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Genetics and Genetic Biomarkers in Sporadic Colorectal Cancer

John M. Carethers[1,*] and **Barbara H. Jung**[2]

[1]Division of Gastroenterology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

[2]Division of G astroenterology, Department of Medicine, University of Illinois Chicago, Chicago, IL, USA

Abstract

Sporadic colorectal cancer (CRC) is a somatic genetic disease in which pathogenesis is influenced by the local colonic environment and the patient's genetic background. Consolidating the knowledge of genetic and epigenetic events that occur with initiation, progression, and metastasis of sporadic CRC has identified some biomarkers that might be utilized to predict behavior and prognosis beyond staging, and inform treatment approaches. Modern next generation sequencing of sporadic CRCs has confirmed prior identified genetic alterations, and has classified new alterations. Each patient's CRC is genetically unique, propelled by 2 to 8 driver gene alterations that have accumulated within the CRC since initiation. Commonly observed alterations across sporadic CRCs have allowed classification into a: (1) hypermutated group that includes defective DNA mismatch repair with microsatellite instability (MSI) and *POLE* mutations in ~15%, containing multiple frameshifted genes and $BRAF^{V600E}$; (2) non-hypermutated group with multiple somatic copy number alterations and aneuploidy in ~85%, containing oncogenic activation of *KRAS* and *PIK3CA* and mutation and loss of heterozygosity of tumor suppressor genes such as *APC* and *TP53*; (3) CpG Island Methylator Phenotype CRCs in ~20% that overlap greatly with MSI CRCs and some non-hypermutated CRCs; and (4) elevated microsatellite alterations at selected tetranucleotide repeats (EMAST) in ~60% that associates with metastatic behavior in both hypermutated and non-hypermutated groups. Components from these classifications are now used as diagnostic, prognostic and treatment biomarkers. Additional common biomarkers may come from genome-wide association studies and microRNAs among other sources, as well as from the unique alteration profile of an individual CRC to apply a precision medicine approach to care.

Keywords

genomic instability; microsatellite instability; chromosomal instability; CIMP

[*]Correspondence: John M. Carethers, M.D., Division of Gastroenterology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, TEL: 734-615-1717, FAX: 734-615-2645, addresses: jcarethe@umich.edu.

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Introduction

Sporadic colorectal cancer (CRC) is defined as cancers that arise from the colorectum without known contribution from germline causes or significant family history of cancer or inflammatory bowel disease. About two-thirds of all colorectal cancers fit this definition, and is the type most commonly seen in clinical practice. Patients often present after 60 years of age, with most cancers originating from precursor initiating adenomas that over 1–2 decades transform into cancer. Generalized screening of this population at age 50 years or older is effective and durable, reducing mortality from CRC, finding earlier staged cancers, and decreasing the incidence of CRC [1–3]. Surgery of the primary cancer and/or limited metastasis is the only approach for attempted cure, with chemoradiation improving outcome in some patients in addition to surgery [4].

Sporadic CRC is a somatic genetic disease that may be influenced by the local colonic environment and the individual's background genetic makeup. Biomarkers that might predict behavior, predict prognosis beyond staging, or direct treatment approaches for patients with sporadic CRC may be expected to come from the consolidated knowledge of the somatic genetics and epigenetics of sporadic CRC. Changes in the nucleic acid structures of the cancer cell might be detected from the fecal stream or the lymphatic or blood circulation. Genomic alterations that occur during the initiation, transformation, and progression of a normal colonic stem cell into a neoplastic, malignant, and metastatic cell are only partially known for sporadic CRC, but the current knowledge of these genetic and epigenetic changes, once detected, can project in some scenarios the future biologic behavior of the cancer. This knowledge has also led to the approach and development of compounds that may target some alterations and/or their functional cell consequences in sporadic CRC to modify tumor behavior and improve patient survival. The knowledge also indicates that some compounds or approaches would not have predictive effectiveness, given specific mutations present in some sporadic CRCs.

The Mutational and Epigenetic Landscape of Sporadic Colorectal Cancer

Driver and passenger genetic alterations

Whole exome or whole genome sequencing of sporadic CRC has confirmed more than 30 years of research on human specimens regarding the understanding of the genetics and epigenetics of this cancer, as well as expanded data with new observations via the utilization of this sequencing technology. Sporadic CRCs are formed through the accumulation of somatic genetic and epigenetic events that are clonal (that is, progeny cells subsequently acquire additional genetic and epigenetic events beyond those of the parental cells they are derived from). These include the loss-of-function defects among selected tumor suppressor genes (which often are mutated through protein-truncating alterations along the length of the gene coupled with allelic loss of the second normal allele, or acquire DNA methylation changes at promoter sequences), and gain-of-function defects in selected oncogenes (which show consistent recurring mutations at one or few amino acid positions along the length of the gene) [5]. These somatic defects may confer a selective growth advantage to a cell, classifying the defect as a "driver" event or lesion and propelling a cell clone to increased

proliferation which progresses towards malignancy [5]. For sporadic CRC, it is estimated that among the ~20,000 identified genes in the human genome there are 138 driver genes (74 tumor suppressor genes and 64 oncogenes) [5]. A typical sporadic CRC, however, may only contain 2–8 driver gene alterations, with the remainder being "passenger" gene defects as a result of genomic chaos and random events, and which have no effect on the neoplastic process [5,6]. This make's each patient's CRC genetically and epigenetically unique [5,7], and is an important item to consider for future approaches regarding precision medicine. The median number of nonsynonymous mutations (that change the translated amino acid) in sporadic colorectal cancers, regardless of pathogenesis, is 66 mutations per tumor [5], with only a handful of driver lesions and a larger number of passenger lesions. Integrated proteomic characterization of CRCs demonstrates the functional context for the observed mutations, with relatively few extending to the protein level [8]. An important concept is that a driver lesion for one patient's tumor might be different compared to another patient's cancer. Common mutations such as seen with *TP53* or *KRAS* are dominant along the mutational landscape as driver mutations, but genes with relatively few mutations numerically outnumber the commonly targeted genes among CRCs [5,7]. Because they are less commonly mutated overall, they are referred as the "tail" of the mutational frequency curve, but these genes are likely important as driver lesions among individual tumors [9]. They might also be considered individual or personalized biomarkers in the future.

Hypermutable and non-hypermutable cancers

Despite inter-patient heterogeneity, there are commonalities among sporadic CRCs. Sporadic CRCs can be grouped into two categories: hypermutated (16% of sporadic CRCs) and non-hypermutated (84% of sporadic CRCs) [7] (Figure 1A). The Cancer Genome Atlas Network characterized hypermutated tumors by mutation rates of >12 per 10^6 bases, and tumors had a median number of 728 total non-silent mutations; non-hypermutated tumors had mutation rates of <8.24 per 10^6 bases, and tumors had a median number of 58 non-silent mutations [7]. The etiology for hypermutated tumors was largely driven by the presence of the biomarker called microsatellite instability (and CIMP) as a result of defects in a DNA mismatch repair (MMR) gene, specifically somatic hypermethylation of the *hMLH1* promoter [7]. Other hypermutated tumors demonstrated somatic mutation of the DNA MMR genes *hMSH6*, *hMSH2*, *hMSH3* and *hMLH3*, as well as mutations in the gene encoding DNA polymerase ε (*POLE*) [7] (Figure 1A). All of these defects would be expected to drive up the mutation rate within cancers as these genes provide repair or editing of DNA after DNA synthesis. Both hypermutated and non-hypermutated cancers demonstrated 15 and 17, respectively, commonly reported mutated genes among tumors, but show unique and near complete differences between the two groups. Hypermutated sporadic CRCs show mutations largely in genes that contain intrinsic coding microsatellites [7] (Figure 1B). Non-hypermutated sporadic CRCs demonstrate mutations classically described over decades of research [7,10,11] (Figure 1C). In addition to the observation of microsatellite instability, any of these mutations might potentially serve as biomarkers for sporadic CRCs if they have prognostic value. Although hypermutated cancers and non-hypermutated cancers progress through different sequences of genetic events, there is some overlap of pathways affected. For instance, *APC* is mutated among both groups of tumors, consistent with its role as a gatekeeper mutation in CRC [5] (Figure 1B,C). Two activin receptor genes, *ACVR2* in

hypermutated tumors and *ACVR1B* in non-hypermutated cancers affect activin signaling pathway, demonstrating its importance overall as a target for inactivation across both groupings of sporadic CRCs [12–14]. Overall, there are ~25 different genes commonly affected by somatic mutation in sporadic CRCs, with tumor suppressor genes to oncogenes at a 4:1 ratio [7]. It has been suggested and largely demonstrated that driver gene defects affect one of three general cell functions: cell fate, cell survival, and genome maintenance, that are subdivided into 12 pathways (DNA damage control, transcriptional regulation, chromatin modification, APC, hedgehog, notch, cell cycle/apoptosis, RAS, PI3K, STAT, MAPK, and TGFβ) that give the affected cell a growth advantage [5]. Indeed, detailed analysis among sporadic CRCs demonstrated consistent activation of Wnt signaling (93% of all hypermutated and non-hypermutated tumors among 16 different Wnt genes in the pathway), receptor tyrosine kinase/RAS signaling, and PI3K signaling, while demonstrating consistent inactivation of TGFβ signaling and TP53 function [7]. Additionally, nearly 100% of tumors showed changes in MYC transcriptional targets, suggesting an important role for *MYC* in sporadic CRC [7].

Non-hypermutated cancers were more frequently associated with somatic copy-number alterations, indicating that this group is more likely to show chromosomal and subchromosomal changes. Chromosome arm gains (1q, 7p and q, 8p and q, 12q, 13q, 19q, and 20p and q), and deletions (18p and q including *SMAD4* in 66% of tumors, 17p and q including *TP53* in 56% of tumors, 1p, 4q, 5q, 8p, 14q, 15q, 20p, and 22q), including recurrent deletion peaks involving *FHIT, RBFOX1, WWOX, SMAD4, APC, PTEN, SMAD3* and *TCF7L2* at chromosome 10p25.2, are common [7]. Amplification of chromosome segments were found at 17 sites, including *USP12* (13q12.13), *CDK8* (13q12), *KLF5* (13q22.1, *HNF4A* (20q13.12), *WHSC1L1* (8q12), *MYC* (8q24) *ERBB2* (17q21.1), and *IGF2* and *miR-483* (11p15.5) [7]. Translocations that create fusion proteins have also been identified. The *NAV2* gene (chromosome 11) joined the 3′ coding portion of *TCF7L1* (chromosome 2) creating a fusion protein that lacks the TCF3 β-catenin binding domain, unregulating β-catenin oncogenic function. The *TTC28* gene (chromosome 22) showed various translocations in 22% of cancers that predict inactivation of its function as a target of TP53 and growth inhibition [7]. While many of these chromosomal alterations show functionality as driver alterations, the majority of translocations may be passenger defects, with breakpoints for translocation among “gene deserts” in the genome [5].

Genome-wide association studies

In addition to the direct genetic and epigenetic analysis of sporadic CRC tissue, potential biomarkers could come from genome-wide association studies (GWAS) in which genes near single nucleotide polymorphisms (SNPs) that are examined from blood may be linked to the risk for CRC on a population scale. These studies attempt to identify DNA variants associated with complex human traits and diseases, with significance at the very stringent level of $P<5\times10^{-8}$. However, GWAS does not determine the cause or mechanism for tumor initiation, progression, or metastasis – it only shows an association with the disease, perhaps at low penetrance that may direct further investigation. Additionally, the associated gene(s) at or near the SNP needs to be proven by pathophysiological studies of its importance in the genesis of sporadic CRC; this has not been done for many of the associated genes.

Table 1 lists GWAS SNPs found to be associated with sporadic CRC [15–35]. It should be noted that these SNPs are found largely from patients of European descent, with some SNPs identified in CRC patients from East Asian descent. Some of the associated genes are plausible for elevated risk of sporadic CRC because there is extensive knowledge of how the gene products work in carcinogenesis. For instance, some associated genes are involved in Wnt signaling and TGFβ signaling, DNA replication, and cell cycle regulation, just as predicted from direct genome analysis of tumors [5]. Other associated genes may highlight pathways not previously understood to be contributory towards sporadic CRC, or may be misidentified based on their nearness to the associated SNP [23,35]. Functional characterization through expression studies and environmental-gene interactions should help determine the importance and involvement of the purported genes [27,35]. Clear identification of involved genes could be used as biomarkers for sporadic CRC risk.

MicroRNAs

While most investigations into the pathogenesis of sporadic CRC deservedly examine affected genes and their associated protein function, post-transcriptional regulation of the affected gene messenger RNAs (mRNAs) has emerged as another level of control of expression that directs sporadic CRC pathogenesis by affecting cell pathways and function without the need for direct genetic or epigenetic defects upon genes. MicroRNAs (miRs) are single-stranded molecules 20–25 molecules in length, derived from non-coding RNAs by RNA polymerase II (or III) that form a distinctive hairpin shape, making them resistant to RNA degradation [36]. Their main function is to bind mRNA via complementary base pairing with the 3′ untranslated region (3′-UTR), or in rare cases the 5′-UTR or coding sequences, causing degradation of that mRNA if the base pairing is fully matched, or silences the mRNA if the base pairing is only partially matched [36,37].

Multiple miRs among the more than >2500 catalogued show altered expression in sporadic CRC [37]. Elevated levels of a miR within sporadic CRC or serum might predict its role as an oncogenic miR (oncomirs), meaning that the miR is accelerating the cancer phenotype or metastasis, whereas reduced levels of a miR within tumors might predict its role as a tumor suppressive miR, meaning that the miR is reducing or preventing the cancer phenotype (Supplemental Table S1). This should be interpreted with caution until the miR has been tested adequately in models such as knockout mice, as elevated levels of a miR might suggest a role on oncogenesis, but could also suggest a response mechanism to the tumor [38]. A few miRs display opposing functions depending on the context [38]. These aspects must be clarified scientifically before specific miRs might be utilized as biomarkers to direct prognosis or therapy. Many miRs map to regions of the genome that are known to be altered in cancer, and their regulation of cell pathways place miRs as an important mechanism to alter those pathways. For instance, miR-155 and miR-21 can downregulate several DNA mismatch repair proteins resulting in tumor microsatellite instability [39,40]. MicroRNA detection for diagnosis of sporadic CRC has potential because of the miR stability in fecal material, making screening for this relatively non-degradable molecule a possibility for the future [41].

Because miRs are encoded, they can be subject to genetic and epigenetic events that can alter their expression, just like any other gene [37]. Polymorphisms and mutations are present in miRs that affect their normal function. The machinery that processes precursors to miRs - from primary-microRNAs (which are capped and polyadenylated for stability) into pre-microRNAs (~60–100 nucleotides in length) in the nucleus, through specific nuclear export proteins (exportin-5) into the cytoplasm where the DICER protein cleaves the pre-microRNA into mature miRs, and it association with the RNA-induced silencing complex (RISC) to directly bind mRNA – all can be affected by mutational events that alter the processing of miRs and alter the pathogenesis of cancer cells [36,38,42]. These alterations can have profound effects on tumor behavior.

MicroRNAs offer additional potential therapeutic options not afforded by many altered genes. Because of their stability, size and low antigenic profile, miRs can be engineered for effective human use [38]. Antagomirs can be designed against oncomirs to eliminate their expression. Tumor suppressor miRs can therapeutically replace their native, non-expressing counterparts. Targeted miRs towards multi-drug resistance genes to improve sensitization of cancer to chemotherapy have been shown to be possible [38]. Levels of miRs as biomarkers can also predict therapeutic response. For instance, high miR-21 levels were predictive of poor 5-fluorouracil treatment response in CRC patients [40]. The utilization of miRs for the approach to and treatment of sporadic CRC is just beginning to be explored.

Lessons from familial cancer

Discovering the genetic basis of hereditable CRC syndromes will continue to provide key insights into sporadic CRC. Often, the hereditable syndrome is the extreme case of its sporadic version. For instance, mutations within the *APC* gene were discovered as the genetic cause of familial adenomatous polyposis, and later, inactivation *APC* and other components of Wnt signaling were identified as the gatekeeper lesion for sporadic CRC [11]. Similarly, the discovery of germline mutations in the DNA MMR genes as the cause for Lynch syndrome helped later identify that one of the DNA MMR genes *hMLH1* was a target for hypermethylation involved in ~15% of sporadic CRCs [11]. Mutations in *POLE* and *POLD1* have now been identified in familial polymerase proofreading-associated polyposis as well as a cause of hypermutated sporadic CRCs [7,43]. Mutations in *PTEN* cause the PTEN Hamartoma Syndrome (including Cowden's disease and Bannayan-Riley-Ruvulcaba sydromes), and *PTEN* inactivation (as well as its counterpart *PIK3CA* mutational activation) is seen in one-third of sporadic CRCs [44–46]. Mutations among TGFβ superfamily members (e.g. *SMAD4, BMPR1A*) are seen in the germline of patients with juvenile polyposis, and these as well as other members of this family (e.g. *SMAD4, SMAD2, ACVR2*, etc.) are commonly involved in sporadic CRCs [7,12–14,44,47,48]. As one-third of all CRC has a familial component but only a small portion of genetic causes overall identified, these human conditions should continue to provide information that may apply to sporadic CRC and its pathogenesis, with some as potential biomarkers for use for precision medicine.

Heterogeneity within and between cancers

The mutational genetic landscape for sporadic CRC is heterogeneous because of clonality. Heterogeneity can be at the interpatient level, meaning that no two patients have perfectly identical tumors. Heterogeneity can be intratumoral, meaning that multiple clones are represented within the gross tumor, each with varying degrees of mutational accumulation that dictate its proliferation and potential progression [5,11]. Heterogeneity can also be intermetastatic, meaning metastatic clones derived from a primary CRC may each have different mutational spectrums between them; and can be intrametastatic, meaning that individual metastatic clones derived from a primary CRC has further evolved within the metastatic origin to diversify its clonality and subsequent mutational genetic makeup [5,49]. These considerations must be taken into account for future application of precision medicine. Varying clones may respond to one treatment, whereas another may be resistant and grow into a recurrent tumor subsequently. Specific therapies such as EGFR inhibitors will affect a clone with wild type *KRAS* differently than another clone that has acquired an activating mutation in *KRAS*.

Characterized Molecular Pathways for Sporadic Colorectal Cancer

The normal-to-adenoma-to-carcinoma-to-metastatic sequence

Sporadic CRCs generally are thought to originate from an initiating genetic event in a normal colonic stem cell involving overactivation of Wnt signaling (gatekeeper lesion), allowing that cell to outgrow surrounding cells to form a dysplastic aberrant crypt focus (ACF). In some instances, a non-dysplastic ACF (also called hyperplastic ACF) can form with initial activating mutations in *KRAS*, but these lesions are believed to be precursors of hyperplastic polyps; they, however, can acquire Wnt signaling activation generally through acquiescence of a *APC* mutation becoming a dysplastic ACF and primed to progress towards a serrated adenoma and carcinoma. Once Wnt signaling is incessantly activated, these early adenoma epithelial cells grow into a physical mass that outgrows but affects the supporting mesenchymal tissue into a grossly observable adenomatous polyp. This “tumor initiation” step for which observable lesions are still benign may take anywhere from 30 to 60 years in a patient’s life depending on the genetic pathway that the adenomatous polyp may acquiesce (Figure 2A). Contingent on the driver mutations that dominate specific individual clones within the adenomatous polyp, clonal outgrowth occurs based on specific clones that acquire mutational events that allow it to proliferate faster than other “lesser fit” clones, creating a heterogeneous polyp in which one clone will acquire the makeup to transform into malignancy. This “tumor progression” step is faster than tumor initiation, and depending on the genetic pathway taken, may be as short as 1–5 years after tumor initiation to as long as 2 decades after tumor initiation (Figure 2A). Even faster is the time to ability to metastasize, which may be as short as near simultaneous with completion of malignant transformation of the primary CRC to as long as a few years (Figure 2A). As the neoplasm moves from initiation to progression to carcinoma to metastasis, there is an accumulation of genetic and epigenetic alterations that define that tumor and which we sample from, providing a snapshot of mutational landscape for that tumor and potential use for precision medicine approaches.

The relative ease of sampling the colon via colonoscopy has provided neoplasms from the various stages of initiation and progression, allowing the scientific community to investigate and propose the natural mutational history for the normal-to-adenoma-to-carcinoma-to-metastatic sequence. It has also allowed insight and characterization of three identified molecular pathways that highlight progression to carcinoma: microsatellite instability (wholly within the hypermutated category), chromosomal instability (wholly in the non-hypermutated category), and CpG Island Methylator Phenotype (CIMP; within both the hypermutated and non-hypermutated categories) (Table 2). These molecular pathways may dictate the timing of tumor progression and metastasis (Figure 2A), as information regarding the epidemiology, mutational events, and immune response differ for each pathway. Treatment approaches may vary as well. A modulator for the three molecular pathways, identified by the biomarker “elevated microsatellite alterations at selected tetranucleotide repeats” or EMAST, has recently been shown predictive of patient survival [50,51] (Table 2). Among sporadic CRC patients, these described pathways have not been able to predict if a metachronous adenoma or cancer will follow the previous pathway in the same patient; clinical surveillance is highly important in finding and removing metachronous lesions.

Hypermutated Sporadic Colorectal Cancers

Microsatellite Instability (MSI-H) Pathway—Microsatellite instability-high (MSI-H), defined by an NCI consensus panel as >30% of microsatellite markers demonstrating frameshift mutation, is a biomarker for defective DNA MMR function within a CRC [11,52,53]. DNA MMR recognizes and directs repair of nucleotide mispairs (or mismatches) after DNA replication; this ensures the fidelity of the replicated DNA before mitosis into two identical daughter cells [11,53]. When faulty, the transmission of DNA fidelity is lost, and mutations in an asymmetric manner are passed onto both daughter cells and eventually multiple mutations are accumulated in progeny cells. MSI-H is observed in about 15% of sporadic CRCs, consistent with the frequency of hypermutated tumors of which they categorically belong [7]. Although by consensus definition frameshift mutations are detected, multiple other mutations including point mutations occur with defective DNA MMR. The mutational process with inactivation of DNA MMR might be initially random, but ultimately clones that acquire mutations in several key regulatory driver genes gain the growth advantage that promotes the formation of the carcinoma (Figure 1B).

The cause for the defect in DNA MMR in sporadic CRC is, for the most part, aberrant bi-allelic hypermethylation of the DNA MMR gene *hMLH1*, preventing its gene transcription [54–56]. Since hMLH1 is a component of hMutLα (a heterodimer between hMLH1 and hPMS2 proteins), the main execution complex for human DNA MMR that binds to one of two recognition DNA MMR complexes (hMutSα, hMSH2-hMSH6; or hMutSβ, hMSH2-hMSH3), complete DNA MMR function is lost without *hMLH1* transcription [11,53]. There is overlap with many CIMP tumors, defined by multiple hypermethylated genetic loci, and MSI-H tumors, since *hMLH1* can be one of multiple methylation targets (Figure 2A).

Sporadic MSI-H CRCs accumulate mutations in driver genes that contain intrinsic coding microsatellites that can be subject to frameshift mutation, such as *ACVR2, TGFBR2, hMSH3* and *hMSH6*, among others (Figure 1B and Figure 2B). The frameshift mutation causes a

stop codon creating a truncated transcript and protein that are neo-antigenic to the patient's immune system [57], while simultaneously inactivating a key signaling pathway that normally holds uncontrolled cell proliferation in check through regulation. Activating oncogenic mutation of *BRAF* (*BRAF*^{V600E}) is another common feature of sporadic MSI-H CRCs, occurring in over 40% of specimens (Figure 1B) despite this gene not containing a coding microsatellite. [11,53,58]. *BRAF*^{V600E} causes incessant signaling of the EGFR/RAS/RAF/MAPK pathway that is known to increase the physical size of adenomas and increase cell proliferation, and typically is genetically seen subsequent to *hMLH1* hypermethylation and activation of Wnt signaling (Figure 2B). MSI-H CRCs show low copy number variation compared to non-hypermutable CRCs, and tend to be diploid (Figure 1A and Table 2). These CRCs have significantly less *TP53* (60% vs. 20%) and *APC* (81% vs. 51%) mutations, compared to CIN CRCs [7]. However, 97% of hypermutable tumors demonstrate a deregulated Wnt signaling pathway [7], confirming this pathway's gatekeeper role for colonic neoplasia even in MSI-H tumors.

MSI-H is an important biomarker for patients with sporadic CRC, with prognosis and treatment value, and is being used today in clinical care. CRCs can be tested via polymerase chain reaction to detect the presence of MSI-H, or more commonly in pathology laboratories, use the proxy of immunohistochemistry (IHC) to note the absence of a DNA MMR protein. Sporadic MSI-H CRCs demonstrate absence of hMLH1 and hPMS2 staining; loss of hMLH1 is due to its hypermethylation and loss of transcription, and loss of hPMS2 is due to protein instability when its partner hMLH1 is absent [59]. MSI-H CRCs histologically are more likely to contain mucin, be poorly differentiated, and possess "Crohn's-like" sub-epithelial lymphoid aggregates and intra-epithelial lymphocytes, thought to be an immune response to the truncated neo-antigens that are produced from the epithelium. The immune response appears to be favorable to the patient; they have improved survival when compared to stage II and III matched patients with microsatellite stable or non-hypermutable CRCs, are more likely to be staged earlier [11,53,60], and are more likely to respond to immune PD-1 immune checkpoint inhibition [61]. There is evidence that patients with MSI-H CRCs do not respond to 5-fluorouracil chemotherapy unlike patients with non-hypermutable CRCs; this is due to lack of DNA MMR function to recognize and execute the normal cytotoxic response to 5-fluorouracil that is incorporated into DNA [62–68] (Figure 1A and Table 2). Algorithms for patients with stage II MSI-H CRC have been developed to discern whether these patients should proceed to chemotherapy or be observed in conjunction with surgery [69,70]. MSI-H CRCs have a propensity for the colon proximal to the splenic flexure, where >70% of these cancers are located [11,53]. Data from a population-based cohort suggests that MSI-H is half as frequent among African American patients compared to Caucasian patients (7% vs. 14%, respectively, $P<0.05$) and may be a partial contributor to the poor outcome in African American cohorts [71]. MSI-H CRCs, based on their hypermutated pattern, appear to progress from a benign to malignant tumor at a rapid pace when compared to CIN tumors [11,53,58,72–74] (Figure 2A). However, the progression to metastasis may be less rapid perhaps due to the immune response incited within MSI-H CRCs [57,71,75] (Figures 1A and 2A).

Although bi-allelic hypermethylation of the *hMLH1* promoter is the most common mechanism to develop a sporadic MSI-H CRC, there is at least one other rare cause for the acquired somatic presence of MSI-H. Half to two-thirds of "Lynch-like" patients, kindreds in which no germline mutation of a DNA MMR has been identified and there is no presence of hypermethylation of *hMLH1* in the MSI-H CRC, show two somatic pathogenic hits to a DNA MMR gene within the tumor [76–80]. Typically, this is by mutation in one DNA MMR gene allele and loss of heterozygosity (LOH) of the other allele. However, the profile of Lynch-like patients suggests they present younger than sporadic MSI-H CRC patients, and germline mechanisms have not been fully ruled out [76–80]. This mechanism was only seen in 13 of 232 (5.6%) of MSI-H CRCs, far less than the occurrence of *hMLH1* hypermethylation [77].

***POLE* mutations—**The initial observation that *POLE* mutations (encoding DNA polymerase ε, one of main enzymes that replicates eukaryotic DNA) cause hypermutable sporadic CRCs came with whole exome sequencing [7]. Seven of 30 (23%) hypermutable CRCs lacked MSI-H, CIMP or *hMLH1* hypermethylation, but showed somatic mutations in *POLE* and missense or nonsense (but not frameshift) mutation in one or more DNA MMR genes (*hMLH1, hMLH3, hMSH2, hMSH6*, and *hPMS2*), and were representative of tumors with the highest mutation rates among all sporadic CRCs [7,78]. Because mutation of the DNA MMR genes were not frameshifts and lacked MSI-H, an initial DNA MMR dysfunction as a trigger is not likely perhaps due to only one allele mutated, and these tumors represent a new class of hypermutated CRCs driven by *POLE* mutation. Although there are some *POLE* mutations in Lynch-like patients with two somatic DNA MMR gene mutations, *POLE* mutation appears not to be the cause of Lynch-like syndrome [78,80]. Indeed, germline mutation in the exonuclease domains of *POLE* and *POLD1* (encoding DNA polymerase δ1) cause a condition termed polymerase proofreading associated polyposis, and tumors are microsatellite stable [43] (Figure 1A). Somatic *POLE* mutations, at present, cause an extremely hypermutable (or ultramutable) tumor that is apparently microsatellite stable unless two DNA MMR alleles of the same gene by chance become mutated. These CRCs to date have no described molecular pathway like MSI-H or CIN tumors, but based on the loss of polymerase ε function, multiple chance mutations likely develop among many of the key signaling pathways, with "best fit" clones outgrowing others to form the tumor.

CpG Island Methylator Phenotype (CIMP) Pathway—CIMP CRCs are often defined by increased or excessive epigenetic methylation of genetic loci that contain CpG islands, typically located in promoter and upstream regulatory regions of genes. Methylation of these regulatory regions abrogates transcription of the affected gene. There is lack of universally accepted defining criteria compared to MSI-H, but investigators often use at least 3 loci methylated from a selected panel of 5 markers to define CIMP (*RUNX3, SOCS1, NEUROG1, CACNA1G, IGF2*) [81]. The etiology for the development of CIMP has been elusive. A number of mechanisms have been investigated including overexpression of DNA methyltransferases [11], mutations in genes that remodel chromatin such as CHD8 [82–84], environmental exposures such as tobacco [85], and mutations in *IDH1* and *TET*, which

cause aberrant methylation in leukemia and gliomas [86]. None of these potential mechanisms are shown consistent for CIMP CRCs.

CIMP can be classified as “high” or “low”, based on the number of markers positive for methylation (≥3 of 5 markers and ≤2 of 5 markers, respectively) [87] or by unsupervised cluster analysis after comprehensive genome-scale DNA methylation profiling [88]. This classification has linked CIMP with subclasses of colonic polyps and cancers. CIMP-high occurs in ~20% of sporadic CRCs, and these tumors demonstrate $BRAF^{V600E}$ mutation and hypermethylation of *hMLH1* for the most part, and are hypermutated tumors [7,81,88]. This group constitutes the majority of MSI-H CRCs (Figure 1A) and mostly arises in the colon proximal to the splenic flexure. CIMP-high is the pattern observed during sessile serrated adenomas pathogenesis [58,89,90] with accumulation of methylation at multiple genetic loci (Figure 2B). CIMP-low occurs similarly in ~20% of sporadic CRCs, but some of these tumors may have been derived from traditional serrated adenomas as they are microsatellite stable and contain *KRAS* mutations, and are non-hypermutable tumors [88,89]. While the majority of CIMP-high and CIMP-low CRCs fit into these subtypes, the classification is not absolute between subtypes of polyps or CRCs. CIMP CRCs overlap with MSI-H and CIN CRC pathways.

The overlap of classification between CIMP-high CRCs and MSI-H CRCs would predict their similar biological behavior to MSI-H CRCs, including proximal colon location, improved survival compared to patients with CIN CRCs, and a muted response to 5-fluorouracil chemotherapy [91–94] (Table 2). Because microsatellite instability assays or immunohistochemistry for the *hMLH1* protein are more easily performed on a CRC sample, these tests are commonly utilized over methylation assays to detect the potential predictive behavior from the tumor. Although CIMP classification maybe useful for understanding how pathogenesis occurs for some CRCs, CIMP status has not yet proven to be a useful clinical tool or biomarker to date for serrated polyps or CRCs. However, the methylation status of specific markers is being utilized as part of a panel of assays for fecal DNA tests for diagnosis the presence of adenomas or CRC [95].

Non-Hypermutated Sporadic Colorectal Cancers

Chromosomal Instability (CIN) Pathway—CIN was the first described pathway among the molecular pathways for CRCs; however, the mechanism that generates CIN is still not clear or known despite this pathway being the most common, affecting ~85% of sporadic CRCs [5,11,96,97]. CIN CRCs are defined by the presence of extensive somatic copy number alterations throughout the genome, and result in an aneuploid tumor [7,11], generally as a result of accumulated asymmetric mitoses (Figure 1A). Chromosome gains and losses (including loss of heterozygosity or LOH events at specific tumor suppressor gene loci), focal gene amplifications, chromothripsis, chromosome rearrangements and base substitutions and deletions are readily identified among cohorts of CIN CRCs, with specific patterns of alterations that affect driver gene pathways and general cell functions [5,7,9,11,98]. A number of potential mechanisms that generate CIN have been proposed, including base excision repair gene mutations, mitotic spindle checkpoint gene mutation, centrosome regulation processes irregularities, DNA checkpoint gene mutations, cell cycle

checkpoint gene and telomerase gene mutation – all of which can drive chromosome alterations – but none of these mechanisms are consistently observed among CIN CRCs [5,11,97]. The loss of both *APC* and *TP53* from human intestinal organoids appears to be sufficient to generate significant aneuploidy, with loss of *SMAD4* and the presence of mutant *KRAS* only minimally additive to the aneuploidy [99]. CIN CRCs are non-hypermutated, with some overlap with CIMP-low CRCs but segregated from hypermutable CRCs that have *hMLH1* promoter hypermethylation (Figure 1A and Table 2).

Clonal outgrowth of dominant cells within the non-hypermutated adenomas and CIN CRCs has identified a predictable pattern of gene alterations that was originally outlined by Fearon and Vogelstein [96] (Figure 1C and Figure 2B). Mutation coupled with LOH of the *APC* gene appears to be the earliest and gatekeeper lesion for tumor initiation (Figure 2B). APC is part of Wnt signaling pathway that regulates cytoplasmic levels of β-catenin, a proto-oncogene that moves to the nucleus and transactivates genes geared towards cellular proliferation [10,11,100,101]. Through regulation of β-catenin, APC is a tumor suppressor, and when inactivated, nuclear β-catenin levels rise to drive increased proliferation. By whole exome sequencing, *APC* is mutated in 81% of non-hypermutated CRCs as the most common etiology for altered Wnt signaling (Figure 1C), which is deregulated in 93% of all non-hypermutable tumors [7]. Oncogenic activation of *KRAS* through mutation at codons 12, 13, or 61 within the gene appears to consistently follow *APC* inactivation during tumor progression [5,11,97,100] (Figure 2B). *KRAS* encodes a protein that binds cyclic nucleotide guanosine tri-or diphosphate to transmit a regulated growth response to the nucleus. Mutant KRAS protein signals this response incessantly, causing increased proliferation and growth in size of the tumor. KRAS is part of the ERBB/KRAS/BRAF/MAPK signaling axis (Figure 3A), in which other members of this axis can nearly mimic the effects of mutant KRAS protein. For instance, mutation or amplification of one of *ERBB1-4* genes (ERBB1 is EGFR, epithelial growth factor receptor) was present in 13% of non-hypermutated CRCs [7]. The prevalence of *KRAS* mutation was 41% among non-hypermutable CRCs, but overall 55% of total CRCs showed activating mutations in *KRAS, NRAS* or *BRAF*, with near mutual exclusivity for mutation in CRCs [7]. Activation mutations in *PIK3CA*, the catalytic subunit of the mitogenic PI3 kinase complex that controls the levels of phosphatidylinositol triphosphate and is antagonized by PTEN function, is seen in 18% of non-hypermutated CRCs [7] (Figure 1C, Figure 2B, Figure 3A). Alterations of PI3 kinase pathway and the ERBB/KRAS/BRAF/MAPK axis (Figure 3A) seem to co-occur in about 33% of tumors, with implications towards simultaneous inhibition therapeutically for a patient to have a beneficial effect [7]. The presence of activated ERBB/KRAS/BRAF/MAPK axis portends a poor prognosis for patients with CRC over patients without those mutations [102–104], but it has been unclear how the presence of mutant *PIK3CA* affects outcome [102,105,106].

Non-hypermutated CRCs also show disruption of tumor suppressor TGFβ signaling pathways during tumor progression, with genomic alterations in *TGFBR1*, *TGFBR2*, *ACVR2A*, *ACVR1B*, *SMAD2*, *SMAD3*, and *SMAD4* in 27% of these CRCs [7,14] (Figure 1C and Figure 2B). As is the case for the tumor suppressor gene *APC*, inactivation is generally by mutation and LOH. Chromosome 18q21 LOH, the location of *SMAD2* and *SMAD4*, is selected for in 80% of stage IV CRC patients [107,108].

Mutation and subsequent LOH of the tumor suppressor gene *TP53* is a key event in the pathogenesis of non-hypermutated CRCs, coinciding with tumor genomic chaos and conversion from benign to malignancy [99,109] (Figure 2B). *TP53* encodes a critical growth suppressive protein that is triggered with DNA damage to regulate the cell cycle and stimulates repair of DNA, the so-called "guardian of the genome" [5,7,11,110], and loss of TP53 function deregulates these processes. *TP53* alterations were found in 60% of non-hypermutated CRCs (Figure 1C), with many of the alterations bi-allelic, and an additional 7% of these CRCs showed alteration in *ATM*, a kinase that activates TP53 after DNA damage [7]. *TP53* mutations confer poor prognosis for patients with CRC [110,111].

Molecular phenotypes and patient outcome

The molecular pathways observed within a sporadic CRC outlining distinct histopathological and molecular subsets may predict patient outcome and survival. Assaying for $BRAF^{V600E}$ and *KRAS* codon 12 and 13 mutation, and the presence or absence of MSI-H and CIMP can group CRCs from which to compare patient outcome [49,103,104]. Two studies, each with over 2000 stage III patients, demonstrated a group of ~11% of MSI-H CRCs that can be dichotomized based on the presence (~7%) or absence (~4%) of $BRAF^{V600E}$ [103,104] (Figure 4A). MSI-H+$BRAF^{V600E}$ +/− CIMP is the molecular phenotype that approximates hypermutated serrated CRC pathogenesis, and MSI-H without $BRAF^{V600E}$ is the typical molecular phenotype observed in patients with Lynch syndrome. Patients in both MSI-H groups have favorable survival outcome compared to patients with CIN CRCs [103,104] (Figure 4A). These two studies identified ~6% of CRCs with CIMP +$BRAF^{V600E}$ but without *hMLH1* hypermethylation approximating non-hypermutated serrated CRCs, and patients show significantly worse outcome compared to patients with CIN CRCs [103,104] (Table 2 and Figure 4A). About 83% of CRCs in these two studies were microsatellite stable that may be a proxy for CIN, and showed the absence (~50%) or presence (~33%) of mutant *KRAS* [103,104] (Figure 4A). The presence of *KRAS* mutation significantly worsened patient survival over microsatellite stable patients without mutant *KRAS*, and *KRAS* mutation was associated with African American CRC patients [103,104] (Table 2). Overall, the characterization of molecular pathways for sporadic CRC can be a tool to predict behavior for patients.

Modulation of hypermutable and non-hypermutable colorectal cancers

One would predict that like tumor initiation and tumor progression, alteration of some genes would be consistently found for a CRC to become metastatic. This could be the case, but to date, no consistent genetic alterations that distinguish metastases from primary CRC have been identified [5].

One consistent theme has been a strong link between the primary and secondary prevention of CRC through the use of anti-inflammatory drugs [112–119]. The level and nature of inflammation in primary CRCs may be complex, but the type, density and intra-tumor location of immune cells can predict patient survival, often better than classical staging [120]. This same inflammation has been associated with initiating elevated microsatellite alterations at selected tetranucleotide repeats (EMAST), a biomarker and form of microsatellite instability caused by isolated loss of the DNA MMR complex hMutSβ

function due to a nucleus-to-cytosol shift of its component hMSH3 protein [50,121–123]. EMAST can be identified in up to 60% of CRCs, making this biomarker more common than MSI-H [124–127] (Figure 1A and Table 2). Because of the repair profile of dinucleotide or larger microsatellite sequences, loss of hMutSβ function appears to be the etiology of MSI-low (defined by mono- and dinucleotide microsatellite markers) as well as EMAST (defined by tetranucleotide microsatellite markers), now equivalent biomarkers identifying hMSH3 dysfunction [122,124–126,128]. Oxidative stress and inflammation, and in particular interleukin-6 signaling, causes hMSH3 to abnormally shift from the nucleus to the cytosol where it cannot participate in DNA repair [122,123]. Importantly, the presence of EMAST in primary CRCs predict advanced stage disease in patients and is associated with poor survival over patients with non-EMAST CRCs [50,51] (Figure 4B). Patients with stage II/III EMAST CRCs (and no functional hMutSβ) appear to still respond to 5-fluorouracil treatment for a survival benefit due to the presence of hMutSα [67,129].

EMAST and its associated hMSH3 dysfunction does not direct oncogenic transformation like the well-described molecular pathways [128]; rather this common biomarker appears to modulate the behavior of both hypermutable and non-hypermutable CRCs towards metastasis [50,51,124] (Figure 1A, Table 2, Figure 2B, Figure 4B). How EMAST modifies the biology towards metastasis is under investigation, but the somatic loss of hMSH3 function is a combined and complex DNA repair defect due to partial loss of DNA MMR function and partial loss of DNA double strand break repair, contributing to both tetranucleotide microsatellite instability and chromosomal instability and aneuploidy [124,128, 130–133]. Thus, EMAST is a common and inflammation-driven biomarker linked to advanced stage and metastatic hypermutated and non-hypermutated CRCs. It would be reasonable to further examine this biomarker in the context of therapeutics for control of inflammation that might affect patient outcome.

Current and Potential Biomarkers for the Sporadic Colorectal Cancer Patient

The molecular classification of sporadic CRCs demonstrates some use of pathway knowledge as predictive biomarkers for patients. Among the three pathways, the presence of MSI-H (or the absence of DNA MMR protein by immunohistochemistry) in the primary tumor has been the most useful and utilized by clinical laboratories due to its ease for assay and its prognostic and treatment value [11,53,60,65,134] (Table 3). CIN and CIMP *per se* are not routinely assayed in clinical scenarios, but components of their pathways are commonly utilized. Methylated markers have been incorporated into FDA-approved fecal DNA diagnostic tests, with 92.3% CRC sensitivity and 42.4% advanced adenoma sensitivity [95] (Table 3). Mutation of *KRAS* or *BRAF* within a primary CRC predicts resistance to the monoclonal antibodies cetuximab and panitumab, used to block ERBB1(EGFR)/KRAS/BRAF/MAPK signaling in stage IV patients [135,136] (Figure 3A and Table 3). These EGFR inhibitor treatments require tumor genotyping and use only in wild type *KRAS* and *BRAF* CRC patients, as mutant versions of these oncogenes provide incessant proliferation signals in spite of EGFR blockade.

Epidemiological information combined with tissue genetic or expression assays have provided data that can be used to predict primary and secondary prevention of CRC with regular aspirin use. Cyclooxygenase-2 (Cox-2) is overexpressed in CRCs in part due to mutant oncogenic *PIK3CA*, driving incessant PI3 kinase signaling [137] (Figure 3B). Aspirin inhibits Cox-2, an enzyme that normally converts arachidonic acid into pro-inflammatory and pro-proliferative prostaglandins (Figure 3B), and can downregulate PI3 kinase function. Regular aspirin use was effective in reducing death among patients whose CRCs contained mutant *PIK3CA* compared to patients not taking aspirin; this improved survival was not observed among patients whose tumors contained wild type *PIK3CA* [138] (Table 3). Thus, aspirin might be used as adjunct therapy for patients with mutant *PIK3CA* cancer. Prostaglandin E_2 (PGE_2), a mediator of proliferation, cell survival, angiogenesis and epithelial-mesenchymal transition, is generated by Cox-2 and subsequent action by microsomal PGE_2 synthase-1. PGE_2 is deactivated by 15-hydroxyprostaglandin dehydrogenase (15-PGDH), abrogating its pro-proliferative capabilities (Figure 3B), but 15-PGDH is downregulated in CRCs, allowing accumulation of PGE_2 [139–141]. Thus, increased Cox-2 and reduced 15-PGDH levels in CRCs allow PGE_2 to accumulate and induce proliferation (Figure 3B). Regular aspirin use was effective for the primary prevention of CRC in patients whose normal colonic mucosa showed high expression levels of 15-PDGH, but not for patients with low expression levels of 15-PGDH [142] (Table 3). This suggests that 15-PGDH might serve as a biomarker to predict CRC primary prevention effectiveness with aspirin.

Additional biomarkers that have clinical applicability will continue to be proposed, tested, and developed from knowledge of the genetic and epigenetic changes in sporadic CRCs. Continued improvement of multi-target fecal DNA panels will likely increase advanced adenoma detection. Sequencing of individual cancer genomes that provide a comprehensive picture of driver mutations within a CRC may become commonplace when costs for this technology are lower and rapid analytic systems are employed. This will be most beneficial with simultaneous development and use of therapies that can address the sequencing findings [143,144]. Micro RNAs, naked DNA, and circulating tumor cells could be utilized routinely for patients with CRC once specific prognostication or therapeutic approaches are vetted [145]. Certain biomarkers from the CRC or tumor immune reaction may lead to individualized immune-vaccines. Additional molecular investigations may shore up recommendations and target populations for the use of anti-inflammatory agents. Further genetic understanding of the metastasis process might identify biomarkers that may be more predictive than imaging, and provide targets to either prevent or limit tumor spread. Indeed, recent investigation of tumor-stromal interactions suggests that patients with poor-prognosis CRC might be driven by tumor-associated stromal cells rather than epithelial tumor cells, and mediated by elevated TGFβ pathway expression upon cancer-associated fibroblasts, increasing metastatic potential [146]. The role of the microbiome in the colon and sporadic CRC, and its influence on the immune system and epithelial biology and behavior is not known. Manipulating the microbiome via several options might prove to shape or change the primary or metastatic behavior of sporadic CRCs [147,148].

Utilizing sporadic CRC biomarkers will become much more frequent in the future. Limitations of biomarkers for clinical decision making include: (a) sample material may not be available for genetic analysis, (b) additional acquired genetic mechanisms with or without treatment may bypass or trump the predicted effects of a mutation, (c) information on genetic or epigenetic information for validity of a purported biomarker may not have been tested in clinical trials, and (d) biomarker information in a patient might not fully predict what occurs in another patient due to non-tumor mechanisms, such as drug metabolism differences, microbiome makeup, or other factors [134]. These limitations should be addressed with appropriate testing and validation in sporadic CRC patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

CRC	colorectal cancer
EMAST	elevated microsatellite alterations at selected tetranucleotide repeats
MSS	microsatellite stable
MSI-H	high levels of microsatellite instability
LOH	loss of heterozygosity
MSI-L	low levels of microsatellite instability
MSI	microsatellite instability
MMR	DNA mismatch repair
CIMP	CpG Island Methylator Phenotype
CIN	chromosomal instability
GWAS	genome-side association study
SNP	single nucleotide polymorphism
miR	microRNA
UTR	untranslated region
ACF	aberrant crypt focus
IHC	immunohistochemistry
RISC	RNA-induced silencing complex
15-PGDH	15-hydroxyprostaglandin dehydrogenase

Cox-2	cyclo-oxygenase-2
5-FU	5-fluorouracil
RISC	RNA-induced silencing complex

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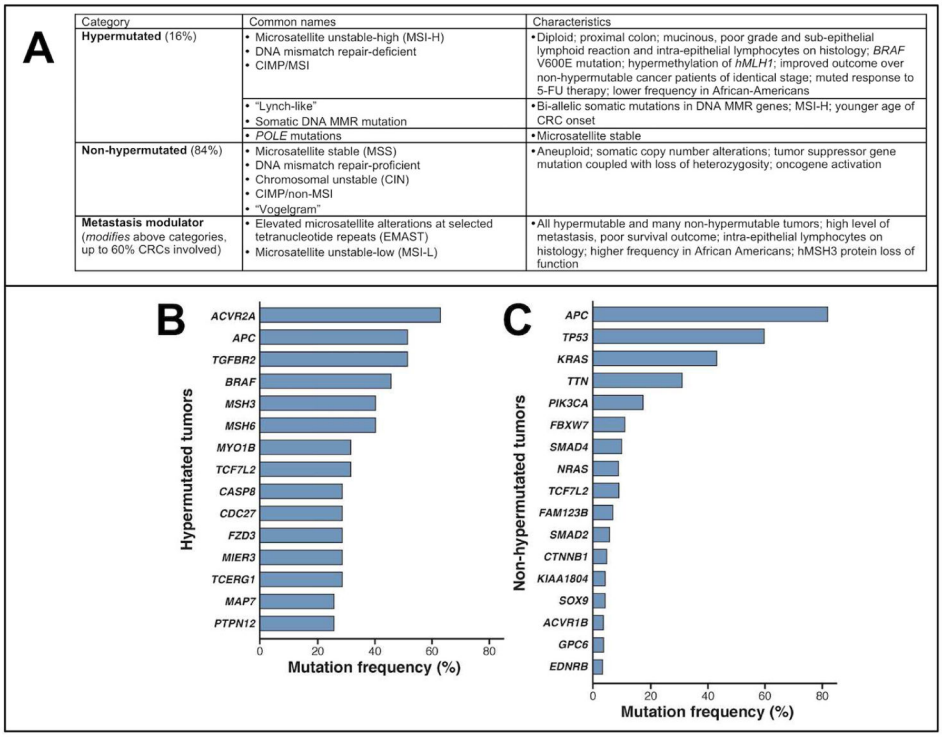

Figure 1.

(***A,B,C***) Mutational landscape and characteristics of sporadic colorectal cancer (CRC). Based on the accumulated number of mutations within a CRC, tumors can be categorized as hypermutated or non-hypermutated. The pattern of gene mutations are different between the two categories, with a few of each as driver mutations in individual cancers. An additional category of "metastasis modulator" caused by EMAST is common after the cancer is formed.

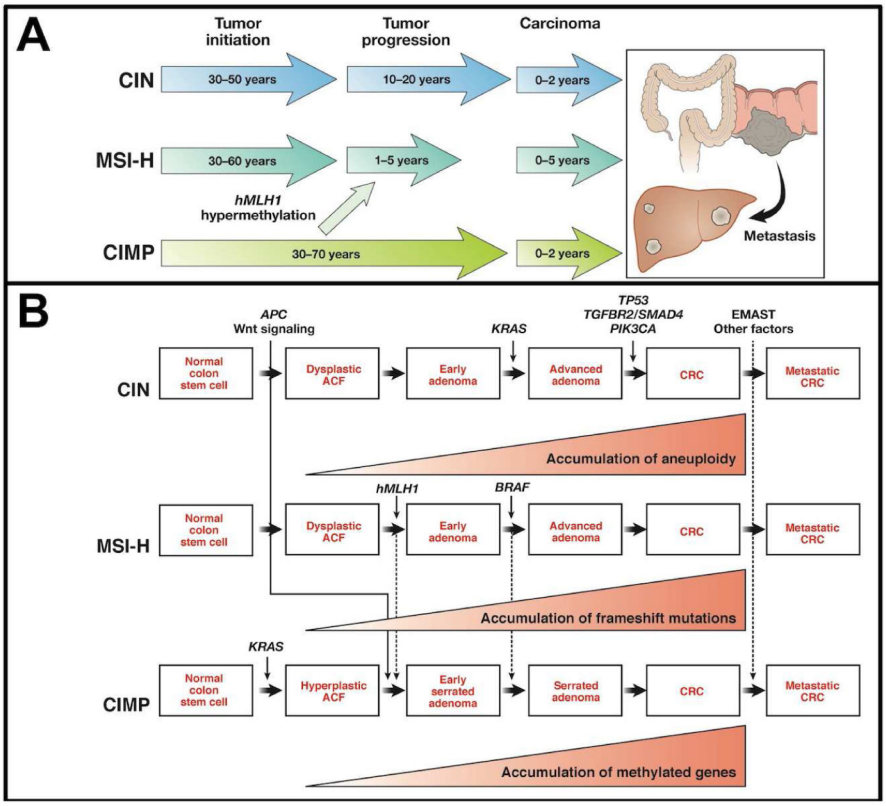

Figure 2.

Timeline for sporadic CRC pathogenesis and its characterized molecular pathways. (***A***) The timelines are based on the average age of CRC for each type. Tumorigenesis can be broken into tumor initiation (development of an adenoma), tumor progression that culminates in a malignancy (carcinoma) that can spread as metastasis. MSI-H tumors are known to have a shortened progression stage. (***B***) Each pathway has its feature of moving from normal to cancer and potentially metastasis, with varying histology. Wnt signaling is the gatekeeper for all 3 pathways. The CIMP pathway contributes to both the MSI-H (through hypermethylation of *hMLH1*) and CIN pathways, and specifically characterizes a serrated pathway. EMAST can modulate all three pathways.

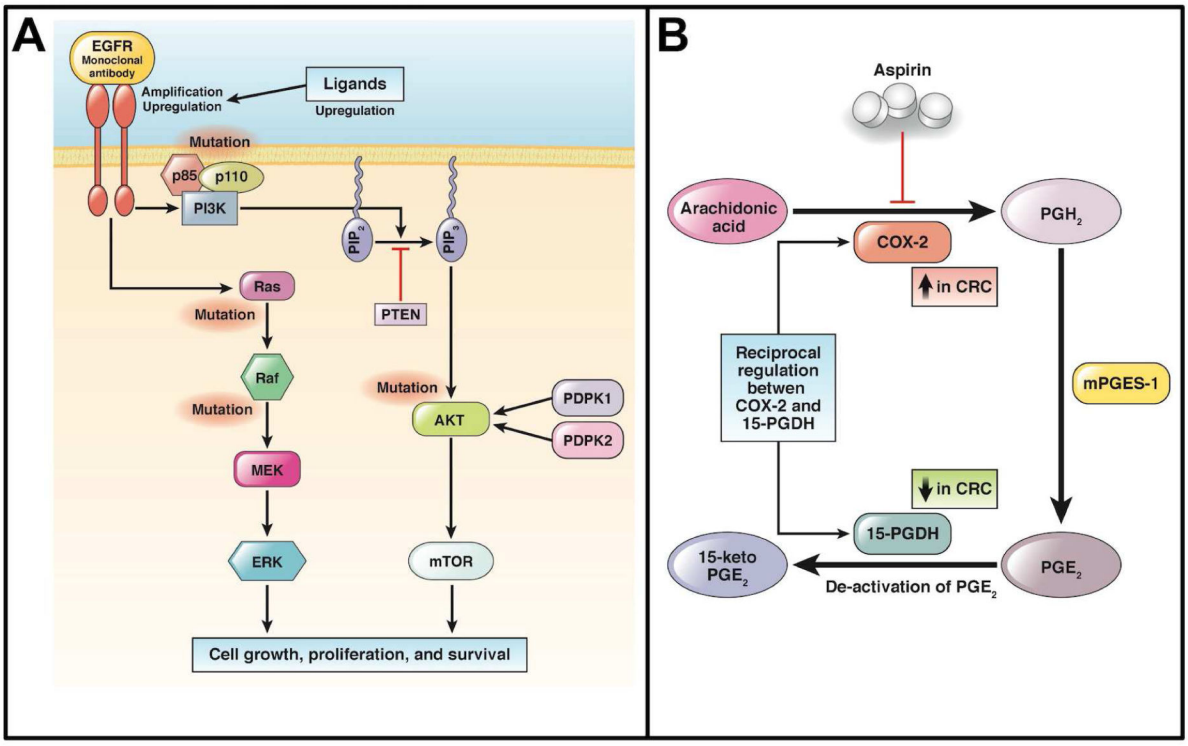

Figure 3.

(***A***) Schematic of the ERBB(EGFR)/KRAS/BRAF/MAPK signaling axis, and its connection with the PI3 Kinase pathway. Common sites for mutation in sporadic CRC are indicated. (***B***) COX and 15-PGDH reciprocal relationship. In sporadic CRCs, Cox-2 is elevated and 15-PGDH is diminished, increasing PGE2 levels that can mediate increased proliferation and angiogenesis. By blocking Cox-2 pharmacologically, and/or selecting patients with high expression of 15-PDGH, levels of PGE2 can be diminished which can help prevent both primary and recurrent CRC.

A

Phenotype	Traditional/CIN	CIN/KRAS	Serrated/CIMP	Serrated/Sporadic CIMP/MSI-H	Familial MSI-H (Lynch)
Cancer Genotype	MSS	MSS/*KRAS* mutant	MSS/*BRAF*^{V600E} mutant	MSI-H/*BRAF*^{V600E} mutant or *hMLH1* hypermethylaton	MSI-H *without* *BRAF*^{V600E} mutant or *hMLH1* hypermethylaton
Frequency among sporadic CRCs	~50%	~33%	~6%	~7%	~4%
Outcome	Reference	Poor 1.5X worse	Poor 1.5-2X worse	Favorable	Favorable 0.3X
Comments	Distal>proximal	Proximal ≅ distal; high representation among African Americans	Proximal>distal, female, older age	Proximal>distal, female, older age	Proximal>distal, young age

B

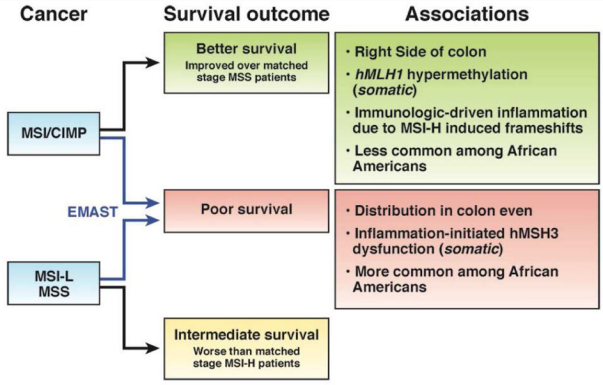

Figure 4.

Outcomes of patients with sporadic CRC. (***A***) Matched phenotype with molecular pathways and patient outcome with sporadic CRC. Data abstracted from references #103 and #104. (***B***) Schematic on patient outcome modulation by EMAST. EMAST does not cause oncogenic transformation, but appears to be induced by inflammation that may modulate metastasis, affecting outcome.

TABLE 1

Genetic loci associated with colorectal cancer risk from various GWAS studies.

Gene(s) at or near locus	Chromosome Locus	GWAS SNP
DUSP10	1q41	rs6687758, rs6691170
LAMC1	1q25.3	rs10911251
NABP1/SDPR	2q32.3	rs11903757
MYNN	3q26.2	rs10936599
FSTL5	4q32.2	rs35509282
PITX1/H2AFY	5q31.1	rs647161
SRSF3/CDKN1A	6p21	rs1321311
TRPS1/EIF3H/UTP23	8q23.3	rs16892766
POU5F1P1, SRRM1P1, MYC	8q24	rs6983267, rs10505477, rs7014346
TPD52L3/UHRF2/GLDC	9p24	rs719725
KRT8P16/TCEB1P3	10p14	rs10795668
ZMIZ1-AS1	10q22.3	rs704017
ABCC2/MRP2	10q24	rs1035209
VTI1A	10q25	rs12241008
TCF7L2	10q25.2	rs11196172
HSPA12A	10q26.2	rs1665650
MYRF, FEN1, FADS1, FADS2	11q12.2	rs174537, rs4246215, rs174550, rs1535
POLD3	11q13.4	rs3824999
LOC120376, FL45803, c11orf53, POU2AF1	11q23	rs3802842
CD9	12p13.31	rs10849432
CCND2	12p13.32	rs3217810, rs3217901, rs10774214
LARP4/DIP2B, ATF1	12q13.13	rs7136702, rs11169552
TBX3	12q24.21	rs59336
BMP4/ATP5C1P1/CDKN3/MIR5580	14q22.2	rs4444235, rs1957636
SCG5, GREM1, FMN1	15q13	rs4779584, rs16969681, rs11632715
CDH1/ZFP90	16q22.1	rs9929218
NXN	17p13.3	rs12603526
SMAD7	18q21	rs4939827, rs7229639
RHPN2	19q13.1	rs10411210
TGFB1, B9D2	19q13.2	rs1800469, rs2241714
BMP2/HAO1/FERMT1	20p12.3	rs961253, rs4813802, rs2423279
HAO1/PLCB1	20p13.3	rs2423279
LAMA5	20q13.33	rs4925386
SHROOM	Xp22.2	rs5934683

TABLE 2

Comparison of some features of MSI-H, CIN, CIMP and EMAST colorectal cancers.

	CIN	CIMP	MSI-H	EMAST
Genomic Instability	Mutation and copy number variation; aneuploid; MSS	Hypermethylation at DNA loci	Microsatellite instability (MSI); diploid	Mostly MSS and MSI-L, includes MSI-H
Germline etiology	Mutation of *APC* in Familial Adenomatous Polyposis	None known	Mutation of DNA MMR gene in Lynch syndrome	None known
Sporadic etiology	Unknown	Unknown	*hMLH1* hypermethylation	Inflammation and loss of function of *hMSH3*
Prevalence in sporadic CRC	~85%	~20%	~15%	Up to 60%
Inflammation	Varied at tumor margin, lamina propria, and intraepithelial locations	Without *hMLH1* hypermethylation: varied	Crohns-like around tumor (tumor margin)	Associated with tumor nests around epithelial components
Immune Reaction	Unknown; varied	Unknown	Neo-peptide driven; favorable	Unknown; unfavorable
Prognosis	Referent	Poor survival without *hMLH1* hypermethylation	Better survival; early stage	Poor survival; later stage
Pathogenesis	Mutation and Loss of Heterozygosity	Without *hMLH1* hypermethylation: unknown	Target gene frameshift mutation; $BRAF^{V600E}$	Combined defect of target gene frameshift mutation and chromosomal instability?
Race	African Americans present younger, more likely have proximal CRC	Unknown	½ frequent in African Americans	Twice as frequent in African Americans
Response to 5-Fluorouracil	Responsive	Without *hMLH1* hypermethylation: responsive	Completely muted	Appears responsive

TABLE 3

Some biomarker examples from sporadic colorectal cancer patients that modify the clinical approach to care. CRC=colorectal cancer.

Biomarker	Material/Tissue	Clinical Approach
NDRG4 and *BMP3* methylation; *KRAS* mutation (NextGen Multitarget v3.0 Fecal DNA Test)	Stool	Adenoma and CRC screening in average risk patients; if positive, perform colonoscopy
KRAS mutation; *BRAF* mutation	Primary CRC (stage IV)	Avoid use of EGFR inhibitors such as cetuximab and panitumab due to resistance in the ERBB/KRAS/BRAF/MAPK axis with mutation
PIK3CA mutation	Primary CRC	Regular aspirin use effective with *PIK3CA* mutation for secondary prevention
15-PGDH expression	Normal colorectal mucosa	Regular aspirin use associated with lower CRC risk with high levels of 15-PGDH
DNA mismatch repair protein expression (or microsatellite instability)	Primary CRC	Absence correlates with microsatellite instability; may identify sporadic vs. Lynch syndrome patient; predicts improved outcome with absence; absence predicts poor response to 5-fluorouracil chemotherapy