Genetics and Individualized Treatment for Colorectal Cancer

The clinical benefit of the genomics revolution is particularly evident in oncology. Genetic characterization of patients’ tumors has revealed that cancer is fundamentally a heterogeneous disease whose complex clinical course can be correlated to specific molecular profiles. Now, genomic data and tools are beginning to be translated from the laboratory to the clinic where physicians and patients can use them to assess risk, predict outcomes, and individualize treatment. Although this transition is still in its infancy, its early fruits are particularly striking in colorectal cancer (CRC) where several genetic tests are available as standard of care for prognosis and prediction of treatment response and emerging technologies promise to significantly improve patient care in the near future.

INTRODUCTION

As the second leading cause of cancer mortality in the United States, CRC is clearly a devastating disease. Although management of this disease has improved significantly in the past twenty years with five-year survival for patients with localized CRC now at 90%, this number drops to a dismal 10% in patients with metastases. As discussed elsewhere in this Series, advances in screening have largely been responsible for recent progress in CRC management. Screening has the potential to further dramatically reduce CRC mortality by facilitating detection of tumors at a curable stage, yet adherence to screening guidelines is problematic. Unfortunately nearly 50% of those at highest risk for developing CRC (individuals over the age of 50) are not screened and most cancers are only diagnosed at symptomatic presentation after they have significantly progressed. This situation highlights the need for ongoing innovations in CRC treatment.

Advances in individualized treatment ultimately hold out the best hope for improvements in patient survival when screening and prevention fail. A persistent challenge facing innovation in cancer therapeutics has been overcoming a “one size fits all” model in order to tease out prognostic, predictive, and druggable tumor markers in specific patients. This individualization is accomplished to some degree by standard clinical and histologic diagnosis. When patients progress to surgery, diagnosis is based on an evaluation of the tumor’s

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features via the TNM (Tumor, Node, Metastasis) staging system, which describes the primary tumor size and invasiveness, lymph node involvement, and the presence of metastases. These tumor characteristics help determine prognosis and inform a therapeutic approach, yet they do not always correlate directly with tumor behavior because homogeneous TNM characteristics can belie an underlying variability. Thus, while surgical and therapeutic strategies are effective in low-grade tumors, curative measures in advanced disease are rare. The challenge of treating advanced disease has in recent years been met by new technologies and significant advances in molecular analysis and bioinformatics to reveal the underlying genomic traits of cancer. The sequencing of cancer genomes has now confirmed longstanding clinical suspicions of underlying variability and shown unequivocally that cancer is a heterogeneous genetic disease with substantial differences in tumors even of the same subtype. This individualized, genetic understanding of cancer has birthed a new treatment paradigm in which tumor- and patient-specific mutations are exploited for their prognostic value, for risk assessment in hereditary CRC syndromes, and for prediction of treatment response.

The Genetic Basis Of Colorectal Cancer

Cancer is a genetic disease. It develops via a stepwise evolutionary process in which an initiating mutation occurs in the genome of one normal cell (or, in the case of hereditary cancer, the preliminary mutation is already present in all cells) and the subsequent progressive accumulation of mutations in that cell and its progeny leads to tumor formation. In cancer development these mutations drive the transformation of normal epithelial tissue first into a benign polyp and eventually into an invasive colorectal tumor. This multistep process typically proceeds over decades as mutations accumulate, deactivating critical tumor suppressor genes that keep cell growth in check and activating tumor promoter genes known as oncogenes that drive excessive growth, invasion into normal tissue, and spread to distant sites. Over the past 30 years, specific mutations have been correlated to specific stages of disease in CRC in particular, allowing for the creation of a generalized model of CRC progression based on genotype (Figure 1). In this model several important events include inactivation of the adenomatous polyposis coli gene (APC) which promotes transformation of normal colonic mucosa into an adenoma, activation of the KRAS or BRAF oncogenes which stimulates further aberrant growth of the adenoma, and genetic deletions of chromosome 18q followed by mutations in the p53 gene to facilitate the final transition from adenoma to carcinoma. The understanding that cancer is a disease resulting from the accumulation of such a core set of mutations in a cell has provided a framework for the individualization of cancer medicine through the correlation of these mutations with specific phenotypes.

This picture of cancer genetics is further complicated by the finding in large-scale cancer genome sequencing projects that many mutations can drive the formation of cancers and that not all tumors of the same type contain the same complement of mutations. The mutations listed above, though the most common mutations associated with certain developmental milestones in CRC formation, are nonetheless only present in 40-50% of CRCs. Further, a single tumor from one patient may contain dozens of mutations and cells within that tumor may even have different mutational profiles. This underlying genetic variability, evident in the varying therapeutic response rates of tumors with otherwise indistinguishable clinical presentation, is not apparent upon standard clinical or even histologic examination. Research is just now beginning to correlate specific mutations with response to therapy, ushering in the era of individualized medicine.

Individualized Standard Of Care Tests

As cancer genomics projects continue to correlate genetic profiles with tumor behavior, the long-prevailing “one size fits all” paradigm of cancer management is quickly fading. In CRC in particular, clinicians can now employ several patient-specific tests to assess risk, enhance prognosis, and predict response to therapy. In hereditary CRCs, this individualized approach is achieved via genetic testing whereas in sporadic CRCs it is achieved via tests for tumor-specific prognostic and predictive biomarkers. The distinction between prognostic and predictive markers is important for both patients and physicians to understand when considering adjuvant therapy. Prognostic markers provide information on how a disease develops in the absence of treatment by evaluating the aggressiveness and likelihood of recurrence whereas predictive markers guide the selection of a therapy that will likely provide the greatest benefit for a particular patient. An individual
biomarker may have both prognostic and predictive clinical value. Diagnostic markers are utilized to detect the presence of a tumor, monitor response to therapy and detect recurrence. Currently, the detection of predictive and prognostic biomarkers is performed by tumor tissue analysis. In the future, blood-based methods to detect biomarkers will simplify this process offering patients easy and accurate tests.

**Hereditary CRC**
Genetic testing represents the first major advance in individualizing the management of CRC through the use of genomic information. An important clinical goal of genetic testing is disease prevention through the identification of patients at high risk for developing CRC. Presymptomatic genetic testing of at-risk individuals can differentiate affected members from those unaffected by familial cancer. The accurate and early identification of individuals at increased risk for hereditary CRC syndromes is vitally important for the implementation of an effective cancer surveillance strategy for the affected individual and their family. Once a syndrome is identified, immediate screening of the family can eliminate the majority of cancer risk associated with hereditary CRC.

Germline genetic testing is available for hereditary CRC syndromes such as FAP, Lynch syndrome or hereditary non-polyposis colorectal cancer (HNPCC), MYH-associated polyposis, juvenile polyposis, and Peutz-Jeghers syndrome. Clinically, germline genetic testing is considered a risk assessment tool to determine whether or not an individual has inherited a genetic mutation that predisposes them to a greatly increased lifetime risk of developing colorectal cancer. This information can be used to help guide screening recommendations and risk-reduction measures early in life, largely eliminating the risk of developing colorectal cancer. Germline genetic testing is not typically used for the general public or for every patient diagnosed with colorectal cancer. The decision to use this type of risk assessment tool is made within the setting of a physician and genetic counselor and is based on an individual’s family history and clinical symptoms, including the number of and age of polyp development as well as extracolonic manifestations.

**Sporadic CRC**
Thus far, the extensive and growing list of mutations and mutational profiles associated with specific colorectal tumor types, their clinical course and response to therapy, has produced three predictive standard of care tests that are recommended by the NCCN Clinical Practice Guidelines in Oncology for Colon Cancer for use in clinical practice: KRAS mutation analysis, BRAF mutation analysis, and MMR testing. These tests will be discussed in terms of their clinical context, their
biology, and the evidence for their recommended use. KRAS mutations tend to occur early in colorectal carcinogenesis and are present in approximately 37% of CRCs. Mutations at particular sites in the KRAS gene have specifically been proven to be predictive of non-response to anti-epidermal growth factor receptor (EGFR) therapy in metastatic disease. EGFR is a cell surface receptor that transmits growth signals through a set of intracellular proteins regulated by KRAS to the nucleus instructing a cancer cell to reproduce and metastasize. Therapies targeting EGFR such as cetuximab (Erbitux, Bristol-Myers Squibb) or panitumumab (Vectibix, Amgen) function by blocking factors that stimulate EGFR thereby preventing the transmission of growth signals to the nucleus. It has been repeatedly shown that for tumors expressing a somatic KRAS mutation, these drugs have little to no effect. Studies of genetic expression have revealed that when KRAS is mutated, it is constitutively active and effectively turned "on" so that growth signals are transmitted to the nucleus regardless of EGFR status. While patients with mutated KRAS are unlikely to benefit from anti-EGFR therapy, there is no guarantee that those with KRAS wild-type will definitely respond, only that they may have a reasonable opportunity to derive clinical benefit. KRAS genotyping of primary or metastatic tumors is strongly recommended in patients with metastatic CRC and should be administered as a standard of care test, since there is minimal benefit for patients with mutated KRAS to endure the toxicity and expense of treatment with cetuximab or panitumumab. No specific methodology for assessing KRAS status is recommended by the NCCN guidelines, only that the test be performed by a laboratory certified for Clinical Laboratory Improvement Amendments (CLIA) in molecular pathology. KRAS status can be evaluated on formalin fixed paraffin embedded (FFPE) tissue from primary CRCs or metastases. If KRAS is wild type, BRAF testing should be considered as discussed below.

BRAF is an oncogene that is mutated in about 5-10% of CRCs and downstream of KRAS in the same growth pathway activated by EGFR signaling. BRAF mutations are also early events in CRC tumorigenesis and are typically mutually exclusive with KRAS mutations. For these reasons, when it became clear that KRAS could predict non-response to anti-EGFR therapy, retrospective studies were performed to explore the possibility that BRAF mutations could achieve the same goal. Although data from single-arm studies suggested this to be the case, further evaluation has shown that anti-EGFR treatment in the first-line setting is potentially beneficial regardless of BRAF status. After first-line therapy, a BRAF mutation may predict non-response to anti-EGFR, but data is still limited in this regard. Randomized controlled trials suggest, however, that BRAF mutation is a strong negative prognostic factor in metastatic disease. BRAF testing can also be performed on FFPE tissues in a CLIA-certified molecular pathology laboratory, usually via PCR and traditional sequencing. In summary, BRAF genotyping is useful prognostically, but not predictively, is optional in patients with metastatic disease and should only take place after wild type KRAS status is confirmed.

Microsatellite instability (MSI) is a colorectal cancer phenotype and form of genetic instability caused by alterations or loss of the DNA mismatch repair (MMR) system. The MMR system normally functions to recognize and repair nucleotides mismatched by DNA polymerase. A defective MMR system leads to stretches of repetitive DNA sequences (microsatellites) and instability (MSI). All patients with hereditary Lynch Syndrome and approximately 15% of sporadic CRC patients exhibit MSI and a defective MMR system. In Lynch syndrome the loss of mismatch-repair function is due to an inherited germline mutation in one of the MMR genes (MLH1, MSH2, MSH6 or PMS2). In sporadic CRC, MSI appears to be due to epigenetic modification of the MLH1 gene. MSI tumors present with distinct clinical and histologic features including the tendency to occur predominantly in the proximal colon, poor differentiation, lymphocytic infiltration and a signet-ring mucinous histology. In both sporadic and familial tumors, the loss of MMR function is readily assessed by either molecular MSI testing with PCR or immunohistochemical analysis (IHC). Molecular MSI analysis is performed by comparing a panel of five microsatellite markers from both tumor and normal tissue. The size of the microsatellite fragments are evaluated and assigned a level of stability according to criteria established by the National Cancer Institute (NCI). Samples with instability in two or more markers, or <30% of the markers, are defined as MSI-H (high-frequency MSI), samples with one unstable marker are designated MSI-L (low-frequency MSI) and samples with no instability in any of the markers are microsatellite stable (MSS). IHC analysis can also be used to determine MMR function by confirming the
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presence or absence of each of the four MMR gene protein products from a tumor tissue sample. Molecular MSI testing is currently considered the gold standard, though IHC offers good sensitivity and specificity. Both tests should be performed in a CLIA certified lab experienced with these methods.

MSI-H status has traditionally been used as a positive predictor to select patients for germline genetic testing for Lynch syndrome, however it has also recently been shown to correlate with patient survival and serve as a positive prognostic marker in sporadic CRC. Multiple studies have shown that patients with MSI-H tumors had better prognosis than those with MSS tumors. MSH-H was shown to be an independent prognostic marker associated with tumors that were less prone to lymph node involvement and metastases, indicating a favorable outcome compared to tumors with MSS. Determination of MSI status is useful for stage II CRC patients, for whom the benefit of adjuvant therapy is often unclear, given their low risk of recurrence. Overall, MSI-H status has shown excellent clinical utility in identifying stage II patients with good prognosis who are unlikely to derive benefit from 5-fluorouracil adjuvant chemotherapy.

Other Individualized Tests
There are several additional marketed tests based on genetic and molecular profiling that are intended to provide prognostic or predictive information for the individualization of CRC management, but whose utility in the clinic is still controversial. Such tests include Theraguide 5-FU, Oncotype Dx, and ColoPrint. Theraguide 5-FU from Myriad Laboratories categorizes risk of 5-FU toxicity into “high” (7-fold increased risk of toxicity), “moderate” (1.4-2.5-fold increased risk of toxicity), “low” (No increased risk of toxicity), or “indeterminate” based on detection of variations in two genes involved in fluoropyrimidine metabolism. The test is indicated for patients experiencing severe toxicity after receiving 5-FU (although some proponents advocate its use before treatment) in order to adjust dosage or choose an alternative regimen. Overall, the use of Theraguide 5-FU is controversial as there are no data from prospective randomized trials to demonstrate its impact on improving toxicity without compromising efficacy, thus the test has not yet been widely adopted in the clinic. Unlike the predictive Theraguide 5-FU, Oncotype Dx and ColoPrint are prognostic tests that use gene expression profiling for stage II CRC. These tests evaluate expression of specific gene collections in CRCs that can be correlated to tumor behavior to

Table 1. Available Somatic Tumor Mutation Tests With Clinical Utility

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<thead>
<tr>
<th>Test</th>
<th>Positive Result</th>
<th>Clinical Utility</th>
<th>Frequency/Incidence</th>
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<tbody>
<tr>
<td>KRAS</td>
<td>Mutation in codons 12,13, or 61 in exons 2 and 3</td>
<td>Tumors with mutations exhibit no response to anti-EGFR therapy.</td>
<td>35-45%</td>
</tr>
<tr>
<td>BRAF</td>
<td>V600E point mutation</td>
<td>Tumors with wild-type KRAS should be tested for BRAF mutations. BRAF mutation positive tumors exhibit no response to anti-EGFR therapy.</td>
<td>10%</td>
</tr>
<tr>
<td>MSI</td>
<td>Loss of any one of MLH1, MSH2, MSH6 or PMS2 protein products via IHC or instability in &lt;30% of microsatellite markers (MSH-H) via MSI analysis</td>
<td>MSI-H identifies Stage II patients with good prognosis who would not benefit from 5-FU based adjuvant therapy.</td>
<td>15%</td>
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evaluate risk of recurrence and possibly select low-risk patients who can be spared chemotherapy. They require tissue and molecular pathology laboratories as do the current standard of care tests. Although an Oncotype DX gene expression profile has been accepted by ASCO and the NCCN as standard of care in breast cancer for the prediction of recurrence in specific contexts, neither of these tests has experienced widespread clinical adoption for CRC since their absolute prognostic and predictive value is still unclear in this disease. Both require further validation in prospective trials. Circulating tumor cell (CTC) analysis identifies and quantifies CRC cells in the plasma through the identification of tumor specific cell surface markers. Though the US Food and Drug Administration has approved certain methods for identifying CTCs, current guidelines do not recommend using CTC’s to assess prognosis or to guide therapy selection due to their low sensitivity, specificity and lack of clinical data. Ongoing evaluations may yet confirm some of these tests for standard of care.

Meanwhile, emerging technologies such as those based on tumor specific genomic alterations detectable in the plasma have the potential to meet some of the shortcomings of the first generation of individualized tests. Through the administration of a simple blood draw, plasma based tests promise to offer highly accurate and sensitive tumor status analysis. An example of next-generation individualized biomarkers lies in technologies such as PARE (Personalized Analysis of Rearranged Ends), in which patients’ tumors are individually sequenced in search of chromosomal rearrangements specific to their tumors that can be used in an exquisitely tailored manner to monitor tumor progression, therapeutic response, and to detect residual and/or recurrent disease. A DNA detection technology known as BEAMing (Beads, Emulsions, and Amplification), which may be nearing clinical availability, is also able to provide a highly specific blood-based test to detect the mutational profile of tumors and monitor therapeutic responses with extremely high sensitivity. This test exploits the fact that as tumor cells die, they release mutant gene fragments into circulation. Through the precise and highly sensitive detection and quantification of these mutations in peripheral blood, clinicians will be able to select therapies specific to the mutational profile of a patient’s tumor without the need for tissue biopsy analysis as well as monitor response to therapy in real-time. As costs for the genomic analysis of blood and tissue DNA decreases and more prospective clinical data is generated, emerging technologies such as PARE and BEAMing promise to truly individualize the management of CRC.

CONCLUSION

An individualized approach to managing colorectal cancer is gradually becoming an integral component of care. This will eventually result in more effective treatment with fewer side effects and will promote a more active role for patients in making decisions related to their health care. Future research promises to improve our understanding of the differences between those patients whose cancers respond favorably to certain therapeutics and those that do not. Just as pathology has been used to determine the stage of a colorectal cancer and subsequent prognosis for patients, molecular tools will be increasingly utilized to provide highly specific information to accurately diagnose and treat colorectal cancer. The advent of individualized medicine, while relatively new to the field of colorectal cancer, presents patients and physicians with a complex set of tools that when properly utilized, can lead to better treatment outcomes for those with cancer and provide important information for managing and largely eliminating the risk of cancer in hereditary conditions.

References