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Molecular Prognostic and Predictive Markers in Colorectal Cancer: Current Status

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Abstract

In parallel with our growing understanding of the molecular pathways underlying colorectal neoplasia, significant advances have been made in the treatment of colorectal cancer (CRC). For the past few decades, 5-fluorouracil-based therapy has been the cornerstone of adjuvant therapy. More recently, additional cytotoxic drugs and molecular-targeted therapies have provided additional clinical benefit in certain patient populations. Unfortunately, overall survival remains about 45%. Notably, our understanding of why certain patients do or do not respond to treatment remains limited. Thus, as therapeutic options for CRC continue to expand, there is now an even greater imperative to identify reliable biomarkers that have the potential to predict prognosis as well as response to chemotherapy. In this review, we will summarize the current status of such molecular prognostic and predictive biomarkers in CRC and assess their usefulness in tailoring therapeutic options.

Keywords

Colorectal cancer; Prognostic; Predictive; Markers; Biomarkers; Chemotherapy; Targeted therapies

Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths in the United States and a common cause of morbidity and mortality worldwide. In 2009, 146,970 new cases of CRC were diagnosed, and 49,920 patients died from this disease [1]. Over the past few decades, significant progress has been made in the diagnosis and treatment of CRC through advances in molecular biology, endoscopy, surgery, and chemotherapy. However, despite these improvements, the overall 5-year survival rate is approximately 45% [2]. Thus, CRC remains a devastating disease and a major global health concern.

The cornerstone of treatment for nonmetastatic CRC is surgical resection of the primary tumor. However, following surgical resection, there is considerable risk for tumor recurrence in patients with stage III and high-risk stage II CRC. In the absence of

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postoperative or adjuvant therapy, about 50% of such patients who undergo potentially curative surgery ultimately relapse and die of metastatic disease. With the introduction of 5-fluorouracil (5-FU) over 50 years ago, adjuvant therapy for CRC has been used to help diminish the risk of metastasis. Adjuvant chemotherapy has since evolved to 5-FU with the addition of leucovorin and oxaliplatin (FOLFOX), a regimen that is associated with a higher 5-year disease-free and overall survival compared with 5-FU alone in stage III CRC patients [3]. In addition, FOLFOX has been shown to significantly reduce recurrence rates and increase overall survival in high-risk stage II CRC patients [3].

In patients with stage IV or metastatic CRC (mCRC), treatment goals are mainly palliative and the 5-year survival rate is less than 10% [2]. With 5-FU adjuvant treatment, overall survival has been shown to be around 12 months. However, the addition of cytotoxic drugs such as irinotecan and oxaliplatin with 5-FU and leucovorin (LV), has significantly improved overall survival to about 20 months [4]. More recently, the development of molecular-targeted therapies, such as vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) antibodies, have provided additional clinical benefit to patients with mCRC [5, 6••].

Despite improvements in both the treatment and knowledge of the molecular basis of CRC, our understanding of why patients do or do not respond to chemotherapy remains poor. With expanded therapeutic options and emerging chemotherapeutic agents for CRC, it is essential to identify reliable biomarkers that would improve both the assessment of prognosis and the ability to predict response to chemotherapy. According to the US National Institutes of Health Biomarkers Definitions Working Group, a biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacological response to a specified therapeutic intervention [7]. Biomarkers are categorized as either prognostic or predictive. A prognostic biomarker provides information on clinical outcome at the time of diagnosis, independent of therapy. Such markers are usually indicators of a patient's overall cancer outcome, disease aggressiveness, and likelihood of recurrence. In contrast, predictive biomarkers provide information about the likelihood of response to a given therapeutic modality based on marker status, and therefore could be used to guide treatment. In this review, we will summarize the current status of molecular prognostic and predictive biomarkers in CRC and assess their clinical usefulness in practice (Tables 1 and 2). In addition, we will discuss the future direction of prognostic and predictive biomarkers.

Molecular Biology of Colon Cancer

The development of CRC is a complex multistep process involving an accumulation of multiple genetic and epigenetic alterations that transform normal colonic epithelium into adenocarcinoma. In 1990, Vogelstein et al. [8] postulated that the cumulative effects of oncogene activation (eg, *KRAS*) and tumor suppressor gene inactivation (eg, *APC, DCC, p53*) are required for colonic neoplastic transformation. As a result of these alterations, a malignant tumor can arise, providing additional growth advantages that lead to clonal expansion. In the well-described adenoma-carcinoma sequence, the accumulation of genetic alterations is induced by genomic instability. Classically, there are two well-described pathways of genomic instability that lead to the development of CRC: 1) chromosomal instability (CIN), which accounts for about 80% to 85% of sporadic CRC and is the underlying mechanism associated with familial adenomatous polyposis; and 2) DNA microsatellite instability (MSI), which is the phenotype associated with 15% to 20% of sporadic CRC and almost all cases of Lynch syndrome (hereditary nonpolyposis colorectal cancer) [9•]. More recently, alternative pathways of tumorigenesis have been characterized,

the most notable of which is the CpG island methylator phenotype (CIMP), mediated through aberrant methylation of tumor suppressor genes [9•].

Molecular Prognostic and Predictive Biomarkers

Microsatellite Instability

Microsatellites are short repetitive DNA nucleotide sequences prone to frameshift mutations and base-pair substitutions during replication in the setting of a defective DNA mismatch repair (MMR) system. In Lynch syndrome, the MSI phenotype is primarily due to mutations in DNA MMR genes, most often *hMLH1*, *hMSH2*, *hMSH6*, and *PMS2* [9•]. In contrast, for the vast majority of sporadic CRC with an MSI phenotype, defects in MMR arise from transcriptional silencing of the *hMLH1* gene caused by promoter hypermethylation [9•]. Currently there are three well-established categories to classify MSI using a reference panel of 5 to 10 microsatellite loci: MSI-high (MSI-H), instability at ≥30% of loci; MSI-low (MSI-L), instability at 10% to 30% of loci; and microsatellite stable (MSS) for cases that display no instability.

Numerous retrospective studies have studied the relationship between MSI and CRC-related prognosis with compelling and consistent results. In a retrospective analysis of five randomized clinical trials of patients with stage II and III colon cancer, Ribic et al. [10] demonstrated that patients with MSI-H tumors had greater 5-year overall survival compared with patients with MSS tumors (hazard ratio [HR] = 0.31; 95% confidence interval [CI] = 0.14–0.72, *P*=0.004). In a meta-analysis of 32 trials, Popat et al. [11] showed that patients with MSI-H CRC had improved disease-free and overall survival, irrespective of disease stage, compared to patients with MSS or MSI-L CRC. Recently, the PETACC III trial confirmed these retrospective findings by demonstrating prospectively that MSI-H is a strong prognostic factor for relapse-free and overall survival in patients with stage II and III CRC. A subgroup analysis suggested a stronger association of MSI-H with survival among patients with stage II than in stage III CRC [12]. In another recent prospective study, encompassing five randomized clinical trials, Sargent et al. [13••] showed that defective MMR (dMMR) or MSI-H was associated with improved disease-free (HR = 0.51; 95% CI = 0.29–0.89, *P*=0.009) and overall survival (HR = 0.47; 95% CI = 0.26–0.83, *P*=0.004) compared to proficient MMR (pMMR) or MSS/MSI-L in untreated stage II and III CRC patients.

While evidence strongly favors MSI status as a significant prognostic marker in CRC, the value of MSI status as a predictive marker of response to adjuvant therapy is less clear. In the Ribic et al. analysis, 5-FU-based adjuvant chemotherapy did not improve 5-year overall survival among stage II and III patients with MSI-H tumors (HR = 1.07; 95% CI = 0.62–1.86, *P*=0.80) but did benefit patients with MSS/MSI-L tumors (HR = 0.72; 95% CI = 0.53–0.99, *P*=0.04) [10]. A separate analysis of multiple prospective clinical trials of stage II and III CRC also demonstrated that 5-FU-based adjuvant chemotherapy did not benefit patients with dMMR tumors (multivariate HR for DFS = 1.39; 95% CI = 0.46–4.15, *P*=0.56), but did benefit patients with pMMR tumors (multivariate DFS HR = 0.67; 95% CI = 0.48–0.93, *P*=0.02) [13••]. In contrast, the CALGB 89803 study showed that stage III CRC patients with dMMR tumors treated with irinotecan and 5-FU/LV had a higher DFS compared with those with intact MMR proteins (HR = 0.76; 95% CI = 0.64–0.88, *P*=0.03). This relationship was not observed among patients treated with 5-FU/LV alone, suggesting that MSI status might predict response to irinotecan in stage III colon cancer [14]. The PETACC III prospective clinical trial reported no predictive effect of MSI status in stage II and III patients treated with 5-FU/LV alone or 5-FU/LV and irinotecan [12]. Additional data may be forthcoming from an ongoing randomized trial (ECOG 5202), which is incorporating assessment of tumoral MSI and LOH 18q status among patients with stage II colon cancer

prior to consideration of treatment. In this study, patients with high-risk tumors (MSS/MSI-L with 18qLOH) are randomized to two different chemotherapy regimens, while patients with low-risk tumors (MSI-H or MSS/MSI-L with retention of 18q) are observed.

KRAS

KRAS, a proto-oncogene, encodes a GTPase that is involved in facilitating cellular response to extracellular stimuli. Point mutations within the *KRAS* gene have been found in about 40% of CRC, resulting in constitutive activation of downstream signaling pathways and resistance to inhibition of cell surface receptor tyrosine kinases, most notably EGFR [15]. Studies evaluating the role of *KRAS* mutations as a prognostic marker in patients with CRC have had conflicting results. In two large studies of patients with stage III CRC, *KRAS* mutations increased the risk of recurrence ($P<0.001$) and death ($P=0.004$) and had worse disease-free ($P=0.008$) and overall survival ($P=0.02$) [16, 17]. However, recent prospective analyses from the CALGB 89083 (stage III colon cancer) and PETACC III (stage II and III CRC) trials demonstrated that *KRAS* mutation was not a prognostic marker for patients treated with adjuvant 5-FU-based chemotherapy [15, 18]. Moreover, the National Cancer Institute of Canada Clinical Trials Group CO.17 showed that *KRAS* mutation status had no prognostic effect for overall survival in previously treated mCRC patients receiving best supportive care [6••].

As a predictive marker in the adjuvant setting, most studies report no association between *KRAS* mutations and response to standard chemotherapy in all stages of CRC. However, *KRAS* mutation status has emerged as a predictive marker to identify patients with mCRC that may benefit from EGFR inhibitors. The first large study to analyze the effect of *KRAS* mutational status with mCRC patients treated with EGFR inhibiter monotherapy was conducted with patient tumor samples from the panitumumab registrational trial [19••]. In this randomized trial, patients with chemo-refractory mCRC were randomized to either panitumumab or supportive care. The results of this study showed that in patients with wild-type (WT) *KRAS* tumors, progression-free survival was improved with panitumumab compared with supportive care (2.8 months vs. 1.7 months, $P<0.0001$). In contrast, no benefit was observed in patients with mutant *KRAS* tumors, regardless of treatment. Consistent results were demonstrated in a large randomized controlled trial using cetuximab monotherapy in mCRC patients [6••]. In this study, 572 chemo-refractory mCRC patients were randomized to cetuximab monotherapy or supportive care. Among *KRAS* WT patients treated with cetuximab monotherapy, the progression-free (3.7 months vs. 1.9 months, $P<0.001$) and overall (9.5 months vs. 4.8 months, $P<0.001$) survival was significantly higher compared to patients treated with supportive care. However, among patients with mutant *KRAS* tumors, there was no significant improvement in progression-free or overall survival associated with cetuximab [6••].

Several studies have evaluated *KRAS* mutation status in patients with mCRC who were treated with EGFR inhibitors combined with standard chemotherapy. In the OPUS trial, patients with *KRAS* WT mCRC receiving cetuximab with FOLFOX had a higher overall response rate (61% vs. 37%, $P=0.011$) compared to patients receiving FOLFOX alone [20]. In the CRYSTAL trial, the addition of cetuximab with FOLFIRI in patients with *KRAS* WT mCRC resulted in a nonsignificant increase in median progression-free (9.9 months vs. 8.7 months, $P=0.07$) and overall (24.9 months vs. 21 months, $P=0.44$) survival compared to patients with *KRAS* WT mCRC receiving FOLFIRI alone [21]. Moreover, at the 2009 European Cancer Organization/European Society of Medical Oncology meeting, Van Cutsem and colleagues [22] showed a significant difference in overall survival for patients with *KRAS* WT mCRC treated with FOLFIRI plus cetuximab versus FOLFIRI alone (23.5 months vs. 20 months, $P= 0.0094$). Currently, both the National Comprehensive Cancer Network (NCCN) and the American Society of Clinical Oncology (ASCO) recommend

KRAS mutation testing in all mCRC patients in order to reserve treatment with EGFR inhibitors to patients with *KRAS* WT tumors.

BRAF

BRAF encodes a serine-threonine protein kinase that acts as a downstream effector of the KRAS signaling pathway. Various studies have revealed that an activating mutation of *BRAF* (*BRAF* V600E) occurs in about 34% to 70% of sporadic MSI-H CRCs and about 10% of unselected CRCs [23]. In the adjuvant setting, *BRAF* mutation status appears to be a valid prognostic marker. Several studies, including two large retrospective series, have shown that *BRAF* mutations were associated with poor clinical outcome, especially in patients with MSS/MSI-L colon cancer [24, 25]. However, pooled results from the PETACC III, EORTC 40993, and SAKK 66–00 trials demonstrated that *BRAF* mutation was not prognostic for relapse-free survival (RFS), but was prognostic for overall survival, particularly in patients with MSI-L and MSS tumors (HR = 2.2; 95% CI = 1.4–3.4, *P*=0.0003) [18]. The observation that *BRAF* has a prognostic value in overall survival but not in relapse-free survival may be due to the small sample size. More recently, in the 2010 ASCO meeting, *BRAF* mutations were shown to be a prognostic indicator of worse outcomes in *KRAS* WT patients from the CRYSTAL trial [26]. In this study, median overall survival after treatment with FOLFIRI chemotherapy regimen was 25.1 months for patients with both *KRAS* and *BRAF* WT, but only 14.1 months for *KRAS* WT patients with a *BRAF* mutation [26].

Currently, the NCCN Colon Cancer Guideline Update 2010 recommends that testing for *BRAF* mutations should only occur in mCRC patients when *KRAS* testing is normal. However, other societies have not provided similar guidelines. Therefore, additional data are needed before definitive conclusions can be drawn regarding the prognostic value of *BRAF* tumor mutation status. Associations of *BRAF* mutation status as a predictive marker of response for EGFR inhibitors are also an active area of investigation. Recently, data from CRYSTAL trial, presented in abstract form at the 2009 ASCO annual meeting, did not confirm *BRAF* as a predictor for resistance to anti-EGFR monoclonal antibody treatment [21].

Loss of Heterozygosity 18q

Loss of heterozygosity (LOH) has been implicated as an important mechanism of tumor suppressor gene inactivation. Chromosome 18q allelic loss is one of the most well-studied molecular prognostic biomarkers, occurring in up to 70% of CRC [27]. Many retrospective studies have demonstrated that LOH 18q is associated with poor survival in advanced stage II and III CRC patients [28]. In a study focused on stage III colon cancer patients, Watanabe et al. [29] found a significantly higher relative risk of death in MSS tumors with LOH of 18q versus those with retained 18q alleles (RR = 2.75; 95% CI = 1.34–5.65, *P*=0.006). Moreover, a meta-analysis of 2189 patients showed that LOH 18q was associated with poor overall survival (HR = 2.0; 95% CI = 1.49–2.69, *P*<0.0001) [27].

Despite showing promise as a prognostic marker in CRC, two recent prospective studies reported contrasting results. In the CALGB 89803 study, stage II colon cancer patients with LOH 18q had lower 5-year disease-free (0.78 vs. 0.93, HR = 0.39; 95% CI = 0.16–0.94, *P*=0.03) and overall (0.85 vs. 0.98, HR = 0.25; 95% CI = 0.07–0.83, *P*= 0.01) survival rates than patients whose tumors had 18q intact [30]. In contrast, the PETACC III group showed that LOH 18q status was not prognostic in stage II CRC, although a prognostic effect was observed in stage III CRC on univariate analysis [12]. A possible explanation for these conflicting results may be due to the lack of standardization of assay methodology. For example, studies may use alternative LOH scoring methods or genetic markers on different regions of chromosome 18q, which can lead to variation in the interpretation of LOH.

Despite the numerous studies suggesting the prognostic effect of LOH 18q, there are limited studies in the literature on the predictive nature of LOH 18q with CRC treatment. As previously described, the E5202 trial is using pretreatment LOH 18q status (as well as MSI status) in resected stage II colon cancer to stratify patients to observation or to two different arms of adjuvant chemotherapy.

TP53

TP53, a tumor suppressor gene, is mutated in about 40% to 60% of CRC [31]. Located on chromosome 17p, TP53 acts as a checkpoint protein that monitors the integrity of the genome. In the setting of DNA damage, TP53 blocks cell proliferation, stimulating DNA repair or promoting apoptotic cell death in the case of defective repair function. The relationship between *TP53* mutation status and patient prognosis has been studied extensively, and the results are inconsistent and inconclusive. In one study, *TP53* mutation was associated with lower RFS rates compared to intact *TP53* (RFS RR = 1.49; P=0.01) in patients with stage II and III colon cancer [32]. However, there was no difference in overall survival [32]. In patients with stage III colon cancer, Westra et al. [33] showed that *TP53* mutation was associated with a shorter DFS, both in univariate (P=0.009) and multivariate analyses (P=0.018). In the TP53 Colorectal Cancer International Collaborative Study comprised of 3583 CRC patients, *TP53* mutation was associated with lower overall survival (P<0.05) [31]. However, more recently, in a prospective blinded analysis, Popat et al. [34] demonstrated that *TP53* mutation status was not associated with worse overall survival (HR = 0.98; 95% CI = 0.78–1.23, P=0.9) in 967 stage I to III CRC patients treated with adjuvant chemotherapy.

The contradictory nature of these studies may reflect differences in the methodologies used to assess *TP53* mutation status, including different antibodies, immunohistochemical staining techniques, and immunohistochemical scoring systems. Unlike the prognostic nature of *TP53* mutation status, data regarding the value of *TP53* mutation status as a predictive marker of adjuvant therapy are more consistent. Several prospective studies have failed to demonstrate any correlation between *TP53* mutation and response to adjuvant chemotherapy [31, 32]. Currently, the European Group of Tumor Markers and ASCO recommend against the use of *TP53* mutation analysis for prognosis and predicting response to adjuvant therapy in CRC.

Thymidylate Synthase

Thymidylate synthase (TS) plays an essential role in DNA synthesis by catalyzing the reductive methylation of deoxyuridylate (dUMP) to thymidylate (dTMP). Inhibition of TS by 5-FU (pyrimidine analog) blocks dTMP production, and therefore rapidly shuts off DNA synthesis and repair, triggering apoptosis. TS expression status has been well studied as a prognostic marker; however, the results are conflicting. Several studies, including a meta-analysis, have shown that high TS expression is associated with poorer overall and disease-free survival in CRC patients [32, 35, 36]. However, Soong et al. [37] reported that low expression of TS is associated with worse prognosis in stage II and III CRC patients treated with surgery alone. More recently, in a prospective, blinded analysis, Popat et al. [34] showed that TS expression had no prognostic value in stage II and III CRC patients treated with adjuvant chemotherapy.

In regard to the predictive utility of TS, the results are also conflicting. Many studies have shown that high TS expression is associated with longer survival in CRC patients receiving 5-FU-based adjuvant therapy [35, 38]. However, other studies found no predictive value of response to adjuvant chemotherapy for TS expression in stage II and III CRC [32, 33]. Given the lack of a standardized assay and significant heterogeneity in outcomes of studies

in the adjuvant setting, TS expression is currently not recommended for routine use as a prognostic or predictive marker in CRC. Additional prospective studies with consistent methodology are needed to define the precise prognostic and predictive value of TS.

VEGF

VEGF is a potent proangiogenic factor whose downstream signaling events include endothelial cell proliferation, migration, and vascular permeability. In CRC, several retrospective studies have shown that increased VEGF expression is associated with tumor aggressiveness, poor disease-free and overall survival, distant metastatic spread, and decreased response to preoperative radiotherapy [39]. At the 2009 ASCO annual meeting, El-Khoueiry and colleagues [40] showed that patients with low *VEGFR1* gene expression had significantly longer time to tumor recurrence compared to those with high *VEFGR1* gene expression. Moreover, high *VEGFR2* gene expression in patients with mCRC treated with 5-FU or capecitabine, oxaliplatin, and bevacizumab was associated with longer progression-free survival than low *VEGFR2* gene expression levels [40]. Despite these promising results, data on VEGF expression status as a prognostic biomarker are limited; therefore, more studies are needed to confirm these findings.

Due to its primary role in promoting tumor growth, angiogenesis, and metastasis, VEGF has become a promising target for therapeutic intervention. In 2004, Hurwitz et al. [5] demonstrated that bevacizumab, a recombinant monoclonal antibody that targets VEGF-A, significantly improved overall survival in patients with mCRC. As a result, bevacizumab has been approved as first-line treatment of mCRC in combination with 5-FU-based chemotherapy. Despite its success in the treatment of mCRC, there are currently no predictive biomarkers of anti-VEGF therapy with bevacizumab in CRC.

Additional Biomarkers

There are additional prospective molecular biomarkers beyond those described above that are currently being investigated for use in CRC, some of which may also constitute targets of molecular-targeted therapy. These include cyclooxygenase-2 (COX-2 or PTGS-2), DNA hypomethylation as measured by long interspersed nucleotide element-1 (LINE-1), and fatty acid synthase (FASN). COX-2 is a key enzyme that promotes inflammation and cell proliferation that has been implicated in colorectal carcinogenesis. Several prospective studies have shown that COX-2 overexpression is significantly associated with worse outcomes in patients with CRC [41, 42]. Moreover, investigation into the value of COX-2 as a predictive marker is especially important given the ready availability of drugs with COX-2 inhibitory activity such as celecoxib and aspirin. Recently, we showed in a large prospective study of 1279 patients with stage I to III CRC that regular aspirin use after diagnosis was associated with a lower risk of CRC-specific mortality, especially among patients who had primary tumors that overexpressed COX-2 (multivariate HR = 0.39; 95% CI = 0.20–0.76) [43]. To further evaluate COX-2's prognostic and predictive potential, the CALGB is currently planning a prospective study, which includes celecoxib as adjuvant therapy among patients with stage III colon cancer (CALGB 80702). In addition to COX-2, markers of aberrant DNA methylation have become increasingly promising. In a large prospective study, genome-wide DNA hypomethylation as measured by LINE-1 was associated with an increase in colon cancer-specific mortality (multivariable HR = 2.37; 95% CI = 1.42–3.94, $P<0.001$) and overall mortality (multivariable HR = 1.85; 95% CI = 1.25–2.75, $P<0.002$) [44]. Lastly, energy balance has been shown to play an etiopathogenic role in CRC [45]. Thus, molecular markers of energy balance are significant areas of investigation. In one study, FASN overexpression was associated with a significant reduction in colon cancer-specific mortality by both univariate and multivariate analyses (adjusted HR = 0.41; 95% CI

= 0.19–0.89) and an insignificant trend toward improved overall mortality (adjusted HR = 0.75; 95% CI = 0.50–1.13) [46].

Future Directions

Although prognostic or predictive value of an individual biomarker may be significant, in the future it is likely that a panel of several biomarkers may be utilized to provide even greater information. Since several complex molecular pathways drive the development of CRC, analysis platforms that simultaneously interrogate multiple genomic and transcriptional changes in tumors may be particularly helpful in identifying unique prognostic and predictive expression signatures. Several studies have already shown that gene expression profiles can predict risk of recurrence, death, and poor clinical outcomes in patients with colon cancer [47, 48]. Recently, application of quantitative reverse transcription polymerase chain reaction of colon cancer tissue in four large independent populations was used to develop multigene algorithms for estimating recurrence risk and differential benefit from 5-FU/LV in patients with stage II colon cancer. The study identified 48 genes that were significantly associated with risk of recurrence and 66 genes associated with 5-FU/LV benefit [49]. Lastly, genome-wide association studies (GWAS) have been successful in identifying at least 10 germline susceptibility loci for the initial development of CRC [50]. Although these loci have not been shown to be prognostic among patients with established CRC [51], future GWAS and genome-sequencing efforts will undoubtedly help uncover new variants that may be both prognostic and predictive for survival among CRC patients.

Conclusions

In summary, significant advances have been made in the treatment and understanding of the molecular pathways of CRC over the past two decades. As a result, an increasing number of chemotherapeutic and molecular-targeted agents are available to treat this disease. In this review, we have summarized several molecular prognostic and predictive biomarkers that are currently used in the management or treatment of CRC. These biomarkers include angiogenesis factors, tumor suppressor genes, genomic instability markers, and growth-factor receptors.

Currently, there are two established molecular biomarkers in CRC: MSI status for prognosis and *KRAS* mutation status as a predictive biomarker for EGFR-targeted treatment in mCRC. Other potential prognostic and predictive biomarkers have either failed to demonstrate clinical utility or have been inadequately studied. In addition, many studies of biomarkers have yield inconsistent results, possibly due to methodological issues including small sample sizes, heterogeneous study populations, and the lack of consistent control groups. In addition, variations in assay techniques may further explain the discrepant, and sometimes contradictory, data in the literature.

With increasing understanding of CRC biology, additional molecular biomarkers are likely to be developed in the near future. Until recently, most biomarker discovery has been focused on molecular markers in tumor tissue. However, further study is needed into additional biomarkers, based on germline genetic susceptibility loci that may also have prognostic and predictive value. Nonetheless, any biomarkers of prognosis and prediction must be validated in carefully designed large, prospective clinical trials, using standardized assay techniques. The discovery and validation of prognostic and predictive biomarkers will hopefully achieve the therapeutic promise of an individualized, molecular medicine approach to tailor patients' treatment regimen, thereby maximizing the efficacy of treatment while minimizing associated hazards.

References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. CA Cancer J Clin. 2009; 59:225–49. [PubMed: 19474385]
2. O'Connell JB, Maggard MA, Ko CY. Colon cancer survival rates with the new American joint committee on cancer sixth edition staging. J Natl Cancer Inst. 2004; 96:1420–5. [PubMed: 15467030]
3. Andre T, Boni C, Navarro M, et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSCIA trial. J Clin Oncol. 2009; 27:3109–16. [PubMed: 19451431]
4. Goldberg RM. Therapy for metastatic colorectal cancer. Oncologist. 2006; 11:981–7. [PubMed: 17030638]
5. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004; 350:2335–42. [PubMed: 15175435]
6••. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med. 2008; 359:1757–1765. This article demonstrated KRAS' predictive value in patients with mCRC receiving cetuximab, an EGFR inhibitor. [PubMed: 18946061]
7. Atkinson AJ, Colburn WA, DeGruttola VG, et al. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001; 69:89–95. [PubMed: 11240971]
8. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N Engl J Med. 1988; 319:525–32. [PubMed: 2841597]
9•. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology. 2008; 135:1079–1099. This article is a comprehensive review of genetic and epigenetic changes that occur during CRC development and metastasis. [PubMed: 18773902]
10. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med. 2003; 349:247–57. [PubMed: 12867608]
11. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol. 2005; 23:609–18. [PubMed: 15659508]
12. Tejpar S, Bosman F, Delorenzi M, et al. Microsatellite instability (MSI) in stage II and III colon cancer treated with 5FU-LV or 5FU-LV and irinotecan (PETAC 3-EORTC 40993-SAKK60/00 trial). J Clin Oncol. 2009; 27:15s. [Abstract: 4001]. Presented at the 2009 Annual Meeting of the ASCO; Orlando, Florida. May 29–June 2, 2009;
13••. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol. 2010; 28:3219–26. This article demonstrated dMMR's prognostic value in stage II and III CRC. [PubMed: 20498393]
14. Bertagnolli MM, Niedzwiecki D, Compton CC, et al. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: cancer and leukemia group B protocol 89803. J Clin Oncol. 2009; 27:1814–21. [PubMed: 19273709]
15. Ogino S, Meyerhardt JA, Irahara N, et al. *KRAS* mutation in stage III colon cancer and clinical outcome following intergroup trial CALGB 89802. Clin Cancer Res. 2009; 15:7322–9. [PubMed: 19934290]
16. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. J Natl Cancer Inst. 1998; 90:675–84. [PubMed: 9586664]

17. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the "RASCAL II" study. Br J Cancer. 2001; 85:692–6. [PubMed: 11531254]

18. Roth AD, Tejpar S, Delorenzi M, et al. Prognostic role of *KRAS* and *BRAF* in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60–00 trial. J Clin Oncol. 2010; 28:466–74. [PubMed: 20008640]

19••. Amado RG, Wolf M, Peters M, et al. Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008; 26:1626–34. This article demonstrated KRAS' predictive value in patients with mCRC receiving panitumumab, an EGFR inhibitor. [PubMed: 18316791]

20. Bokemeyer, C.; Bondarenko, I.; Hartmann, JT., et al. Biomarkers predictive for outcome on patients with metastatic colorectal cancer treated with first-line FOLFOX4 plus or minus cetuximab: updated data from the OPUS study [Abstract: 428]. Presented at the 2010 Gastrointestinal Cancers Symposium; Orlando, Florida. January 22–24, 2010;

21. Kohne C, Stroiakovski D, Changchien C, et al. Predictive biomarkers to improve treatment of metastatic colorectal cancer; outcomes with cetuximab plus FOLFIRI in the CRYSTAL trial. J Clin Oncol. 2009; 27:15s. [Abstract: 4068]. Presented at the 2009 Annual Meeting of the ASCO; Orlando, Florida. May 29–June 2, 2009;

22. Van Cutsem E, Rougier P, Kohne C, et al. A meta-analysis of the CRYSTAL and OPUS studies combining cetuximab with chemotherapy as 1st line treatment for patients with metastatic colorectal cancer: results according to *KRAS* and *BRAF* mutation status. Eur J Cancer. 2009; 7:324. [Abstract: 6077]. Presented at the 2009 ECCO/ESMO Multidisciplinary Congress Meeting; Berlin, Germany. September 20–24, 2009;

23. Minoo P, Moyer MP, Jass JR. Role of *BRAF*-V600E in the serrated pathway of colorectal tumorigenesis. J Pathol. 2007; 212:124–33. [PubMed: 17427169]

24. Samowitz WS, Sweeney C, Herrick J, et al. Poor survival associated with the *BRAF* V600E mutation in microsatellite-stable colon cancers. Cancer Res. 2005; 65:6063–9. [PubMed: 16024606]

25. Ogino S, Nosho K, Kirkner GJ, et al. CpG island methylator phenotype, microsatelite instability, *BRAF* mutation and clinical outcome in colon cancer. Gut. 2009; 5858:90–6. [PubMed: 18832519]

26. Van Cutsem E, Lang G, Folprecht M, et al. Cetuximab plus FOLFIRI: final data from the CRYSTAL study on the association of *KRAS* and *BRAF* biomarker status with treatment outcome. J Clin Oncol. 2010; 28:15s. [Abstract: 3570]. Presented at the 2010 Annual Meeting of the ASCO; Chicago, Illinois. June 4–8, 2010;

27. Popat S, Houlston RS. A systematic review and meta-analysis of the relationship between chromosome 18q genotype, DCC status and colorectal cancer prognosis. Eur J Cancer. 2005; 41:2060–70. [PubMed: 16125380]

28. Sun XF, Rutten S, Zhang H, et al. Expression of the deleted in colorectal cancer gene is related to prognosis in DNA diploid and low proliferative colorectal adenocarcinoma. J Clin Oncol. 1999; 17:1745–50. [PubMed: 10561211]

29. Watanabe T, Wu TT, Catalano PL, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med. 2001; 344:1196–206. [PubMed: 11309634]

30. Bertagnolli MM, Niedzwiecki D, Hall M, et al. Presence of 18q loss of heterozygosity (LOH) and disease free and overall survival in stage II colon cancer: CALGB Protocol 89803. J Clin Oncol. 2009; 27:15s. [Abstract: 4012]. Presented at the 2009 Annual Meeting of the ASCO; Orlando, Florida. May 29–June 2, 2009;

31. Russo A, Bazan V, Iacopetta B, et al. The TP53 colorectal cancer international collaborative study on the prognostic and predictive significance of *p53* mutations: influence of tumor site, type of mutation, and adjuvant treatment. J Clin Oncol. 2005; 23:7518–28. [PubMed: 16172461]

32. Allegra CJ, Paik S, Colangelo LH, et al. Prognostic value of thymidylate synthase, Ki-67, and p53 in patients with Dukes' B and C colon cancer: a national cancer institute-national surgical adjuvant breast and bowel project collaborative study. J Clin Oncol. 2003; 21:241–50. [PubMed: 12525515]

33. Westra JL, Schaapveld M, Hollema H, et al. Determination of TP53 mutation is more relevant than microsatellite instability status for the prediction of disease-free survival in adjuvant-treated stage III colon cancer patients. J Clin Oncol. 2005; 23:5635–43. [PubMed: 16110022]

34. Popat S, Chen Z, Zhao D, et al. A prospective, blinded analysis of thymidylate synthase and p53 expression a prognostic markers in the adjuvant treatment of colorectal cancer. Ann Oncol. 2006; 17:1810–7. [PubMed: 16971666]
35. Edler D, Glimelius B, Hallstrom M, et al. Thymidylate synthase expression in colorectal cancer: a prognostic and predictive marker of benefit from adjuvant fluorouracil-based chemotherapy. J Clin Oncol. 2002; 20:1721–8. [PubMed: 11919227]
36. Popat S, Matakidou A, Houlston RS. Thymidylate synthase expression and prognosis in colorectal cancer: a systematic review and meta-analysis. J Clin Oncol. 2004; 22:529–36. [PubMed: 14752076]
37. Soong R, Shah N, Salto-Tellez M, et al. Prognostic significance of thymidylate synthase, dihydropyrimidine dehydrogenase and thymidine phosphorylase protein expression in colorectal cancer patients treated with or without 5-fluorouracil-based chemotherapy. Ann Oncol. 2009; 19:915–9. [PubMed: 18245778]
38. Johnston G, Fisher ER, Rockette HE, et al. The role of thymidylate synthase expression in prognosis and outcome of adjuvant chemotherapy in patients with rectal cancer. J Clin Oncol. 1994; 12:2640–7. [PubMed: 7989939]
39. Zlobec I, Lugli A. Prognostic and predictive factors in colorectal cancer. Postgrad Med J. 2008; 84:403–11. [PubMed: 18832400]
40. El-Khoueiry A, Pohl A, Danenberg K, et al. Wt Kras and gene expression levels of VEGFR2, EGFR, and ERCC-1 associated with progression-free survival (PFS) in patients with metastatic colorectal cancer treated with first line 5-FU or capecitabine with oxaliplatin and bevacizumab (FOLFOX/BV or XELOX/BV). J Clin Oncol. 2009; 27:15s. [Abstract: 4056]. Presented at the 2009 Annual Meeting of the ASCO; Orlando, Florida. May 29–June 2, 2009;
41. Soumaoro LT, Uetake H, Higuchi T, et al. Cyclooxygenase-2 expression: a significant prognostic indicator for patients with colorectal cancer. Clin Cancer Res. 2004; 10:8465–71. [PubMed: 15623626]
42. Ogino S, Kirkner GJ, Nosho K, et al. Cyclooxygenase-2 is an independent predictor of poor prognosis in colon cancer. Clin Cancer Res. 2008; 14:8221–7. [PubMed: 19088039]
43. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA. 2009; 302:649–58. [PubMed: 19671906]
44. Ogino S, Nosho K, Kirkner GJ, et al. A cohort study of tumoral LINE-1 hypomethylation and prognosis in colon cancer. J Natl Cancer Inst. 2008; 100:1734–8. [PubMed: 19033568]
45. Chan AT, Giovannucci EL. Primary prevention of colorectal cancer. Gastroenterology. 2010; 138:2029–43. [PubMed: 20420944]
46. Ogino S, Nosho K, Meyerhardt JA, et al. Cohort study of fatty acid synthase expression and patient survival in colon cancer. J Clin Oncol. 2008; 26:5713–20. [PubMed: 18955444]
47. Wang Y, Jatkoe T, Zhang Y, et al. Gene expression profiles and molecular markers to predict recurrence of Dukes' B colon cancer. J Clin Oncol. 2004; 22:1564–71. [PubMed: 15051756]
48. Smith JJ, Deane NG, Wu F, et al. Experimentally derived metastasis gene expression profile predicts recurrence and death in patients with colon cancer. Gastroenterology. 2010; 138:958–68. [PubMed: 19914252]
49. O'Connell MJ, Lavery I, Yothers G, et al. Relationship between tumor gene expression and recurrence in four independent studies of patients with stage II/III colon cancer treated with surgery alone or surgery plus adjuvant fluorouracil plus leucovorin. J Clin Oncol. 2010; 28:3937–44. [PubMed: 20679606]
50. Tomlinson IP, Webb E, Carvajal-Carmona L. A genome-wide association study identifies colorectal cancer susceptibility loci on chromosome 10p14 and 8q23.3. Nat Genet. 2008; 40:623–30. [PubMed: 18372905]
51. Tenesa A, Theodoratou E, Din FV, et al. Ten common genetic variants associated with colorectal cancer risk are not associated with survival after diagnosis. Clin Cancer Res. 2010; 16:3754–9. [PubMed: 20628028]

Table 1

Summary of prognostic biomarkers for colorectal cancer

Biomarker	Alteration	General comments
Microsatellite instability	MSI-H	MSI-H or dMMR tumors are associated with longer DFS and OS. Evidence favors MSI-H as a strong prognostic biomarker. NCCN and ASCO guidelines recommend testing for MSI status on stage II CRC.
KRAS	Mutation	Prospective studies regarding the prognostic value of *KRAS* mutation are inconsistent.
BRAF	Mutation	Several studies demonstrate that *BRAF* mutation status is associated with shorter OS in MSS/MSI-L and *KRAS* WT tumors; however, only the NCCN recommends *BRAF* mutation testing in *KRAS* WT mCRC.
Loss of heterozygosity 18q	LOH 18q	Prospective studies regarding the prognostic value of LOH 18q are inconsistent. The ongoing E5202 will help provide additional data.
TP53	Mutation	Prospective studies regarding the prognostic value of *TP53* mutation are inconsistent. EGTM and ASCO recommend against *TP53* mutation analysis for prognosis.
Thymidylate synthase	Overexpression	Prospective studies regarding the prognostic value of thymidylate synthase overexpression are inconsistent.
VEGF	Overexpression	Data on VEGF expression status are limited; therefore, more studies are needed to determine VEGF expression status as a prognostic biomarker.

ASCO American Society of Clinical Oncology; *CRC* colorectal cancer; *DFS* disease-free survival; *dMMR* defective mismatch repair; *EGTM* European Group of Tumor Markers; *LOH* loss of heterozygosity; *mCRC* metastatic colorectal cancer; *MSI* microsatellite instability; *MSI-H* microsatellite instability-high; *MSI-L* microsatellite instability-low; *MSS* microsatellite instability-stable; *NCCN* National Comprehensive Cancer Network; *OS* overall survival; *WT* wild type

Table 2

Summary of predictive biomarkers for colorectal cancer treatment

Biomarker	General comments
Microsatellite instability	Several studies have shown that 5-FU adjuvant chemotherapy does not benefit patients with MSI-H or dMMR tumors. However, there are a few notable studies which demonstrate contrasting results, including a secondary analysis of a trial which suggested that irinotecan + 5-FU treatment may be of greater benefit to patients with MSI-H or dMMR tumors.
KRAS	Prospective studies have shown longer PFS and OS in *KRAS* WT mCRC treated with EGFR inhibitors alone or combined with 5-FU-based adjuvant therapy. NCCN and ASCO guidelines recommend *KRAS* mutation testing in all mCRC patients prior to EGFR inhibitor therapy.
BRAF	There are limited studies on the predictive nature of *BRAF* mutation status with CRC treatment. Currently, *BRAF* mutation status is an active area of investigation as a predictive marker of response for EGFR inhibitors.
Loss of heterozygosity 18q	There are limited studies on the predictive nature of LOH 18q with CRC treatment. The E5202 trial is currently investigating the role of LOH 18q as a predictive biomarker.
TP53	Several prospective studies have failed to demonstrate any correlation between *TP53* mutation status and response to adjuvant therapy. EGTM and ASCO recommend against *TP53* mutation analysis for predicting response to adjuvant therapy in CRC.
Thymidylate synthase	Prospective studies regarding the predictive value of thymidylate synthase overexpression are inconsistent.
VEGF	There are limited studies on the predictive nature of VEGF expression with CRC treatment. The predictive value of VEGF expression remains to be determined.

5-FU 5-fluorouracil; *ASCO* American Society of Clinical Oncology; *CRC* colorectal cancer; *dMMR* defective mismatch repair; *EGFR* epidermal growth factor receptor; *EGTM* European Group of Tumor Markers; *LOH* loss of heterozygosity; *mCRC* metastatic colorectal cancer; *MSI-H* microsatellite instability-high; *NCCN* National Comprehensive Cancer Network; *OS* overall survival; *PFS* progression-free survival; *WT* wild type