Update on Sporadic Colorectal Cancer Genetics

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Abstract

Our understanding of the genetics of colorectal cancer has changed dramatically over recent years. Colorectal cancer can be classified in multiple different ways. Along with the advent of whole-exome sequencing, we have gained an understanding of the scale of the genetic changes found in sporadic colorectal cancer. We now know that there are multiple pathways that are commonly involved in the evolution of colorectal cancer including Wnt/β-catenin, RAS, EGFR, and PIK3 kinase. Another recent leap in our understanding of colorectal cancer genetics is the recognition that many, if not all tumors, are actually genetically heterogeneous within individual tumors and also between tumors. Recent research has revealed the prognostic and possibly therapeutic implications of various specific mutations, including specific mutations in *BRAF* and *KRAS*. There is increasing interest in the use of mutation testing for screening and surveillance through stool and circulating DNA testing. Recent advances in translational research in colorectal cancer genetics are dramatically changing our understanding of colorectal cancer and will likely change therapy and surveillance in the near future.

Keywords
- colorectal cancer
- exome sequencing
- hyper-mutated tumors
- intra-tumor heterogeneity

Many changes in our understanding of colorectal cancer genetics have taken place since the widespread availability of DNA sequencing. While family history is a well-known risk factor for colorectal cancer[1] and some familial cancer syndromes are well described,[2–4] the majority of colorectal cancers are actually sporadic. The precursor to colorectal adenocarcinoma has previously been thought to be only the adenoma. However, there is emerging evidence that some sporadic adenocarcinomas may evolve via an alternate pathway from serrated polyps.[5,6] Sporadic colorectal cancers occur as a consequence of the accumulation of genetic defects over time. As these defects accumulate, masses form and then become adenocarcinomas.

The genetic defects found in colorectal cancers are typically mutations in the DNA, small deletions or insertions, and copy number variation (CNV). In addition to these changes in the genetic sequence and number of copies of a gene in colorectal cancer, epigenetic alterations to the DNA are thought to play a critical role in tumorigenesis which are discussed elsewhere.[7] Through exome sequencing and whole-genome sequencing, we now know that each tumor contains many mutations and each mutation can be categorized by its known or predicted functional outcome. If the functional outcome is significant for the protein encoded by the gene, and if it results in a growth advantage for the tumor, this will be selected for and is called a *driver mutation*.[8] Any given tumor typically has few driver mutations and many passenger mutations. Passenger mutations are those alterations in the DNA that neither help nor hamper the fitness of a tumor but are present because cancer cells undergo poorly controlled replication resulting in mutations. Because driver mutations are those that are critical for the progression and survival of cancer cells, they are typically of prognostic and therapeutic significance. Driver mutations are often nonsense mutations, which code for a stop codon and result in truncation of the protein, but they can also be frameshift mutations where small insertions or deletions result in disruption of the reading frame and the protein is thus dramatically altered. Drivers can also be missense mutations where a single nucleotide is changed that results in a different amino acid being encoded which can result in a nonfunctional protein or altered protein function. Passenger

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mutations are often silent. Silent mutations are those that do not result in a change in the amino acid sequence of the coded protein.

CNVs are common in colorectal cancer. There are three types of alterations: amplifications, deletions, and loss of heterozygosity (LOH). In normal human DNA, there are two copies of each chromosome of the DNA with one originating from each parent. In cancer, alterations in large or small sections of chromosomes are called CNVs. These CNVs can be amplifications where there are more than one and sometimes many copies of a portion of a chromosome or even just a single gene. Another type of CNV is a deletion in a portion of a chromosome. Again, this can be loss of a whole chromosome or a portion or even just a single gene. The third type of CNV is LOH where one copy of a section of a chromosome is lost. The tumor can still be copy number neutral (retain two copies of a given segment) if the remaining copy is duplicated, so there are two copies that are the same.

Genetic Classification of Sporadic Colorectal Cancer

Over time many classification schema have been proposed for colorectal cancers. Examples of different classification systems are by location (proximal vs. distal, colon vs. rectal), familial or sporadic, histopathologic, and molecular. This review will focus on molecular genetics and thus will discuss the most recent classifications of genetic alterations leading to sporadic colorectal cancers.[9–11]

The first pathway is the chromosomal instability pathway (CIN), which is thought to be important in approximately 70% of tumors.[12,13] In CIN tumors, there is an accumulation of CNV with varied karyotypes from cell to cell and LOH leading to loss of tumor suppressor genes. These patients also have mutations in key driver genes, but whether this causes the chromosomal instability or is the cause of it is unclear. Additionally, the mechanism for CIN is not known but is likely contributed to by chromosomal segregation defects and telomere dysfunction.[13] These tumors typically have *APC* mutation and loss as well as mutation and loss of *TP53*.

The next commonly described pathway to sporadic colorectal cancer is the microsatellite instability (MSI) pathway. Microsatellites are short nucleotide repeats that occur throughout the genome and are prone to errors with DNA replication. It is the function of the mismatch repair genes to fix these errors. When they are not fixed, they accumulate, leading to MSI tumors. MSI can be measured, hence the name, but the lack of DNA repairs also leads to the accumulation of many other mutations. These tumors are hyper-mutated containing greater than 700 mutations each and sometimes several thousands. MSI tumors make up 15% of sporadic colorectal cancer and are caused by hyper-methylation of the promotor of the mismatch repair gene, *MLH1*, causing silencing.[12] This should not be confused with hereditary nonpolyposis colorectal cancer, which is MSI-high due to germline mutations (rather than hyper-methylation) in the mismatch repair genes. Additional mechanisms leading to hyper-mutated MSI sporadic tumors are mutations in other mismatch repair genes (*MSH6, MSH2*, and *MLH3*). Sporadic MSI tumors commonly have DNA methylation and *BRAF* mutations. These are also common in serrated polyps and there is growing evidence that serrated polyps serve as the precursor lesion to sporadic MSI colorectal cancers.[5,6,10]

The third category of colorectal cancer is the CpG Island Methylation Pathway (CIMP).[14] About 15% of sporadic colorectal cancers fall into the CIMP category, whereby epigenetic methylation of genes containing CpG islands are methylated leading to gene silencing. These tumors are hyper-mutated and have a high incidence of *BRAF* mutations. However, the mechanism for CIMP is not clear. It is defined by having several CpG islands methylated on analysis of DNA-wide methylation profiling.[15] There is clear overlapping between the CIMP and MSI pathways in sporadic colorectal cancers. CIMP and MSI typically are hyper-mutated with 700 or more mutations, while CIN tumors are non–hyper-mutated with around 60 to 100 mutations per tumor.

Genes and Pathways Altered in Sporadic Colorectal Cancer

When Fearon and Vogelstein put forth their genetic model for colorectal tumorigenesis in 1990, they proposed a pathway whereby adenomas progressed to carcinomas via a series of specific genetic alterations including mutation or loss of *APC*, mutation of *KRAS*, and mutation of *TP53*.[16] In fact, only 6.6% of colorectal cancers contain mutations in all three of these genes and 38.7% of tumors contain mutations in only one of these.[17] Fearon et al additionally proposed that colorectal cancers were clonal—meaning that the entire tumor is the same.[18] As will be discussed in the section on tumor heterogeneity, deep multiregional sequencing has shown that this is an oversimplification with some mutations being clonal and others not. Each tumor contains 60 to 1,000s of exome mutations, but there are certainly recurrently mutated genes and pathways in colorectal cancer. Hyper-mutated tumors contain a higher incidence of mutations in *BRAF*, *PIK3CA*, and *PTEN*, while non–hyper-mutated tumors have a higher incidence of *APC*, *KRAS*, and *TP53* gene mutations.[19] What follows is a description of commonly mutated genes and pathways in somatic colorectal cancer. In colorectal cancer, multiple pathways can be activated or altered at once driving tumor progression and diminishing response to drugs that target individual pathways. Combination therapies may be more promising.

Wnt Pathway: The Wnt signaling pathway is the most commonly altered pathway in colorectal cancer. In the comprehensive Cancer Genome Atlas publication assessing 224 tumors in depth, the authors found that the Wnt pathway was altered in 93% of tumors.[20] They found 16 different altered Wnt pathway genes. The most common was biallelic inactivation of *APC*, either through LOH or mutation. Other common alterations were activating mutations in *CTNNB1*; mutations in *SOX9*; deletions in *TCF7L2*; and *AXIN2, FBXW7, ARID1A*, and *FAM123B*. The Wnt pathway is critical in homeostasis in intestinal epithelial cells through multiple downstream partners. Probably the best described is the β-catenin

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signaling pathway, whereby destruction of β-catenin is regulated by Wnt binding. If Wnt does not bind its ligand, then a destruction complex made up of APC, axin, CKI, and GSK3 binds to β-catenin, causing it to be degraded by a ubiquitin-mediated proteasome pathway. If the receptor is bound by Wnt, then the free pool of β-catenin in the cytoplasm is increased and it translocates to the nucleus where it activates transcription of TCF-regulated target genes.[9] In colorectal cancers, when APC is inactivated via mutation or loss, the net effect is increased, unregulated gene transcription.

TP53: TP53 is lost or mutated in approximately 60% of colorectal cancers. The p53 protein is a transcriptional regulator of the cell cycle and apoptosis, and thus mutations allow unregulated growth.[9] The majority of *TP53* mutations are missense. Approximately 70% of tumors show 17p LOH which is the location of the *TP53* gene.

TGF-β: The TGF-β pathway is important in adult tissue homeostasis regulating such important functions as modulating cell proliferation and immune function. Mutations in members of this pathway are common in colorectal cancer, including *TGFBR2, SMAD2,* and *SMAD4* [9]. About 30% of colorectal cancers have mutations in TGFBR2.

PI3K: The PI3K signaling cascade is altered in 50% of sporadic colorectal cancers[20] and the *PIK3CA* gene itself is altered in 20% of cases.[21] This pathway is crucial for cellular growth, proliferation, and survival. Alterations in this pathway in colorectal cancer lead to constitutive activation through mutations in positive regulators such as *HER2, EGFR,* and RAS family members or loss of function mutations in negative regulators such as *PTEN*.[22]

KRAS: KRAS is the most commonly affected RAS family member in colorectal cancer with mutations found in 40% of tumors.[9] Tumors that harbor KRAS mutations are not sensitive to EGFR inhibitor therapy.

BRAF: About 10% of tumors contain mutations in *BRAF*. These sporadic tumors are typically MSI-high. BRAF mutation is another predictor of poor response to EGRF inhibitors. BRAF inhibits are not effective in colorectal cancer, likely due to a rich cross-talk among parallel pathways allowing tumors to evade therapy.[23]

Copy Number Changes in Sporadic Colorectal Cancer

CNV is common in colorectal cancer and certain chromosomal defects occur frequently. These changes often result in loss of the genes for *APC, TP53*, and *SMAD4*, which are all tumor suppressor genes. Tumors with widespread chromosomal imbalance are classified as CIN, as discussed previously. These chromosomal abnormalities occur due to asymmetric mitosis, focal or broad chromosomal gains or losses, chromothripsis, and chromosome rearrangements.[12] CNV can affect any amount of a chromosome, from the whole thing, to an arm, to a single gene. One example of this in colorectal cancer is *EGFR* amplification, where a tumor can have many copies of this gene increasing the amount of this receptor improving the fitness of the tumor.[24] Commonly affected chromosomal arms are as follows: gains in 1q, 7p and q, 8p and q, 12q, 13q, 19q, and 20p and q as well as deletions in 18p and q, and 17p and q.[20] 17p loss is seen in a high percentage of colorectal cancers likely improving tumor fitness due to the loss of *TP53* which is located here.[9] Another commonly deleted region is 18q which is where the *SMAD2* and *SMAD4* genes are located.[25] This leads to dysregulated growth because SMAD2 and SMAD4 proteins are downstream in the TGF-β–signaling pathway. Genes such as FHIT, RBFOX1, WWOX, SMAD4, APC, PTEN, and SMAD3 can all be focally deleted as well.[20]

Whole-Genome and Whole-Exome Sequencing in Colorectal Cancer

Whole-genome and whole-exome sequencing have revealed that each tumor contains multiple CNVs and 50 to 300 mutations unless a tumor is microsatellite unstable, in which case it can contain greater than 700 mutations.[20] With the advent of widespread whole-exome sequencing and the Cancer Genome Atlas Network (TCGA), came the most comprehensive characterization of colorectal cancer to date.[20] The TCGA colorectal cancer publication presented the analysis of 224 colorectal cancers. It highlighted multiple new driver genes such as *FAM123B*, *ARID1A*, *SOX9*, and *FZD10* and further emphasized the importance of activation of the Wnt signaling pathway and downregulation of the TGF-β pathway. In addition, researchers noted that any given tumor will generally contain only two to three of these known drivers and many other private mutations. In addition to the sequencing performed on these tumors, CNV was also assessed using SNP arrays and methylation profiling for epigenetic changes was also performed. Since the TCGA publication, there have been multiple other publications reporting sequencing of specific groups of colorectal patients. One such study sequenced colorectal tumors found in 31 patients with inflammatory bowel disease and found more *TP53* mutations and less *APC* and *KRAS* mutations than in other sporadic tumors in addition to novel, recurrent mutations in *SOX9, EP300, NRG1*, and *IL16*, highlighting the different mechanism to tumorigenesis in this population.[26] These types of sequencing studies contain a wealth of information that we are just starting to be able to mine and utilize.

Genetic Heterogeneity in Colorectal Cancer

Inter-tumor heterogeneity: Very few gene mutations are shared across a high percentage of tumors. Most genes mutated in cancer are mutated in a low proportion of cancer, but discovering these genes requires sequencing many tumors.[27] Any given colorectal cancer contains 60 to 1,500 mutations. Only a few of these mutations are in known drivers and the rest are mutations specific to that individual tumor. Thus, there is substantial heterogeneity between tumors in terms of what genes are mutated. The same can be said with regard to CNV where some chromosomal changes (like 17p loss) are recurrent, but most changes found in a given tumor are unique to that tumor.

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Intra-tumor heterogeneity: The theory of clonal evolution in tumor growth is longstanding,[28] but only recently have the tools necessary to understand tumor evolution in depth become available.[29–32] Over the past few years, the concept of genetic intra-tumor heterogeneity with tumor genetic subclones has itself evolved from being theoretical to being well accepted. Descriptive studies have been published for many types of cancer, providing evidence for the concept of branched, rather than linear, evolution of tumors where individual tumors contain intra-tumor heterogeneity with multiple subclonal populations.[33–39] In CRC, mutational heterogeneity had been reported upon analysis of few mutations in distinct areas of individual tumors in the past.[39–42] Recently, one in-depth analysis of 15 colon cancers and adenomas revealed the existence of widespread early heterogeneity, where each gland appeared to be a subclone.[39] We have also recently reported evidence of genetic subclones in rectal cancer.[43] We found that up to 30% of the mutations vary from one spatial region to the next in individual rectal cancers and that each region is likely made up of multiple shared and private subclones. Based on these studies, it appears that tumors are made up of inter-mixed subclones, whereby any given spatial location contains multiple genetic subclones. Because of tumor mixing, subclones are usually defined with the assistance of sophisticated bioinformatics algorithms, as the biopsy samples from the tumors are generally an admixture of subclones. These algorithms cluster groups of mutations together based on their allele frequency to define subclones. These allele frequencies are adjusted for the mutation site–specific CNV because the copy number state at the mutation site will alter the allele frequency. Because CNVs are so common in CRC, the allele frequency of a given mutation needs to be adjusted for its copy number state to obtain the cellular prevalence of a mutation. This type of adjustment can only be performed by using matched CNV and mutational analysis. The clinical importance of intra-tumor heterogeneity in colorectal cancer is largely unknown. The implications of this heterogeneity are widespread and likely have implications to tumor testing, therapeutic resistance, and metastasis. For instance, when testing a tumor to assess whether a patient is a candidate for directed chemotherapy, if the resistant subclone is not found, the patient may be subjected to chemotherapy that is not helpful. In addition, intra-tumor heterogeneity is seen when comparing primary tumors to their matched metastasis in stage 4 patients when either CNV or specific mutations are assessed, raising concerns that therapies chosen based on the mutational status of the primary tumor may or may not target the metastasis.[44,45]

Clinical Utility of Genetic Alterations in Colorectal Cancer

Specific DNA mutations may be useful for screening or surveillance in colorectal cancer. The first stool DNA–based screening test for colon cancers and adenomas is now available and is called Cologuard. It tests for 11 distinct DNA markers including *KRAS* mutations, aberrant *NDRG4* and *BMP3* methylation.[46] In the initial studies, the sensitivity was 92% for detection of colorectal cancer and 42% for advanced adenomas.

Another emerging technology is testing for tumor mutations in the blood, called *ctDNA*, of patients. Thus far, this falls in the arena of research but may very well be used clinically in the near future. Multiple studies have shown that this is not only feasible but may be advantageous over testing of tumors. Measurement of ctDNA may be useful in surveillance and to assess for emergence of mutations causing resistance to targeted therapies.[40,47]

Genetic Testing of Tumors to Guide Therapy

Multiple gene tests are currently available clinically to predict response to various targeted therapeutics for use in metastatic colorectal cancer. The tests that are clinically the most useful are *KRAS* testing to predict response to EGFR-targeted therapies. If a patient has a *KRAS* mutation, he or she does not respond to EGFR-targeted therapy.[48] EGFR-targeted therapies are useful in metastatic colorectal cancer. Unfortunately, resistance to EGFR-targeted therapies has also been associated with *PIK3CA, BRAF*, and *NRAS* mutations in various studies.[48,49] *KRAS*, *BRAF*, and *NRAS* mutations activate the RAS-RAF-MAPK pathway, which is downstream of EGFR leading to the resistance to EGFR inhibitors. *PIK3CA* mutations can activate the PI3 kinase-PTEN-Akt, which is downstream from the EGFR pathway and thus can cause resistance to EGFR therapy but not in all studies. Combination inhibitors that affect these pathways are being studied that may overcome the resistance.

While 10% of colon cancers do harbor *BRAF* mutations, BRAF inhibitors have not improved outcome for patients with colorectal cancer. Some preliminary studies raise the possibility that this is due to crosstalk with other pathways and that combinations of targeted inhibitors may be effective.[23]

Emerging data have shown that although immune-enhancing therapies do not improve outcome for most colorectal cancers, they may be beneficial in MSI tumors. Le and colleagues recently showed improved disease-free survival in patients with metastatic MSI tumors treated with the immune checkpoint inhibitor, pembrolizumab.[50]

Unfortunately, while targeted therapies are available for multiple genes and pathways altered in colorectal cancer, alone, most have not been thus far effective. It is likely that combinations of targeted therapies will play a larger role in colorectal cancer therapy in the future.

Conclusion

Over the past several years, researchers have found that the genetics of colorectal cancer is more complex than we ever imagined. This complexity is due to the discovery of alterations in many genes and pathways as well as intra- and inter-tumor heterogeneity (►**Table 1**). Ultimately, greater knowledge of the genetic mechanisms causing colorectal cancer will lead to advances in diagnostics and therapy.

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Table 1 Major advances in colorectal cancer genetics

Advancement	Description
Classification schema	CIN: accumulation of CNV with varied karyotypes from cell to cell and LOH leading to loss of tumor suppressor genes and mutations in key driver genes MSI: hyper-mutated tumors due to lack of DNA mismatch repair CIMP: hyper-mutated, BRAF mutation, widespread promoter methylation of DNA
Pathways/genes	Wnt pathway; TP53;TGF-β; PI3K
Inter-tumor heterogeneity	60–1,000s of mutations per tumor Few common mutations between tumors
Intra-tumor heterogeneity	Different regions of tumors contain shared and private mutations and copy number changes

Abbreviations: CIMP, CpG Island Methylation Pathway; CIN, chromosomal instability pathway; MSI, microsatellite instability pathway.

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