OncoTargets and Therapy

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REVIEW

Recent Advances in Our Knowledge of mCRC Tumor Biology and Genetics: A Focus on Targeted Therapy Development

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Abstract: Metastatic colorectal cancer (mCRC) remains a highly lethal malignancy although considerable progress has resulted from characterizing molecular alterations such as *RAS* mutation status and extent of microsatellite instability (MSI) to guide optimal use of available therapies. The availability of gene expression profiling, next generation sequencing technologies, proteomics analysis and other technologies provides high resolution information on individual tumors, including metastatic lesions to better define intra-tumor and inter-tumor heterogeneity. Recent literature applying this information to further customize personalized therapies is reviewed. Current biomarker-based stratification used to select optimal therapy that is personalized to the mutation profile of individual tumors is described. Recent literature using whole exome sequencing of metastatic lesions and primary CRC tumors and other advanced technologies to more fully elucidate the tumor biology specific to mCRC sub-types and to develop more precise therapies that improve outcomes is also reviewed.

Keywords: colorectal cancer, metastasis, mutation, whole exome sequencing, targeted therapy

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related mortality in men and women combined.[1] Patients with metastatic CRC (mCRC) have an extremely poor 5-year survival rate, < 14%.[2] While overall prognosis remains dismal, progress in more specific targeting of therapy to individual tumor profiles has contributed to significantly improve overall survival for mCRC patients, which now exceeds 30 months.[3] Many patients now survive multiple treatments, and treatment with third-line therapy options is increasingly common. Despite these advances, further progress depends on: i) even more detailed delineation of molecular mCRC sub-types that are responsive to specific, targeted therapies; and ii) identifying mutations and other molecular alterations that contraindicate use of established therapies, and developing more effective alternative strategies. Frontline treatment for mCRC continues to rely on fluoropyrimidine (FP)-based chemotherapy that was first shown to provide a survival benefit for post-operative adjuvant chemotherapy in 1988,[4] and later shown to improve survival in mCRC.[5] While still heavily relied upon, FPs are used to treat >2 million cancer patients world-wide, FP-based doublet (5-fluorouracil (5-FU)/leucovorin (LV)) and triplet (eg, FOLFOX) chemotherapy for CRC is now refined and supplemented by anti-EGFR or anti-VEGF therapy that is

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http://doi.org/10.2147/OTT.S242224

based on tumor profiling.[6] An exciting recent development has been the use of microsatellite instability (MSI) profiling to identify patients likely to benefit from immunotherapy.[7] MSI profiling in CRC was initially implemented to identify patients who were unlikely to benefit from FP-based chemotherapy.[8]

The availability of comprehensive mutational profile information from whole exome sequencing (WES)[9] of tumor samples holds great promise for increasing the precision of therapy for mCRC by identifying mutations associated with metastatic progression. Intriguingly, recent studies employing WES,[10] and related technologies,[11] have validated the stepwise continuum model of CRC initiation and progression initially put forth by Vogelstein 30 years ago,[12] but with nuances that enable refined therapeutic approaches. Importantly, these studies validate that in most instances metastasis is a relatively late event, which largely occurs with preservation of the driver oncogenic profile of the primary tumor indicating that biopsy samples from the primary tumor may be used for therapeutic planning.

CRC evolves according to three main pathways: 1) chromosomal instability (CIN); 2) microsatellite instability (MSI); and 3) CPG island mutator phenotype (CIMP).[13] The classic sequential mutational progression described by Vogelstein[12] and extended thru detailed multi-omics profiling of CRC tumors[10,11] is applicable primarily to the CIN pathway, which constitutes the majority of CRC (>70%), and the overwhelming majority of mCRC (95%).[7] Alternatively, CRC can develop due to genetic or epigenetic changes affecting DNA repair capacity. The MSI pathway results from mutations in microsatellites that disable DNA mismatch repair (MMR), resulting in hypermutability.[8] In contrast, the CIMP pathway involves aberrant hypermethylation of CpG sequences in promoter regions of genes involved in DNA repair and oncogenesis, and this results in tumor progression following initial mutation in either *KRAS* or *BRAF*.[14] In general, prognosis and survival for MSI and CIMP CRC is more favorable than for CIN-positive CRC.[15]

In this review, biomarker-based stratification currently used to select the best available treatment for mCRC patients is first reviewed (Figure 1). Recent developments in tumor biology using WES, proteomics, and other advanced technologies that promise to modify current therapeutic paradigms (Figure 2) and improve outcomes for mCRC patients is then reviewed.

Current Biomarker-Based Stratification of mCRC

As with other malignancies, such as lung cancer,[16] there is increasing emphasis on identifying key oncogenic drivers that define molecular sub-types of metastatic colorectal cancer (mCRC) and in developing effective targeted therapies tailored to the oncogenic drivers.[6,17] Epidermal growth factor receptor (EGFR) plays an important role in the normal function of gastrointestinal tissue and a prominent role in the development of neoplasia in colonic epithelium. EGFR regulates proliferation and migration in the intestinal epithelium, which is a highly dynamic tissue.[18] EGFR overexpression occurs frequently in CRC, with 60–80% of tumors estimated to overexpress the receptor,[19] and overexpression is associated with a poor prognosis. Antibodies targeting EGFR (eg, cetuximab and panitumumab) are a preferred front-line treatment for mCRC,[20] provided tumor profiling for biomarkers associated with innate resistance to anti-EGFR therapy does not contraindicate their use.[21] However, biomarker screening may yield ambiguous results with tumor heterogeneity a confounding factor.[22] Further, acquired resistance to anti-EGFR therapy is also a serious concern.[23]

EGFR activates multiple signaling pathways[24] including RAS-RAF-MAPK and PI3K-Akt to execute its oncogenic function. Activating mutations in these downstream signaling pathways negate the efficacy of anti-EGFR therapy necessitating screening to identify patients who are unlikely to respond to expensive therapy.[17] The use of an extended RAS panel that screens not only for the >40% of CRC with activating *KRAS* mutations in exon 2, but also for mutations in exons 3/4 of *KRAS* and exons 1/2/3/4 of *NRAS* (~20% of patients without exon 2 *KRAS* mutations),[25] more broadly detects RAS-related resistance to anti-EGFR therapies,[26] and is recommended by the National Comprehensive Cancer Network. In principle, the activating mutations in *KRAS* that preclude use of anti-EGFR therapy in mCRC could be targeted by RAS-specific agents including AMG510, which targets $KRAS^{G12C}$. While early results with AMG510 and MRTX849 are promising in NSCLC,[27] limited response was detected in mCRC.[28] Lack of response in mCRC was found due to re-activation of EGFR signaling, and combining AMG510 and anti-EGFR therapy was effective in pre-clinical mCRC models.[28] In addition to promoting cell proliferation independent of EGFR activation, the $KRAS^{G12D}$ activating mutation promotes an

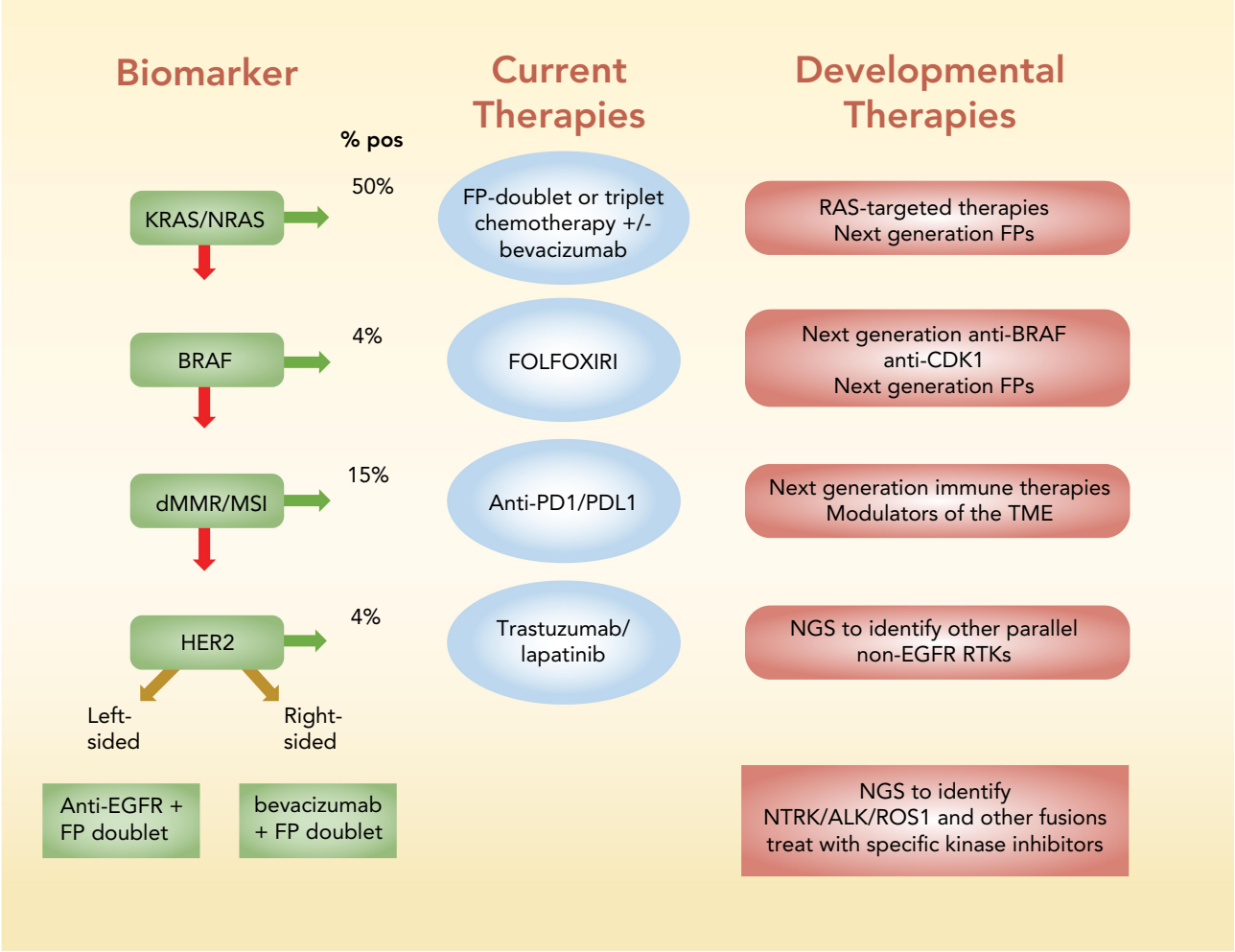

Figure 1 Summary of biomarker-based stratification to identify optimal front-line treatment for mCRC patients. Screening for KRAS/NRAS and BRAF activating mutations and for high MSI and deficiencies in MMR is recommended by the NCCN prior to treatment. %-positive indicates percent of patients that test positive for the biomarker indicated.

Abbreviations: FP, fluoropyrimidine; FOLFOXFIRI, folinic acid, 5-fluorouracil, oxaliplatin, irinotecan; MMR, mismatch repair; MSI, microsatellite instability; NGS, next generation sequencing; PD1, programmed cell death 1; PDL1, programmed death ligand 1; RTK, receptor tyrosine kinase.

immunosuppressive profile in CRC,[29] and is an important modulator of immunotherapy in CRC.[29]

The BRAFV600E activating mutation also is associated with innate[30] and acquired[31] resistance to anti-EGFR therapy and poor prognosis overall (OS ~18.2 months vs >38.4 months in RAS/BRAF-WT).[32] The detection of *BRAF* mutations at diagnosis is important because it may stimulate treatment with more aggressive chemotherapy, such as FOLFOXIRI.[33] However, targeted anti-BRAFV600E therapy with vemurafenib, dabrafenib, or encorafenib is ineffective in mCRC,[34] in part due to increased EGFR signaling thru the MEK/ERK pathway.[35] While dual inhibition of BRAF and MEK was effective in melanoma, it was not in mCRC. This resulted in studies showing elevated CDK1 caused apoptosis suppression in BRAF-mutant CRC. CDK1 inhibitors, including R0-3306 and the non-selective CDK inhibitor dinaciclib,[36] are being evaluated in pre-clinical studies for treatment of BRAF-mutant CRC. CDK inhibitors show promising activity in CRC with combined targeting of CDK1, 2, and 9 considered the most effective strategy.[37]

Activation of the PI3K/Akt pathway, which is frequently mutated in CRC with enrichment in mCRC,[10] also confers resistance to anti-EGFR therapy and to first-line chemotherapy.[38] While multiple clinical trials have evaluated Akt inhibition, Akt-targeted therapies directed in general have not been effective for mCRC treatment.[39] PI3K/mTOR dual inhibitors such as BEZ235 and LY3023414, however, display promising activity in pre-clinical models,[40] and MTORC1/2 inhibitors such as TAK-228

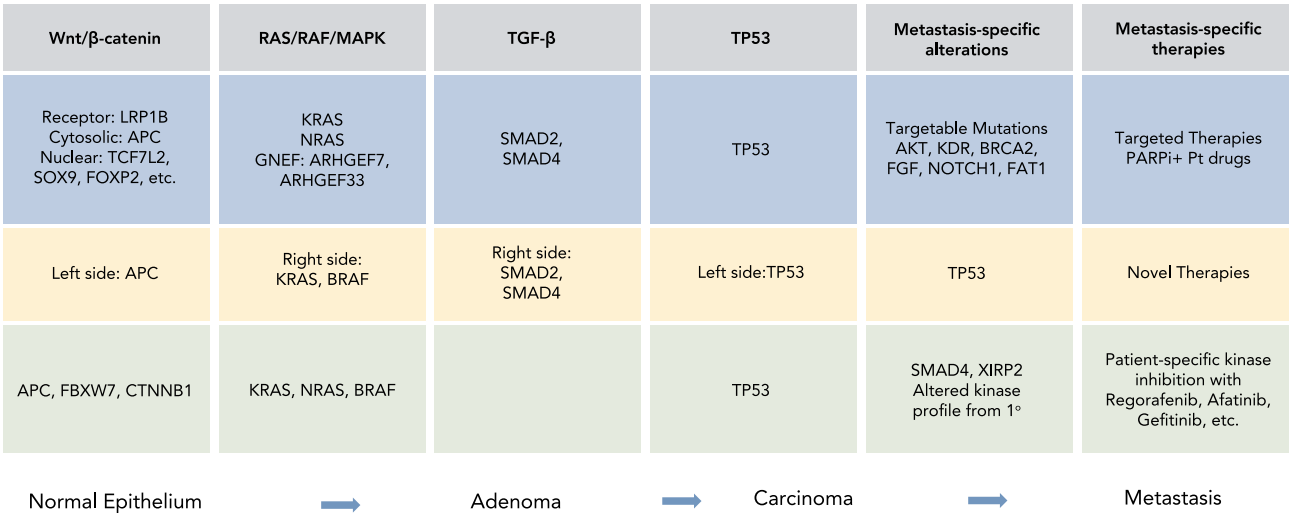

Figure 2 Summary of mutations in the Wnt/β-catenin, RAS/RAF/MAPK, TGF-β, and TP53 pathways that occur during the course of CRC tumor progression from normal epithelium to adenoma to carcinoma and to metastasis are listed. Mutations in light blue were reported by Ishaque et al[10], light yellow from Yaeger et al[11] and light green from Li et al[76]. Specific metastasis-specific alterations identified in each study are highlighted and potential new therapies for mCRC are indicated.
Abbreviations: PARPi, poly ADP ribose polymerase inhibitor; Pt, platinum drugs.

also show promising activity targeting this pathway.[41] Interestingly, MTORC2 activity was not detected in primary human and mouse CRC samples, but was found to be expressed in macrophages where it contributed to anti-tumor activity.[42]

A number of factors other than *KRAS/NRAS* and *BRAF* mutations influence the efficacy of anti-EGFR therapy, including plasma levels of EGFR ligands such as amphiregulin (AREG) and epiregulin (EREG),[43] and these may be monitored to provide an indication of resistance to anti-EGFR therapy.[24] EGFR gene copy number and acquired mutations in extracellular domains also may contribute anti-EGFR therapy resistance in mCRC. Mutations in *Her2, FGFR1, PDGFRA*, and *MAP2K1* also are associated with acquired resistance to anti-EGFR therapy.[44] Resistance to anti-EGFR therapy is associated with gene fusions in actionable targets including *ALK* (eg, ceritinib) and *NTRK1* (eg, larotrectinib), and these may be detected through sequencing of circulating tumor DNA (ctDNA).[45] *ALK* fusions are characteristic of a rare sub-type of mCRC that is highly aggressive, but responsive to ALK inhibitors.[46] Other mutations associated with innate resistance to anti-EGFR therapy include *HER2* and *MET* amplification.[23] *HER2* amplification occurs in 3–5% of mCRC patients[47] and promising results have been obtained with anti-HER2 antibody trastuzumab in combination with either the EGFR/HER2 kinase inhibitor lapatinib[48] or pertuzumab,[49] an antibody that inhibits HER2 dimerization.

MMR Effects on Chemo- and Immunotherapy

Deficiencies in DNA mismatch repair (dMMR) and the occurrence of high microsatellite instability (MSI) occur in about 15% of CRC patients, and the MSI pathway is considered a distinct pathway of oncogenic progression from the more common chromosomal instability (CIN) pathway.[50] Approximately 20% of cases with deficiencies in MMR result from germline mutations associated with Lynch syndrome,[51] and the remainder are due to sporadic mutations, or frequently epigenetic silencing of MLH1 (CIMP). dMMR/MSI is more common in right-sided tumors and is associated with poor differentiation and increased lymphocyte infiltration.[52] Screening for dMMR/MSI has been routinely employed for more than a decade because clinical data indicated dMMR/MSI was associated with lack of response to FP chemotherapy. High MSI is associated with more favorable outcomes for stage II patients, however for patients with mCRC high MSI is associated with worse survival.[53] Variable expression of a mutant form of HSP110 (HSP110ΔE9) is implicated in differential therapy response in MSI tumors[54] and detection of the mutant, while technically challenging, may be beneficial for MSI status determination in ambiguous cases,[55] and as a potential therapeutic target.[56]

A potential explanation for the relationship of MSI with lack of response to 5-FU chemotherapy is that MSI is also associated with elevated thymidylate synthase (TS),[57,58] which is the molecular target of FP chemotherapy.[59]

Elevated TS may also contribute to a pro-metastatic phenotype,[60,61] and its increased nuclear localization is associated with poor outcomes in CRC.[62] CRC tumors responding to 5-FU chemotherapy display low expression levels of TS (*TYMS*), thymidine phosphorylase (*TYMP*), and dihydropyrimidine dehydrogenase (*DPYD*).[63] Germline polymorphisms in *DPYD* significantly affect 5-FU degradation, and approximately 5% of CRC patients are highly vulnerable to 5-FU-induced systemic toxicities which can be life threatening.[64] *DPYD* testing prior to FP treatment is considered essential in Europe,[65] but not yet in the US. Polymeric FPs[66,67] are being developed that may overcome limitations of current FPs resulting from elevated TS, altered *DPYD*, and other factors.

Encouraging results have been obtained using immune checkpoint blockade to treat dMMR/MSI CRC,[7] and positive results have largely been restricted to this sub-type.[68] Pembrolizumab, which targets PD1, is now used as front-line treatment for mCRC, while Nivolumab (anti-PD1), alone or in combination with ipilimumab (anti-CTLA-4), is used to treat mCRC that has progressed with chemotherapy. The most important indicators of response to immunotherapy with anti-PD1/PDL1 immune checkpoint blockade is dMMR/MSI status, and screening is routinely implemented prior to therapy. The much higher mutation load in MSI vs MSS disease (1782 vs 73 somatic mutations per tumor) results in cancer-specific antigen presentation and increased lymphocyte infiltration into tumors.[69] A number of clinical trials are ongoing to extend the benefits of immunotherapy to MSS or MMR-proficient (MMR-p) CRC, which constitutes 95% of mCRC, and these are predominantly in Phase II and being evaluated as second line therapies.[7]

While dMMR/MSI status is an important indicator of response to immunotherapy, a number of efforts are ongoing to better define factors affecting response to immune checkpoint blockade and to develop therapies directed at alternative targets. Retrospective analysis of immune, fibroblastic, and angiogenic microenvironment of 1388 CRC tumors from transcriptomic analysis resulted in classification into four subgroups that were differentiated with respect to outcomes based on expression of genes associated with immune populations and tumor progression or an anti-tumor immune response.[70] The lack of utility for existing CRC immune signatures for extending the benefits of immunotherapy beyond MSI resulted in development of the gastrointestinal immune signature (GAIS-42), that identified four immune-metabolic clusters (IM Cluster 1–4) that differed in expression of cancer-associated fibroblasts (CAFs), myeloid derived suppressor cells (MDSCs), and other immune cell populations together with *BRAF* and *RAS* mutations, and metabolic profiling to distinguish tumors and to identify potential therapeutic targets. The authors note the potential for drugs to reprogram tumor metabolism such as pyruvate dehydrogenase kinase 1, or inhibitors of lactate transporters, to increase the capacity of the adaptive immune system to mount an anti-tumor response.[71]

Defining the Genomic Landscape That Promotes Metastatic Progression

The power of next generation sequencing has been applied by multiple studies in the last few years to characterize oncogenic drivers of metastasis and reveal new therapeutic targets for mCRC and other cancers.[72] In general, sequencing studies have demonstrated strong concordance between primary tumors and metastatic lesions with regard to key driver oncogenic mutations.[73] Divergence, when it occurs, is generally consistent with it being a late event in cancer progression, which is consistent with the Fearon-Vogelstein model,[12] which has recently been updated in a combined approach that also uses mathematical modeling, epidemiological studies, and sequencing data.[74] While sequencing studies confirm the validity of the "Vogelstein sequence" of CRC progression with sequential mutations in the *Wnt-, Ras-, TGF*-β, and *TP53* pathways (Figure 2), additional components of these pathways that are vulnerable to activating mutations are still being discovered and as a result classification models are being refined to more accurately predict susceptibility to targeted therapeutic interventions.[75] Interestingly, while metastases in general display good concordance with the primary tumor from which they were derived, significant differences are apparent at the proteomic level, indicating multi-omic analysis may be required to more fully understand metastatic progression and optimize treatments.[76] Sequencing and multi-omic studies complement differential gene expression analysis that resulted in identification of consensus molecular sub-types (CMS) of CRC based on expression data,[77,78] and demonstrated utility in explaining therapeutic response,[79] and metastatic progression.[80]

To identify the genomic determinants of mCRC, Ishaque et al[10] performed whole genome sequencing of metastatic lesions vs corresponding primary tumors and normal samples in 12 mCRC patients.[10] In general there

was concordance between primary and metastases with 65% of all single nucleotide variants (SNVs) shared between primary tumors and metastatic lesions, although their data suggest a higher rate of mutation in metastases relative to matched tumor after truncal separation. Mutations in protein coding regions identified well-established signaling pathways implicated in CRC progression including Wnt/β-catenin, *RAS*, and *TGF*-β being important for promoting progression from normal epithelium to adenoma. Interestingly, recurrent mutations were also detected in *ARHGEF33*, a guanine nucleotide exchange factor, with similar frequency as for *KRAS* (25% vs 29%), implicating it as an alternative mediator of RAS signaling and a potential modulator of EGFR-targeted therapies. Forty-eight genes were found to be mutated in metastases relative to primary tumors and data supported, in general, a late-stage mutagenic process contributing to metastatic progression. Functional annotation of metastasis-specific genes identified extracellular matrix, PI3K-Akt signaling, and focal adhesion-related pathways being dysregulated during metastatic progression. The authors developed an extended progression model of CRC that includes development of metastasis. In this model, potentially targetable mutations in *AKT, BRCA2*, and *NOTCH1* were enriched in metastatic lesions and, based on DNA repair deficiency mutational signatures in metastases, the authors suggested use of PARP inhibitors and platinum-based therapy for treating mCRC. Hepatic fibrosis/stellate genes were also significantly enriched in metastases consistent with metastasized cells activating pathways that foster organ-specific metastatic colonization.

Yaeger et al[11] compared oncogenic genomic alterations in primary tumors from TCGA or from a proprietary cohort sequenced at Memorial Sloan Kettering (MSK) to metastases from patients with mCRC treated at MSK. Sequencing used a hybridization capture-based next generation sequencing assay[81] for targeted sequencing of up to 468 genes at all exons and selected introns and was applied to >1100 patient samples. Forty-seven recurrently mutated genes were identified in the primary tumors with *APC, TP53, KRAS, PIK3CA* and *SMAD4* the most frequently mutated genes. A series of potentially novel recurrently mutated genes including *PTPRS, PIK3CG, FLT4, MAP2K4, IKZF1, JUN, TBX3, FOXP1, INHBA*, and *CDKN1B* occurred in 1–4% of primary tumors. However, only *TP53* mutations were selectively enriched in metastatic disease. Overall, the data indicated strong concordance between primary and metastatic CRC. The study noted significant differences in mutational burden between primary tumors originating in the right and left side of the colon with significant enrichment of *KRAS, BRAF, PIK3CA, PTEN, AKT1, RNF43, SMAD2*, and *SMAD4* in right-sided primary tumors and of *APC* and *TP53* in left-sided primary tumors. >3/4 of right-sided tumors had mutations affecting RAS-MAPK/PI3K pathway activation while the majority of left-sided primary tumors mitogenic pathway activation was limited to EGFR or other receptor tyrosine kinase alterations and survival was longest in such patients. Conversely, RAS mutations which predominated in right-sided primary tumors conferred poor survival and was associated with multiple sites of first metastasis. The authors conclude that the data provide a potential explanation for differential response to EGFR-targeted therapies based on tumor sidedness and suggest inhibitors of other RTKs, such as HER2, may display higher efficacy in left-sided tumors.

Multiple other studies have sought to identify metastasis-related genes affecting survival in colon cancer. For example, whole exome sequencing was performed by Siraj et al[82] to identify mutations associated with CRC metastasis. Thirty paired primary CRC and distant metastases revealed high overall genomic concordance between primary CRC and metastasis consistent with use of a single diagnostic biopsy from the primary tumor to characterize genomic variation of metastases. Divergence between primary tumor and metastatic lesions was generally shown to be a late event supporting late dissemination consistent with the Fearon-Vogelstein multistage progression model. Wei et al[83] identified differentially expressed genes between primary and metastasis colon cancer tissue. The researchers identified a six gene signature with independent prognostic significance of outcomes in colon cancer. The six genes included aldehyde dehydrogenase 2, neural precursor cell expressed, developmentally down-regulated 9, filamin A, lamin B receptor, twinfilin actin binding protein 1 and arginine-rich splicing factor 1.[83] DEG analysis of TCGA data was also undertaken by Zhou et al in a study that identified *REG1B, TGM6, NTF4, PNMA5*, and *HOXC13* as displaying prognostic significance with a potential role in CRC metastasis.[84] Other studies investigating genetic alteration in mCRC were recently reviewed.[85]

While next generation sequencing approaches are providing detailed understanding of mCRC progression and identifying oncogenic driver mutations to guide therapeutic

targeting, proteomic analysis has potential to provide an alternative insight into metastasis-specific characteristics that guide therapeutic intervention. Li et al undertook an integrated Omics analysis of mCRC from 146 CRC patients including 70 with metastases.[76] Analysis of genomic alterations in mCRC relative to non-mCRC identified *SMAD4* and *XIRP2* as enriched in primary tumors from mCRC patients. The investigators further applied non-negative matrix factorization to identify single-base substitution (SBS) signatures as classified by Alexandrov et al[86] and identified catalogue of somatic mutations in cancer (COSMIC) SBS 1 as having a higher contribution to mCRC than to non-mCRC. Genes enriched in SBS1 include *HYDIN, C1QB*, and *COL22A1* which were previously implicated in CRC metastatic progression.[87] Somatic copy number alterations (SCNAs) did not significantly differ between mCRC and non-mCRC.

Omics studies also revealed metastases are similar to the corresponding primary tumor at a genetic level although significant differences emerge upon analysis at a proteome and phosphoproteome levels.[76] To determine if differential protein expression could distinguish mCRC from non-mCRC, consensus clustering of 2440 differentially expressed proteins was performed which resulted in identification of 3 consensus clusters. CC2, which included extra-cellular matrix (ECM) and immune-related pathways, and CC3, which included DNA replication and metabolic pathways, showed slight enrichment in mCRC. The investigators then performed phosphoproteomic profiling and identified sub-clusters in each consensus cluster and found that metastatic tumors had more upregulated proteins compared to non-metastatic tumors with proteins important for ECM-receptor interaction, drug metabolism, focal adhesion, and tight junctions upregulated in metastatic tissue. The investigators performed kinase-substrate enrichment analysis and found enrichment for distinct kinases in the different consensus clusters with metastases differing from primary tumors within the same cluster. The investigators went on to test three kinase inhibitors (afatinib, gefitinib, and regorafenib) in 31 mini-PDX models including nine primary-metastatic pairs. Studies revealed that metastatic tissue could respond differently relative matched primary to the same drug and that phosphoproteomic profiling could identify mCRC sub-types that were responsive to particular drugs.

To identify factors affecting the rate of metastasis development, Ottaiano et al[88] performed genome profiling and immunohistochemical analysis in primary and matched metastatic tissues. The investigators separately considered patients with oligometastatic disease[89] (omCRC) from the more common and more lethal poly-metastatic disease (pmCRC) based on 3 year follow up following detection of primary CRC with synchronous single liver metastasis. There was 91.7% genetic sharing between mutation profile in primary tumors and matched liver metastases. However, there was marked differences in preservation of mutations in key oncogenic drivers in patients with disease that did not progress, including loss of alterations in *KRAS* and *SMAD4* in some patients, while gain of mutations in oncogenic drivers were detected in some patients that developed poly-metastatic disease. The investigators also analyzed differences in immunological microenvironment in matched primary tumor and metastatic disease. $CD3^+$, $CD8^+$, $FoxP3^+$, and $GrzB^+$ were quantified however significant differences related to tumor progression were found only for $CD8^+$ cells at invasive margins of primary tumors and $GrzB^+$ cells in tumor cores of metastatic lesions. The authors concluded that oligo-metastatic disease involves "back" mutations of key driver genes and relatively higher density of $GrzB^+$ T cells while poly-metastatic disease was characterized by "forward" mutations in key oncogenic drivers and low density of $GrzB^+$ T cells.

Discussion

CRC is a heterogeneous disease and considerable progress in extending survival for patients with mCRC has resulted from identification of key molecular driver mutations (eg, *KRAS/NRAS/BRAF*) and other factors (MSI) that characterize specific CRC molecular sub-types, screening for these in individual patients, and tailoring therapy based on these factors. Recent studies indicate that further progress in developing personalized therapies by implementing screening for alternative genetic aberrations that drive cancer progression are possible. For example detection of mutations in alternative receptor tyrosine kinases beyond *EGFR* and *HER2* and additional fusions beyond *ALK* and *NTRK* prior to initiating treatment could further improve outcomes in mCRC. Also, identifying mutations that contraindicate therapy such as *ARHGEF33* that similar to *KRAS/NRAS* activating mutations negates response to anti-EGFR therapy could improve outcomes.

Apart from identifying novel targets thru genomic and proteomic analysis, considerable effort is being dedicated to developing effective therapies targeted at well-established pathways in CRC progression that are currently undruggable. A challenge in developing targeted therapies for improved treatment of mCRC is that many of the oncogenes that are key to driving cancer progression and metastasis are not

amenable to targeted therapy. For example, while Wnt[90] signaling mutations are ubiquitous in CRC, because of its important role in normal physiology, targeting Wnt is problematic. Another example is Src that is differentially expressed in mCRC and thus of potential utility for mCRC treatment but, because of its important role in the immune system, is not readily targeted for mCRC treatment.[91] Detailed analysis of how these pathways are dysregulated in CRC, particularly during metastatic progression, may enable selective targeting that could significantly improve survival.

Acknowledgments

This work was supported by NIH-NCI R21 CA218933, NIH-NCI P30 CA012197 and Wake Forest Baptist Comprehensive Cancer Center.

Disclosure

The author reports no conflicts of interest for this work.

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