46]. In this retrospective mutational analysis, a next generation sequencing approach gene panel, Ion AmpliSeq™ Colon and Lung Cancer Panel (Life Technologies) [47] was utilized to search for mutations in 22 genes involved in CRC, including *ALK*, *EGFR*, *FGFR1-3*, *KRAS*, *PIK3CA*, *BRAF*, and others. A total of 182 tumor samples were assessed by the NGS panel, with at least one gene mutation detected in 124/182 (68.1%) of tested tumors. Other than *KRAS* exon 2 mutations, which encompassed 29/182 (15.9%) samples previously defined as wild type by local assessment, further analyses frequently detected mutations included *KRAS* exons 3/4 (8.8%), *NRAS* exons 2/3 (7.1%), *PIK3CA* exons 9/20 (13.2%) and *BRAF* (8.2%). Overall response rate (ORR) to FOLFIRI plus cetuximab in patients with *KRAS* and *NRAS* wild-type tumors was 62.0% (95% CI, 55.5–74.6%), with median PFS of 11.1 months (95% CI, 9.2–12.8 months), whereas ORR to FOLFIRI plus cetuximab in patients with *KRAS* or *NRAS* mutant tumors was only 46.6% (95% CI, 39.9–57.5%) with median PFS of 8.9 months (95% CI, 7.4–9.6 months). Importantly, NGS analysis in this set of CRC tumors was deemed to be feasible and demonstrated a high level of intra- and inter-tumor heterogeneity. However, it remains to be seen exactly how this tumor heterogeneity will impact on real-time, prospective clinical decision-making processes for CRC therapies.

In another example, massively parallel tumor multigene sequencing was used to evaluate response to panitumumab in the phase III 408 study in which mCRC patients were randomized to panitumumab plus best supportive care versus best supportive care alone [48]. In this study, tumors were analyzed with NGS methods for mutations in *KRAS* codon 61, *NRAS* codons 12/13/61, *EGFR*, *BRAF*, *PTEN*, *PIK3CA*, *AKT*, *TP53* and *CTNNB1*, utilizing formalin-fixed paraffin-embedded tissue that had previously been assessed for *KRAS* codons 12 and 13 mutations. In this population, mutational frequencies were *KRAS* (45%), *NRAS* (5%), *BRAF* (7%), *PIK3CA* (9%), *PTEN* (6%), *TP53* (60%), *EGFR* (1%),

AKT1 (<1%), and *CTNNB1* (2%), consistent with previously reported mutational rates in CRC. Interestingly, of patients with *KRAS* wild-type tumors, 0 of 9 patients with *NRAS* mutations, 0 of 13 patients with *BRAF* mutations, 2 of 10 with *PIK3CA* mutations, 1 of 9 with *PTEN* mutations, and 1 of 2 with *CTNNB1* mutations responded to panitumumab; furthermore, one patient with a *KRAS* Q61H mutation had a partial response to panitumumab. These data indicate that multigene sequencing via NGS methods can provide powerful predictive biomarker information for clinical decision making, and that simple *KRAS* exon 2 mutational status data is no longer sufficient for making therapeutic decisions for patients with mCRC.

Conclusions

Next generation sequencing has increased the speed and efficiency of genomic alteration identification in tumors such as colorectal cancer. High-throughput data collection on a scale not previously seen exponentially increases the knowledge of potential colorectal cancer drivers. However, challenges that remain include understanding the clinical relevance of these genomic alterations, if any, and the practical utilization of these methods to improve real-time cancer care for patients. The high volume of NGS data generation in recent years has outstripped the development of other essential components of the sequencing process; namely, data storage capabilities, quality control and informatics infrastructure and programs with which to analyze this large amount of data. Furthermore, the need for efficient sequencing turnaround is paramount for patients as they wait for their physicians to make appropriate therapeutic decisions regarding their clinical care. Nonetheless, next generation sequencing methods have already begun to revolutionize the way we understand the biology of colorectal cancer, and we are hopeful that this technology will help lead to vast improvements in the outcomes of patients with colorectal cancer.

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Table 1

Examples of next-generation sequencing applications

NGS Types	Source of Genomic Material	Description of Application	Examples of Alterations Identified
Whole genome sequencing [9, 49–51]	Genomic DNA	Identifies sequence of coding and noncoding regions, copy number variation, structural rearrangements	Point mutations Repeats Insertions/deletions DNA copy number changes Translocations
Exome sequencing [52–54]	Exons (protein-encoded genomic DNA)	Identifies sequence of exons, including copy number variation, structural rearrangements	Point mutations Repeats Insertions/deletions DNA copy number changes Translocations
Epigenome sequencing [55–58]	Genomic DNA (targeted)	Identifies DNA methylation and posttranscriptional histone modifications	Transcription-factor binding sites (ChIP-seq) Genetic imprinting (Bisulfite-seq)
Transcriptome sequencing (RNA-seq) [59, 60]	Reverse-transcribed RNA (complementary DNA, or cDNA)	Identifies sequence of transcribed or coding RNA, including splicing variants, copy number, gene expression profiling	Gene fusions Alternative splicing events Point mutations Insertions/deletions