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Mini review

Consensus Molecular Subtypes of Colorectal Cancer and their Clinical Implications

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The colorectal cancer (CRC) subtyping consortium has unified six independent molecular classification systems, based on gene expression data, into a single consensus system with four distinct groups, known as the consensus molecular subtypes (CMS); clinical implications are discussed in this review based on articles relevant to the CMS of CRC indexed in PubMed as well as the authors' own published data. The CMS were determined and correlated with epigenomic, transcriptomic, microenvironmental, genetic, prognostic and clinical characteristics. The CMS1 subtype is immunogenic and hypermutated. CMS2 tumors are activated by the WNT-β-catenin pathway and have the highest overall survival. CMS3 feature a metabolic cancer phenotype and CMS4 cancers have the worst survival and have a strong stromal gene signature. The CMS of CRC may better inform clinicians of prognosis, therapeutic response, and potential novel therapeutic strategies.

Keywords: Colorectal cancer, molecular subtypes, clinical, classification

Although colorectal carcinoma (CRC) remains the third most commonly diagnosed cancer in America with over 135,000 new cases expected in 2017 (1), mortality from CRC has fallen by more than half since 1975 (2), in part, due to dietary modifications, medical prevention, surveillance of high-risk subpopulations, improved surgical care, and treatment with molecularly targeted systemic therapies. However, CRC remains the second most lethal cancer (following lung cancer) with approximately 50,000 CRC-related deaths expected for 2017 (1), highlighting the continued need to study predictive markers for response to available and emerging therapies.

The standard American Joint Committee on Cancer staging system (3) provides prognostic information to help with the clinical management of patients with CRC. Early disease lacking regional lymph node involvement generally is managed with surgical extirpation alone. Cancers deemed to be "high risk" for metastasis or cancers that have metastasized to the regional lymph nodes (high-risk stage II or stage III, respectively) are offered adjuvant systemic therapies for potential survival benefit. Stage IV cancers are offered resection, if appropriate, combined with systemic therapy with the hope of extended progression-free survival. Varied response rates to standard therapeutic regimens suggest that the disease collectively known as "CRC" is molecularly heterogeneous,

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with varying tumor biologies.

Comprehensive genomic analyses have demonstrated that individual CRCs are unique, with a median of 76 non-silent mutations each (4). In an effort to correlate cancer cell phenotype with clinical behavior and guide rational treatment with specific targeted therapies, the CRC subtyping consortium unified six independent molecular classification systems (5-10), based on gene expression data, into a single consensus system with four distinct groups, known as the consensus molecular subtypes (11) (Table 1), with further elaboration based on epigenomic, transcriptomic, microenvironmental, genetic, and clinical characteristics of the tumors. In this review, we will discuss the clinical, prognostic, and treatment implications of these consensus subtypes.

Consensus molecular subtypes (CMS) of colorectal cancer

The six investigative groups within the consortium previously had independently developed subtyping algorithms; in the development of the CMS classification system (11), the different CRC datasets were normalized from the raw formats and using a network-based approach, four subtypes were identified. Approximately 87% of the 4,151 normalized samples from the collaborative efforts of the six groups could be assigned to a CMS, leaving 13% of colorectal cancers molecularly "unclassified". Additional molecular information, including mutations, somatic copy number alterations, promoter methylation status, and post-translational gene regulation, as well as biological characteristics were correlated with the subtypes. Clinical analyses identified significant differences between subtypes in location, gender, histopathological grade and stage at diagnosis, as well as prognostic endpoints such as disease free survival (DFS), relapse free survival (RFS) and survival after relapse (SAR). No subtype was defined solely by a genetic aberration; for example, an activating mutation in the *KRAS* proto-oncogene can be found across all CMS subtypes. Similarly, wild-type *KRAS* can also be found across all four subtypes.

CMS1

CMS1 precursor lesions are also known as serrated polyps. The serrated pathway to carcinoma is characterized by: 1) proximal colon location, 2) high *BRAF*^{V600E} mutation rate, 3) hypermethylation of CpG islands, which causes loss of tumor suppressor function (CpG island methylator phenotype [CIMP]), 4) an association with an impaired DNA mismatch repair (MMR) system, and 5) the infiltration of immunogenic lymphocytes in the tumor microenvironment. Mutations or hypermethylation of the promoter regions of the MMR genes cause microsatellite instability (MSI). MSI cancers are also considered "hypermutated" with approximately 47 mutations per 10^6 bases, compared to microsatellite stable (MSS or CMS2) tumors which average $2.8/10^6$ bases (12). MSI tumors can be sporadic (~12% of all CRC) or hereditary (~3%, Lynch syndrome) (13).

Clinical Implications

Patients with early stage MSI tumors (most CMS1 cancers) have a better prognosis compared to patients with microsatellite stable (MSS) tumors (14). Stage II cancers with MSI have a low recurrence rate and thus are generally not considered for adjuvant chemotherapy. Patients with stage III MSI tumors do not benefit from fluorouracil monotherapy (15) but are responsive to combination fluorouracil, leucovorin, and oxaliplatin adjuvant chemotherapy (FOLFOX) (16). CMS1 tumors have a favorable outcome when detected before disease dissemination (13). In part, the good prognosis may be linked to the presence of specific T-cell populations: $CD8^+$ cytotoxic T lymphocytes, $CD4^+$ activated type 1 T helper cells (Th1), and natural killer cells. However, CMS1 tumors were associated with worse survival after relapse (11, 17).

Due to the strong immunogenicity of these tumors, immunomodulation using checkpoint inhibitors are currently in early clinical trials for advanced disease (18). Cancer cells exploit a

Table 1. Consensus molecular subtypes of colorectal cancer (1, 20, 21)

	CMS1	CMS2	CMS3	CMS4
Alternate name	Microsatellite Instability Immune	Canonical	Metabolic	Mesenchymal
Primary characteristics	Hypermutated, microsatellite unstable and strong immune activation	Epithelial, marked WNT and MYC signaling activation	Epithelial and evident metabolic dysregulation	Prominent TGF–β activation, stromal invasion and angiogenesis. +/- WNT
Incidence	14%	37%	13%	23%
Genomic associations	MSI, high mutation count, low copy number	Chromosomal instability (CIN), low-moderate mutation count and copy number	CIN, moderate mutation count, low-moderate copy number	CIN, low mutation count, high copy number
Precursor lesions	Serrated (low TGFβ microenvironment)	Tubular adenoma	Tubulovillous adenoma with serrated features (21)	Serrated (high TGFβ microenvironment)
Epigenomic associations	High methylation	Low methylation	Moderate methylation	Low methylation
Transcriptomic pathways	Immune activation, JAK-STAT activation, Caspases	WNT targets, *MYC* activation, *EGFR* or *SRC* activation, *VEFG* or *VEGFR* activation, Integrin activation, *TGFB* activation, *IGF* and *IRS2* activation, *HNF4a*, *HER2* and cyclin upregulation	DNA damage repair, Glutaminolysis, lipidogenesis, cell cycle	Mesenchymal activation, complement activation, immunosuppression, integrins
Stroma-immune microenvironment	Few CAF, highly immunogenic, large immune infiltrate, tends towards adaptive immune response	Very few CAF, poorly immunogenic, tends toward innate immune response	Few CAF, highly immunogenic, tends toward adaptive immune response	Many CAF, inflamed, tends toward innate immune response, epithelial to mesenchymal transition
Associated mutations	*MSH6*, *RNF43*, *ATM*, *TGFBR2*, *BRAF*, *PTEN*	*APC*, *KRAS*, *TP53*, *PIK3CA*	*APC*, *KRAS*, *TP53*, *PIK3CA*	*APC*, *KRAS*, *TP53*, *PIK3CA*
Clinical associations Histopathologic associations	Solid, trabecular, mucinous features	Tubular	Papillary	Prominent desmoplasia, stroma
Age (years)	69	66	67	64
Sex	44% M, 56% F	58% M, 42% F	53% M, 47% F	55% M, 45% F
Location	Proximal	Distal	Mixed	Distal
Stage at diagnosis (%)				
I	12	13	17	8
II	44	40	41	33
III	40	39	37	47
IV	4	8	5	12
Grade (%)				
1	15	22	20	9
2	40	73	68	72
3	45	5	12	19

survival mechanism of self-tolerance by expressing the protein programmed death ligand-1 (PDL-1) when bound to T lymphocyte cell-surface receptors called programmed death-1 (PD-1). Inhibitors of

PD-1 (e.g., nivolumab, pembrolizumab) or PDL-1 (durvalumab) are immunostimulatory, increasing the ability of T-cells to recognize tumor cells and destroy them.

CMS2

CRC in the CMS2 category arises from the canonical adenoma-to-carcinoma sequence (19). This gene expression profile is consistent with a differentiated epithelial cell phenotype, typically characterized by the initial loss of tumor suppressor gene *APC*, followed by an activating mutation in *KRAS* and loss of *TP53*. CMS2, 3, and 4 tumors demonstrate high degrees of chromosomal instability (CIN), with losses and/or gains of large portions of chromosomes, loss-of-heterozygosity, and aneuploidy (20). CMS2 and 4 exhibit high somatic copy number alterations, a specific type of chromosomal rearrangement that could include base pair replications or deletions. CMS2 cancers were found to have more frequent copy number gains in oncogenes and copy number losses in tumor suppressor genes. Relative to CMS1, CMS2 cancers had a low mutation rate (defined as non-hypermutated, or <8 mutations per 10^6 bases) (12). One interesting finding revealed by the cancer genome atlas network is that *APC* and *TP53* were relatively less mutated in hypermutated CRC (i.e., CMS1) consistent with the current CMS categories. CMS2 tumors have activated WNT-β catenin and MYC signal transduction pathways.

Clinical implications

Approximately 39% of CMS2 cancers are stage III at the time of diagnosis and treatment and standard adjuvant chemotherapy is recommended for stage III. Five-year overall survival for all stages of CMS2 are the highest of any subtype at 77%, compared with 73%, 75% and 62%, respectively for CMS1, 3 and 4 (11). Additionally, CMS2 cancers were more commonly left sided lesions (59%), with higher survival rates after relapse (35 months); these characteristics are in contradistinction with CMS1 tumors, which are more prevalent in the right colon and exhibit poor survival after relapse (9 months) (11).

CMS3

CMS3, also known as the metabolic subtype, has genomic features consistent with CIN, but has relatively low somatic copy-number alterations (SCNAs) compared with CMS2 or 4. CMS3 also had more MSI than CMS2 and 4 (CIMP-low, intermediate hypermethylation). Approximately 30% CMS3 tumors are considered hypermutated (less common than CMS1 tumors, but more than CMS2 or 4 type tumors). Although *KRAS* mutants were present in every molecular subtype, they were more prevalent among CMS3 CRC (68%). Of all the subtypes, CMS3 appeared the most similar to normal colon tissue at the gene expression level. Recently, it has been suggested that the precursor lesion to *KRAS* mutant CRC (the majority of CMS3 cancers) are tubovillous adenomas with serrated features, a mixed histologic variant between CMS1 and 2 (21). Pathway analyses showed that CMS3 mRNA were enriched for 9 of 10 metabolic pathways investigated, including glutamine, fatty acid, and lysophospholipid metabolism.

Clinical Implications

In a subgroup analysis among patients treated with FOLFOX for stage III colorectal cancer, *KRAS*-mutant cancers (specifically, codon 12 mutation) and distal location of the tumor were shown to be associated with shorter time to recurrence and poor prognosis (22). For metastatic CRC, the higher frequency of *KRAS* mutations among these tumors limits standard chemotherapeutic options as mutant *KRAS* is typically an indicator of poor response to epidermal growth factor receptor (EGFR) monoclonal antibodies (mAb; e.g., cetuximab) (23). In those CMS3 tumors that do not demonstrate *KRAS* (nor *BRAF* and *PIK3CA*) mutations, EGFR mAbs may prove useful.

Future treatments for these cancers will likely target the characteristically overexpressed molecular targets in this group. For instance, approximately 3 and 5% of CMS3 and 4 CRC,

respectively, show high copy number for human epidermal growth factor receptor 2 (*HER2*). Tyrosine kinase inhibitors (TKI) against human epidermal growth factor receptors such as neratinib and dacomitinib are currently in clinical trials and may be used in conjunction with trastuzumab to target *HER2*-expressing tumors, which are not amenable to EGFR mAb treatment (18). Preclinical studies show that CRC cell lines with *HER2* mutations are resistant to EGFR mAbs; this resistance was overcome by combination therapy to pan-EGFR TKIs (24). In EGFR mAb-resistant CRC cell lines with *KRAS* mutations, a combination of pan-RAF and MEK inhibitors may be considered (25).

Additionally, since the reprogramming of cellular metabolism is an established hallmark of cancer (26), preclinical studies have shown efficacy using inhibitors that target many metabolic processes, such as glucose transporters, glycolytic enzymes (e.g., pyruvate dehydrogenase kinases) and fatty acid synthase (27). Our laboratory has shown that cystathionine-β-synthase (CBS), and its product, the gasotransmitter hydrogen sulfide, are upregulated in CRC compared to normal colonic mucosa; CBS overexpression contributes to tumor proliferation, angiogenesis, and bioenergetics (28). Furthermore, CBS upregulation in a premalignant colonic cell line induces metabolic reprogramming and an invasive phenotype (29). Most CMS3 cancers do not have identifiable therapeutic gene targets, such as *HER2*, but rather have a metabolic phenotype. Inhibition of cancer cell anabolic metabolism with CBS inhibitors may prove to be a useful treatment target in *KRAS* mutant CRC (28, 30).

CMS4

In experimental studies utilizing premalignant human organoid cultures with the genetic background of a serrated adenoma (*BRAF*^{V600E}), the cells developed into a CMS4 (mesenchymal) or CMS1 (MSI) phenotype in response to high or low transforming growth factor β (TGFβ) in the microenvironment, respectively (31). Although CMS4 precursor lesions have a gene signature consistent with the serrated pathway (6), CMS4 tumors exhibit extremely low levels of hypermutation, MSS status, and very high SCNA counts. CMS4 CRC displayed a mesenchymal phenotype with gene signatures consistent with an activated stroma: angiogenesis, integrin binding to matrix proteins, TGFβ signaling characteristic of carcinoma-associated fibroblasts (CAF), and an inflammatory microenvironment with prominent innate immune cells (11). In contrast to the anti-tumor immune environment of CMS1 cancers, the CMS4 tumor microenvironment is pro-inflammatory, with the presence of Treg cells, T helper 17 cells, myeloid-derived suppressor cells, and tumor promoting macrophages. The presence of immunosuppressive cytokines such as IL-23 and IL-17 link CMS4 cancers to colitis-associated colorectal carcinoma, where *TP53* inactivation occurs early in the transformation to dysplasia (32), which is distinct from CMS2 precursor lesions, where loss of TP53 tumor suppressor function occurs late in the adenoma-to-carcinoma sequence.

Clinical Implications

CMS4 cancers, often diagnosed at advanced stages, have a poor prognosis with the worst 5-year overall survival (62%) and relapse-free survival (60%) of any molecular subtype (11). Although standard adjuvant therapy (FOLFOX) for stage III is recommended, CMS4 cancers show no benefit from systemic adjuvant treatments (8). For metastatic disease, CMS4 cancers are resistant to anti-EGFR therapy, independent of *KRAS* mutation status (6). Anti-angiogenesis therapies such as bevacizumab are standard additions for stage IV disease (33); however, other stromal elements such as CAF and pro-tumorigenic immune cells such as tumor-associated macrophages are not specifically targeted. For these reasons, targeting the peritumoral microenvironment may emerge as novel therapies in the future. For example abituzumab, a monoclonal antibody against tumor cell surface integrin αvβ6

which binds to fibronectin, shows promise in an early phase I/II clinical trial (34).

Conclusion

Comprehensive transcriptomic analysis has allowed for the identification of four consensus molecular subtypes of colorectal carcinoma into which most CRC can be categorized based on their genomic signature. These subtypes aid in prognostication as well as determining treatment strategies for individual patients based not just on the mutations and activated pathways in those tumors, but also based on the phenotypic characteristics and responses to treatment of other tumors with similar signatures. Novel targeted therapeutic strategies, such as immune checkpoint blockade and metabolic normalization can be applied in highly individualized treatment regimens to improve life expectancy even in advanced cases of colorectal carcinoma.

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Conflict of interest

M.R.H., C.S., and C.C. are officers and/or shareholders of CBS Therapeutics Inc., an UTMB spin-off company involved in research and development of H_2S biosynthesis inhibitors for the therapy of cancer. The other authors declare no potential conflicts of interest.

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