



Back to the Colorectal Cancer Consensus Molecular Subtype Future

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Abstract

Purpose of Review This review seeks to provide an informed prospective on the advances in molecular profiling and analysis of colorectal cancer (CRC). The goal is to provide a historical context and current summary on how advances in gene and protein sequencing technology along with computer capabilities led to our current bioinformatic advances in the field.

Recent Findings An explosion of knowledge has occurred regarding genetic, epigenetic, and biochemical alterations associated with the evolution of colorectal cancer. This has led to the realization that CRC is a heterogeneous disease with molecular alterations often dictating natural history, response to treatment, and outcome. The consensus molecular subtypes (CMS) classification classifies CRC into four molecular subtypes with distinct biological characteristics, which may form the basis for clinical stratification and subtype-based targeted intervention.

Summary This review summarizes new developments of a field moving “Back to the Future.” CRC molecular subtyping will better identify key subtype specific therapeutic targets and responses to therapy.

Keywords Consensus molecular subtypes · CMS · Colorectal cancer · RNAseq

Advances Which Have Impacted the Classification of Colorectal Neoplasia

The Data Sets of Life

The central dogma is a biological principle that describes the two-step molecular information transfer involving transcription and translation [1]. This principle involves transcribing DNA information contained in genes that flows through RNA and is translated into proteins: DNA → RNA → protein. Early work on the chemical nature of the substance that transferred the ability to transform pneumococcal subtypes was identified as DNA [2]. The independent functions of DNA versus

protein were discovered sometime later [3]. The distribution density and molecular ratios of nucleotide information present in DNA were then revealed [4]. It was unclear how molecular biologic information was maintained until the proposal of the double helix structure of DNA [5]. It was not until 25 years later that the first sequencing methods emerged [6, 7]. Furthermore, there were no desktop computers to help decipher the complexities of molecular information exchange at that time, and computations were usually done on slide rules.

The informatic focus of those times was on protein. The major data exchange of that day was on 3D X-ray diffraction identification of crystallographic structures. The first of these three-dimensional model of a protein using this technology was the whale myoglobin molecule [8]. The initial sequencing of proteins involved peptide sequencing [9], and the first structure elucidated was the amino-acid sequence in the glycycl chain of insulin [10]. Margaret Dayhoff emerged as the founder of bioinformatics by extensively using computational methods in collaboration with Robert S. Ledley, to apply computing resources to biomedical problems that led to the development of COMPROTEIN [11, 12, 13]. COMPROTEIN was used to elaborate protein primary structure from Edman peptide sequencing data coded on FORTRAN punch cards.

The data sets of life have expanded far beyond the central dogma [14, 15] and may have even expanded into the realm of “Personomics” [16], clearly expanding the data sets taken into

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consideration as part of precision medicine [17•]. We now are considering epigenetic alterations such as DNA methylation defects and aberrant covalent histone modifications that occur in cancer initiation and progression. The extremely high selective pressure brought to bear during natural history of tumor formation are very dynamic. In the case of heterogeneous tumors like CRC, tumor heterogeneity [18, 19, 20•, 21–23] and immunity [19, 24–27] add additional selective pressures. These epigenetic changes are likely to be considered if detectable throughout the cancer continuum including early onset, progression, and ultimately metastasis, therapeutic resistance, and recurrence [19, 20•, 21, 27–31].

The launching of The Human Genome Project (HGP) on October 1, 1990 involved an international collaborative research program designed to generate a complete map of all the genes of human beings. While DNA sequencing [6, 7] and other technologies were initially time consuming and costly, they have progressed over time to be much faster and less expensive [32•]. Then came the early development of RNA sequence-based methods for transcriptome characterization [33]. We now have high-throughput DNA sequencing methodology (next-generation sequencing; NGS) [32•] that has rapidly evolved over recent years with new methods that are continually emerging [34], and we are now entering the third revolution in sequencing technology [35•]. As one example of this progress, digital droplet PCR is advancing our analysis of mutated circulating tumor DNA [36, 37]. As another, single-cell sequencing is helping to elaborate clonal tumor evolution and heterogeneity [38•, 39••, 40].

The Internet, World Wide Web, and Bioinformatics

Advances in molecular biology, sequencing, and computer science set the stage for modern bioinformatics. The parallel emergence of these advances is elegantly detailed elsewhere [41]. The Internet began with the concept of wide area networks (WANs) in computer science laboratories in the USA, UK, and France. This enabled the expansion beyond the local area network (LAN) that interconnected computers within a limited physical site like a laboratory, university campus, or office building. A US Department of Defense contract helped establish Advanced Research Projects Agency Network (ARPANET), an early packet switching network that implemented the protocol suite transmission control protocol/internet protocol (TCP/IP), forming the foundations of the Internet. This allowed end-to-end data transfer and communication across WANs. ARPANET governance was transferred to the National Science Foundation (NSFNET) and then to commercial network providers of the present day, who are connected to one or more of the network access points (NAPs).

Within this same timeframe, Tim Berners-Lee's work at the Conseil Européen pour la Recherche Nucléaire (CERN)

helped create the World Wide Web as a global information exchange system for interlinked data. This made it possible for bioinformatics to advance further with the ability to exchange numerous forms of data and informatics tools [41]. These included the world's first nucleotide sequence database, the European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Data Library, SWISS-PROT, and REBASE [42]. GenBank database also became the responsibility of the National Center for Biotechnology Information (NCBI) and made additional informatics tools publicly available. One of these was the rapid sequence database search tool known as basic local alignment search tool (BLAST) [43]. It was more efficient than the FASTA tool used prior to that. FASTA operated by first rapidly searching for matched data or hash sequence structure followed by applying a dynamic programming algorithm in the same sequence area [44•]. BLAST, by contrast, applied mathematical statistics and the ability to identify structure shared by sequences of high-scoring segment pairs (HSPs) [45]. Current methods using highly sophisticated algorithms and bioinformatic approaches based on NGS are now identifying genomic alterations in human somatic cells, including point mutations, chromosomal rearrangements, gene fusions, epigenetic profiles, and structural variations (SVs) that now provide us with molecular signatures helping to guide precision medicine [46–48] and personalized vaccine development [49].

Early Molecular Signature Efforts of Colorectal Cancer

The prevalence of *ras* gene mutations in human colorectal cancer was first noted by Vogelstein and others [50•]. It was then revealed that the exclusion of the Deleted in Colon Cancer (DCC) gene along with the DCC locus involving a portion of chromosome 18q was accompanied by susceptibility to hereditary nonpolyposis colorectal carcinoma in a kindred analysis [51]. Subsequently, it was revealed that *p53* gene mutations were involved in colorectal neoplasia through inactivation of a tumor suppressor function of the wild-type *p53* gene [52, 53]. The presence and high prevalence of adenomatous polyposis coli (*APC*) mutation in familial polyposis coli (FAP) patients and sporadic CRC was also a key early finding [54•, 55]. These cumulative findings were accompanied by the notion of a progression of accumulated molecular lesions leading to colorectal cancer or the early "Vogelgram" [56•]. Although many of these original findings hold true, a number of advances have been made with the advent of NGS and bioinformatics (Fig. 1).

The Cancer Genome Atlas

Colorectal cancer was one of the many tumor types examined within The Cancer Genome Atlas (TCGA) [57]. The TCGA was established under the purview of a collaboration between

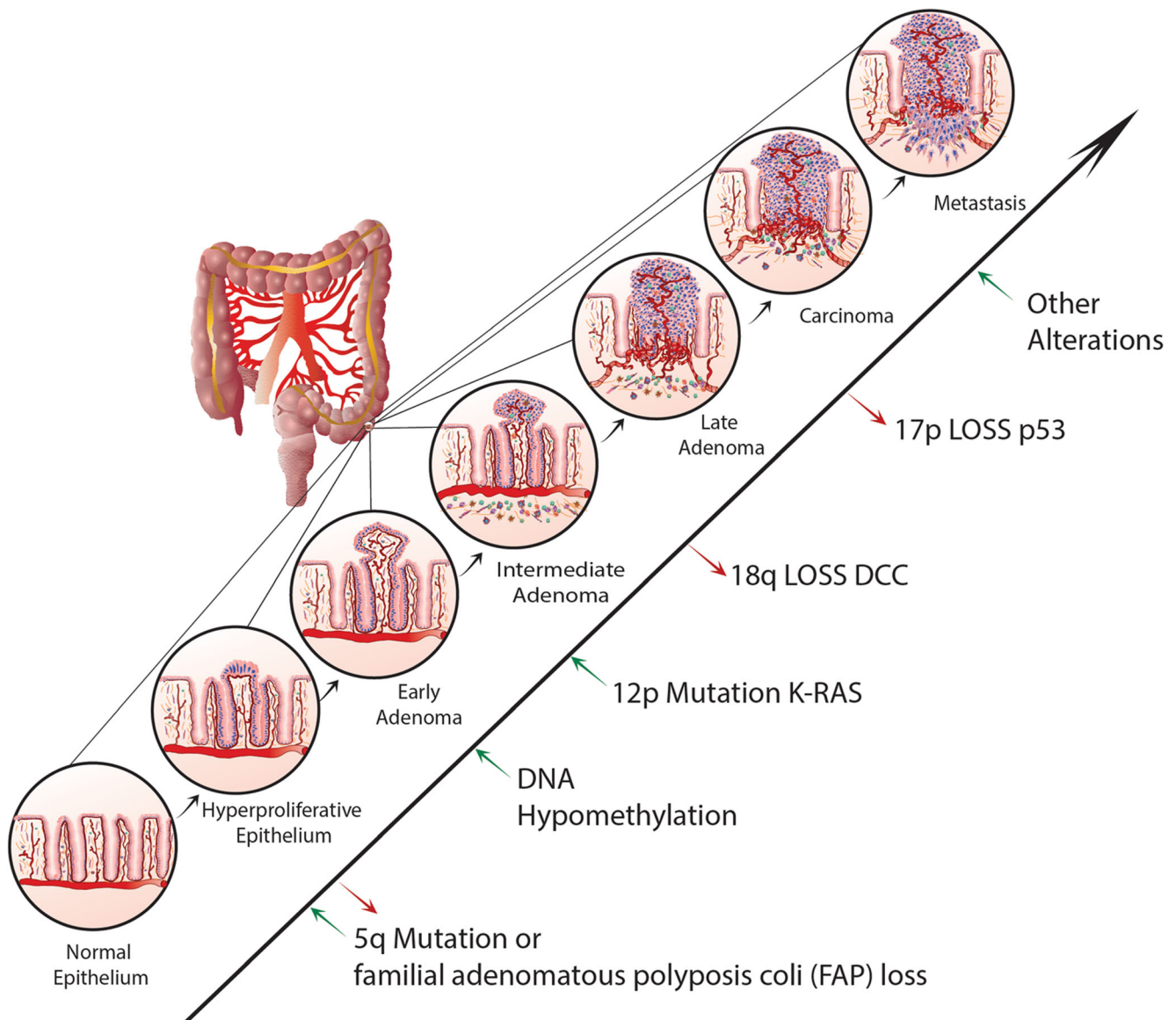


Fig. 1 A genetic model for colorectal tumorigenesis. Adapted from Fearon and Vogelstein Cell, Vol. 61, 759–767, 1990. Tumorigenesis progresses through a series of genetic alterations. These alterations include oncogenes (ras) and tumor suppressor genes (particularly those on chromosomes 5q, 17p, and 18q). Early stages involve 5q mutations or

familial adenomatous polyposis coli loss. Alterations in DNA methylation may follow and then K-ras mutations. Loss of 18q or deleted in colorectal cancer (DCC) follow and then 17p loss (p53). Other alterations may precede metastasis

the National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI). The TCGA was designed to generate comprehensive multi-dimensional data mapping and tools to examine key genomic changes in the analyzed cancer types [58]. The TCGA was scheduled to close, and new genomics initiatives are transitioning to the National Cancer Institute's Center for Cancer Genomics (CCG).

The colorectal TCGA was designed to characterize somatic alterations in colorectal carcinoma. It was conducted on 276 CRC samples [59••]. It analyzed multiple sets of data, including exome sequence, DNA copy number, promoter methylation, messenger RNA, and microRNA expression.

Ninety-seven of these CRC samples were subjected to using low-depth-of-coverage whole-genome sequencing. Of these colorectal carcinoma samples, 16% were found to be hypermutated and three-quarters of these had high microsatellite instability associated with hypermethylation and MLH1 silencing. One quarter exhibited somatic mismatch-repair gene and polymerase ϵ (POLE) mutations.

With the exception of hypermutated tumors, CRC displayed consistent subtype signature patterns of genomic alteration. Significantly mutated genes included APC, TP53, SMAD4, PIK3CA, and KRAS as might be expected, along with those in SOX9, FAM123B, and ARID1A. Copy-number

changes included amplifications ERBB2 and IGF2. Chromosomal translocation patterns included a fusion between NAV2 and TCF7L1. MYC-directed transcriptional activation or repression was also observed in the initial TCGA report [59••].

A number of subsequent studies identified certain molecularly similar subgroups in CRC. In one study, CRC-intrinsic deficient mismatch repair and epithelial-to-mesenchymal transition subtypes were shown to predict chemotherapy benefit [60]. In another study, CRC gene expression subtypes were identified including surface crypt-like, lower crypt-like, CIMP-H-like, mesenchymal, and mixed [61]. In a separate study, genome-wide mRNA expression subtypes were matched to pharmacologically characterized cell line panels to help determine their potential to develop targeted therapies for defined CRC patient sub-populations [62]. Additional efforts revealed three subtypes with improved disease-free survival (DFS) after surgical resection to potentially spare patients from adverse chemotherapy effects when disease was localized [63]. Within these subtypes, a filamin A expression pattern failed to respond to cetuximab, but based on a cMET subtype, receptor tyrosine kinase inhibitors were proposed for potential efficacy [63]. In separate analyses, chromosomal-unstable and microsatellite-unstable cancers were among two molecular subtypes identified, while a third subtype was largely microsatellite stable and contains relatively more CpG island methylator phenotype-positive carcinomas that could not be further separated based on characteristic mutations [64]. Within the same time frame, a classification of CRC into six molecular subtypes was identified, which were hypothesized to arise through distinct biological pathways [65]. Similarly, studies that applied hierarchical clustering identified four robust tumor subtypes with biologically and clinically distinct behavior. These clusters were separated into (1) stromal components, (2) nuclear beta-catenin, (3) mucinous histology, and (4) microsatellite-unstability and BRAF mutations [66]. These studies that identified molecular similarities in certain CRC subtypes were ultimately clarified within our large international consortium, formed to resolve reported subtyping inconsistencies [67••].

Consensus Molecular Subtypes (CMS Classification)

Six groups reporting gene expression-based CRC classifications formed a unique, international consortium that examined shared, large-scale data, and analytics across the expert groups [67••]. This consortium revealed marked interconnectivity among six independent classification systems that ultimately coalesced into four consensus molecular subtypes (CMSs) with distinguishing mRNA expression along with distinct molecular and clinical features (Fig. 2). Samples were primarily Stage II and III tumors with some normal samples for

comparison. Of the four subtypes, CMS2 was named the canonical subtype that made up 37% of all clusters observed and exhibited epithelial characteristics with marked WNT and MYC activated pathway signaling. CMS1 by contrast made up 14% of the categorized molecular clustering and displayed microsatellite instability along with significant immune activation and hypermutated features. The CMS3 subtype made up 13% of the molecular clustering and showed features of epithelial and metabolic dysregulation. The CMS4 subtype was found in 23% of the molecular clusters with epithelial mesenchymal transformation (EMT) characteristics accompanied by prominent stromal invasion and angiogenesis, hallmarked by transforming growth factor- β (TGF- β) activation. The remaining 13% of tumors had molecular features that were mixed and are thought to reflect a transition phenotype or intratumoral heterogeneity, typically with characteristics of multiple CMS. These CMS group classifications are considered the most robust system currently available for CRC that maintain clearly distinct molecular features connected to biological and clinical stratification, which serve as a framework for molecularly targetable interventions. Close collaborations among basic researchers, bioinformaticians, and clinicians may be critical for meeting the challenges of integrating CRC subtyping into routine clinical practice [68], with the goal of maximizing therapeutic response and minimizing adverse side effects for each patient [69].

Continued Molecularly Targeted Mutation and Biomarker Efforts

Since the initial CMS publication, there have been a number of reports focusing on biomarker study designs for individual standard molecular targets such as RAS [70–79] and BRAF [70, 76, 80–82]. Loss of CDX2 has also been paired with BRAF as a criterion for early-stage patients not qualifying for chemotherapy to be reconsidered for such treatment [83]. Separate molecular profile efforts have also focused on EGFR [84–91]. Mutation analysis has also evolved to enable tracking of circulating, free tumor DNA (ctDNA) mutations [84, 89, 92, 93]. The use of patient ctDNA has enabled ~real-time detection of acquired resistance to anti-EGFR therapy in a phase 2 randomized clinical trial [84]. Continued development of molecular tools has enabled additional characterization of ERBB2/ERBB3 in CRC, leading to identification of associations with MSI and co-occurring PIK3CA mutations [93]. These findings may inform therapeutic strategies in the setting of ERBB2/ERBB3 mutations, highlighting the potential clinical impact of molecular tool development.

Continued Consensus Molecular Subtype Efforts

Classically, CRC diagnosis and prognostic stratification are based on histopathologic assessment of cell or nuclear

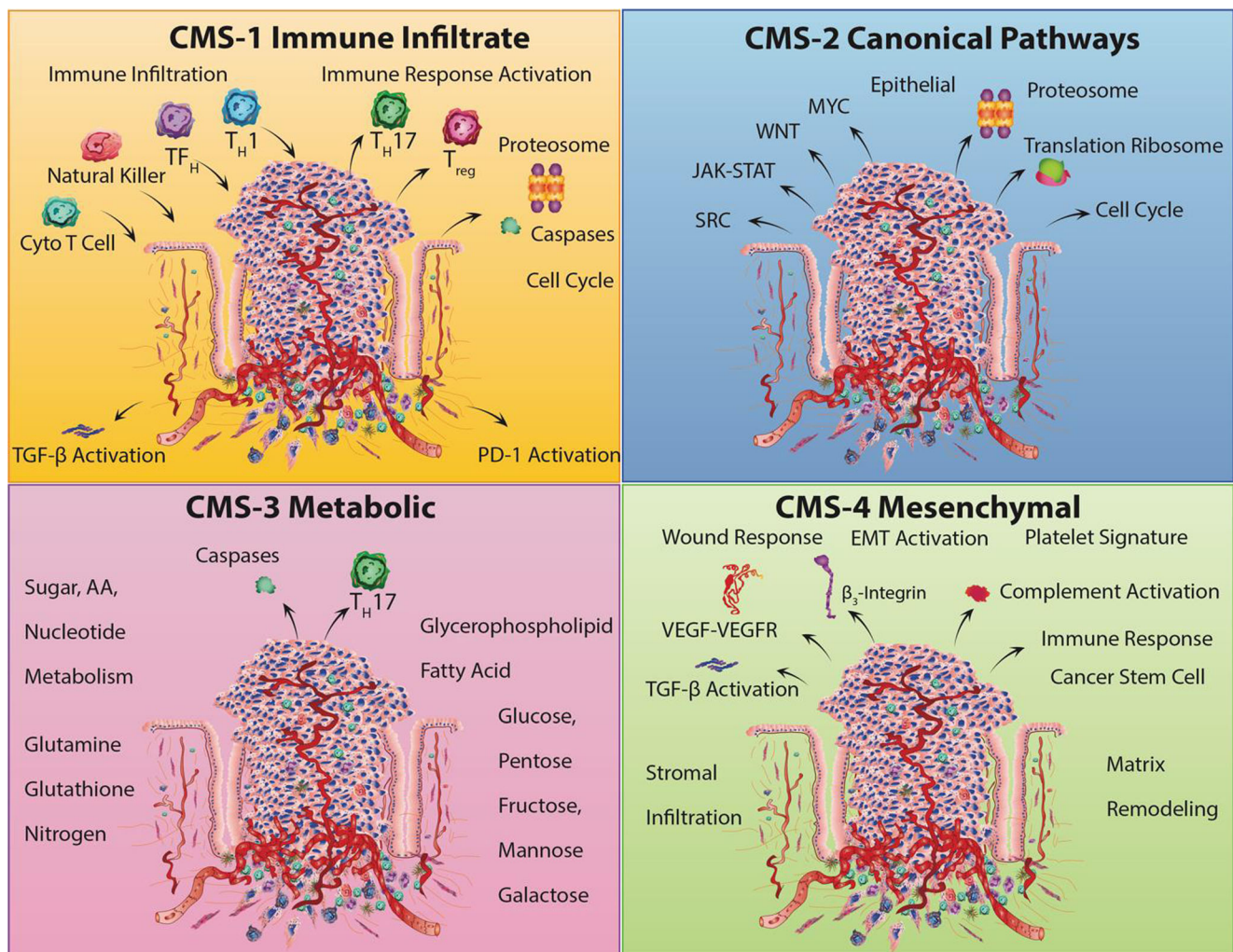


Fig. 2 The consensus molecular subtypes. CMS1 displays subtype clusters that involve hypermutation and microsatellite instability along with increased immune cell infiltrates consisting of Th1 lymphocyte, cytotoxic T cell, NK cell infiltration, and upregulated immune checkpoints such as PD-1. CMS2 clustering involves the upregulation of canonical pathways including WNT and MYC downstream targets. CMS3 clustering is defined by dysregulation of metabolic pathways

including carbohydrate and fatty acid oxidation and the loss of T_H17 cells. CMS4 clustering is referred to as a mesenchymal subtype that involves the upregulation of EMT pathways. CMS4 clustering also shows elevated TGF-β signaling, matrix remodeling, angiogenesis, complement activation as well as integrin-β3 upregulation, stromal infiltration, immune upregulation, and platelet signatures

pleomorphism, aberrant mitotic figures, altered glandular architecture, and other phenomic abnormalities [94]. From this standpoint, complexity may involve oncogenic perturbation of spatiotemporal signaling, leading to disruption at multiple levels of tissue organization. Tumor complexity can extend to morphologic plasticity based on a single molecular signature that generates heterogeneous cancer phenotypes. In contrast, morphologically homogeneous tumors can exhibit substantive molecular diversity. When considering a signaling pathway-based or mechanistic interpretation of omics data in a setting of cancer pathology, CRC-CMS stratification is expected to provide clarity for clinical decision making. In one cohort study involving 608 patients, tumor sidedness was evaluated in relationship to CMS [95]. In this study, the prevalence of TP53, KRAS, BRAFV600, PIK3CA, SMAD4, CTNNB1,

GNAS, and PTEN mutations differed by side and location. Within this context, transverse colon tumors had mutation profiles that more closely resembled left-sided tumors, suggesting that prognostically, transverse and left-sided tumors should be combined, keeping right-sided tumors separate. CMS prevalence also varied by colon location with CMS1 and CMS3 prevalence decreasing and CMS2 prevalence increasing moving from proximal to distal colon. This study also found that the sigmoid-rectal region appears unique and the transverse colon is distinct from other right-sided locations.

In another study, TGFβ signaling was observed to direct serrated adenomas to the mesenchymal colorectal cancer subtype, CMS4 [96]. This study also showed that TGFβ signaling was elevated in a genetically engineered organoid culture

carrying a BRAF(V) (600E) mutation, constituting a model system for sessile serrated adenomas. In a separate study, SMAD4, a key mediator of TGF β signaling, mutation was found to be independently associated with worse outcomes among patients undergoing resection of colorectal liver metastasis [97]. Another study was able to demonstrate that TGF-beta was a classic EMT inductor, causing upregulation of ANXA2 and internalization of both E-cadherin and ANXA2 in CRC cells [98]. This same study revealed that ANXA2 silencing was able to reduce TGF-beta-induced invasiveness, and inhibitors of the Src/ANXA2/STAT3 pathway were able to reverse EMT. Stromal involvement is significant in CMS4 and in one study of colorectal peritoneal carcinomatosis, primary tumors were found to have high stromal content and CMS4 biology [99]. The authors of this study went on to suggest that patients with colorectal peritoneal carcinomatosis may benefit from therapies targeting tumor-stroma interaction in addition to cytoreductive surgery and hyperthermic intraperitoneal chemotherapy treatments [99]. Furthermore, the additional knowledge of somatic mutations may guide the use of preoperative therapy, extent of surgical margin, and selection for ablation [100].

When examining miRNA signatures, other CMS4-related studies showed that the miR-200 family, which negatively regulates EMT, was significantly under-expressed in CMS4 tumors, revealing an additional aspect of CMS4 biology [101]. Studies on the CMS paradigm have also evaluated the influence of multiple parameters, such as the origin, developmental route, and micro environmental regulation influence on CMS [102]. One study utilized a multi-omics approach to examine 34 commonly used CRC cell lines, categorizing them into the four CMS subtypes [103]. This study annotated CMS along with MSI, MSS, POLE, and CpG island methylator phenotype (CIMP) status in this cell line panel. Epigenetics can have additional phenotypic impact on CIMP tumors and comprise 20% of colorectal cancers, and associations have been established with female sex, age, right-sided location, and BRAF mutations [104]. In general, the morphologic appearance of cell lines in CMS1 and CMS4 was mesenchymal, whereas CMS2 and CMS3 cell lines were more epithelial-like. In another study, the presence of KRAS mutations was found to be independently associated with a reduction in immune infiltrates and reactivity in CRC, but the extent of this effect varied by CMS [71]. Another study found that CMS might serve as a predictive factor for the efficacy of chemotherapy against mCRCs [105]. Gene expression analyses have also shed further light on distinct CMSs that are differentially distributed between right- and left-sided CRCs [106]. This study also revealed that greater proportions of the “microsatellite unstable/immune” CMS1 and the “metabolic” CMS3 subtypes are found in right-sided colon cancers.

Preclinical CMS Progress

Preclinically, CRC cell lines, primary cultures, and patient-derived xenografts (PDX) were examined and found robustly assigned to one of the four CMSs, independent of the stromal contribution [107]. In this same study, CMS stratification was examined by functional analyses, identifying mesenchymal enrichment in CMS4 and metabolic dysregulation in CMS3. This study also found an association with sensitivity to chemotherapy-induced apoptosis prevalent in CMS2 and CMS4, which correlated with delays in outgrowth of CMS2, but not CMS4 xenografts. A separate preclinical study found that tumor cell proliferation was associated with successful PDX establishment and was able to distinguish patients with poor clinical outcomes within CMS2 [108]. In another preclinical study, a CMS4-like inducible mouse model was also generated based on a Kras mutant allele and conditional null alleles of Apc and Trp53 (iKAP), providing a potential genetically engineered mouse model of CMS4-like CRC [74]. The advantages, disadvantages, and challenges associated with using PDXs in the identification of targets and drug testing were recently summarized in a Nature feature [109].

Improving the CMS Classifier

Other studies have sought to improve upon the CMS classifier for more refined prognosis predictions [110]. There have also been suggestions that CMS be used to guide precision treatment of CRC, necessitating a high degree of confidence in the CMS classification method [111]. One group retrospectively evaluated CMS as a prognostic factor for stage III CRC patients treated with FOLFOX adjuvant chemotherapy, finding that CMS was predictive in these patients [112]. Another study developed an immunohistochemical-based classifier containing four specific staining profiles involving FRMD6, ZEB1, HTR2B, and CDX2 in combination with cytokeratin [113]. Based on the great potential of CMS clinically and the need for a high degree of confidence in CMS classification, other groups including ours (unpublished results) have continuing, dedicated efforts to improve the CMS classifier, such as CMScaller [114].

CRIS

A separate classifier was recently developed, focusing on a CRC intrinsic signature (CRIS), to cluster samples by patient-of-origin rather than region-of-origin [115]. This classifier was generated only using PDX tissue, thereby limiting the data to only samples that successfully establish these xenografts from patient samples. The purpose was to emphasize the potential of cancer-cell intrinsic signatures to reliably stratify CRC patients by minimizing the confounding effects of stromal-derived intratumoral heterogeneity (ITH). This study

involved 75 RNA transcription profiles, 25 patients' samples at three regions per sample, namely the invasive front (IF), central tumor (CT), and lymph node (LN). The CRIS were determined using the nearest template prediction (NTP) classifier. Cell-type-specific signatures included epithelial, leukocyte, fibroblast, and endothelial cells. CRIS concordance was broken down into CRIS. A through E and patient clustering for CT and IF was at 92% (22/24 samples). In another study by the same group that compared CMS to CRIS using multiple sampling method approaches, the authors concluded that CRIS provides more spatially and temporally robust classification of molecular subtypes compared to CMS [116]. This group combined CRIS transcriptional subtyping and CD8 immunohistochemistry to identify poor prognosis stage II/III colorectal cancer patients who were able to benefit from adjuvant chemotherapy [117].

Immune-Related Consensus Molecular Subtype

Prior to the CMS report, an immune signature called the co-ordinate immune response cluster (CIRC) was proposed involving 28 genes coordinately regulated across CRC patients [118]. Four patient groups were identified by this method. Group A was heavily enriched for patients with microsatellite instability (MSI-H) and POL mutations, and had high CIRC expression, including several inhibitory molecules: CTLA4, PDL1, PDL2, LAG3, and TIM3. RAS mutation by contrast was enriched in patient groups with lower CIRC expression. RAS mutant tumors predicted a relatively poor immune infiltration and low inhibitory molecule expression.

In the case of CMS-directed studies, by contrast, stratified analyses revealed that chemokine-like factor (CKLF) was a potential prognostic marker in the MSI-immune consensus molecular subtype CMS1 [119]. Heterogeneity in immune function in relation to CMS has also been examined in a study of CRC genome-wide expression datasets, including 1597 tumors and 125 adjacent normal colon tissues [120]. CRC clusters were identified using a combination of multiple clustering algorithms and multiple validity metrics. The CIBERSORT algorithm was used to compute relative proportions of 22 human leukocyte subpopulations across CRC and normal colon tissue, identifying five clusters of tumor immune infiltrate (COMMUNAL clusters). Distribution of these clusters was then assessed by CMS, finding that four of the clusters overlapped significantly with the four CMSs. Additional analysis identifying differential expression specific to tumor epithelial cells was able to characterize mechanisms of tumor escape from immune surveillance occurring in particular CRC clusters. Common and cluster-specific influx of immune cells into CRCs was found along with several

deregulated gene targets to help improve of immunotherapeutic strategies in CRC. In a separate, prospective cohort of 1265 patients with stage II/III cancer, TIL/MMR status and BRAF/KRAS mutations were examined along with CMS status on 142 cases [121]. These authors identified that associations with 5-year disease-free survival (DFS), which were evaluated and validated in an independent cohort of 602 patients and concluded that TIL/MMR subtyping was superior compared with histopathological, genomic, and transcriptomic subtypes [121]. Platelets are derived from immune lineage megakaryocytes and may also impact tumor CMS [122].

CMS and the Microbiome

There have also been reports focused on the microbiome patterns that are associated with CMS [123]. In this study, CRC subtypes were identified in 34 samples using RNA-sequencing-derived gene expression in concert with the relative abundances of bacterial taxonomic groups using 16S rRNA amplicon metabarcoding. 16S rRNA analysis revealed the enrichment of Fusobacteria and Bacteroidetes, and decreased levels of Firmicutes and Proteobacteria in CMS1. Further analysis of bacterial taxa focused on non-human RNA-sequencing reads uncovered distinct bacterial communities associated with each molecular subtype. The most highly enriched species associated with CMS1 included *Fusobacterium hwasookii* and *Porphyromonas gingivalis*. CMS2 was enriched for *Selenomas* and *Prevotella* species, while CMS3 had few significant associations. Targeted quantitative PCR validation has also been done, showing an elevation of *Fusobacterium nucleatum*, *Parvimonas micra*, and *Peptostreptococcus stomatis* in CMS1. These results reflected that *Fusobacterium* was associated with a CRC subtype characterized by CpG island methylation, MSI and inflammatory signatures, and higher prevalence in right-sided tumors. These authors also noted the concept of bacterial biofilms as initiators of CRC that may facilitate microbial invasion of the mucous layer [124].

CMS-Related Drug Development and Treatment: "Back to the Future"

Revealing that there are multiple molecular subtypes in a sense is a game changer for the CRC field. If one considers drug development for example, the assumption that this was a single disease that progressed through a common series of molecular steps or accumulation of mutations is rapidly changing. Take the four CMS described to date for the sake of argument. Recall that we now know that there are four CMSs with identifiable features that separate CMS1 (MSI immune, 14%), hypermutated, microsatellite unstable, and strong immune activation; CMS2 (canonical, 37%), epithelial,

marked WNT, and MYC signaling activation; CMS3 (metabolic, 13%), epithelial, and evident metabolic dysregulation; and CMS4 (mesenchymal, 23%), prominent TGF- β activation, stromal invasion, and angiogenesis. Suppose we have a drug targeting the metabolic pathways inherent to CMS3 that lead to a response rate of 50% in this subgroup and greatly enhanced progression free and overall survival, this would be considered an unmitigated success. However, absent knowledge of the CMS, had this drug been tried on the entire CRC population, may only have shown a 6.5% response rate assuming that its effects were CMS3 specific, and this drug would likely have been discarded as ineffective for CRC. The identification of molecularly distinct subtypes can provide distinct subsets of patients on which to test new treatments as well as clues for specific biological pathways to target for these subgroups. In a sense, we seem to find ourselves at a “Back to the Future” crossroads that could be expected to result in the reevaluation of many drugs in CMS-related cell lines or PDX models based on molecular changes of a given CMS in response to drug. This would include assessments prior to, following, and in resistance to treatment settings. The same would seem to apply to tumor biomarker and circulating biomarker analysis and assay development. This potential future would enable us to stratify our treatments based on CMS-related analyses, not exposing CRC patients to toxic or targeted drugs from which they will derive no benefit. Such a future would have a higher probability of developing more precise treatments. A future where NGS and other advanced technologies combine with bioinformatics to serve as “Doc’s” time traveling DeLorean. A future where the McFly’s time continuum is selected for the betterment of the CRC field... and Biff’s?well you know.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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.

Abbreviations CMS, consensus molecular subtype; MSI, microsatellite instability; NGS, bioinformatics, biostatistics, colorectal cancer, targeted therapy, precision medicine

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- Of importance
- Of major importance

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