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Biomarker-directed Targeted Therapy in Colorectal Cancer

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With advances in the understanding of the biology and genetics of colorectal cancer (CRC), diagnostic biomarkers that may predict the existence or future presence of cancer or a hereditary condition, and prognostic and treatment biomarkers that may direct the approach to therapy have been developed. Biomarkers can be ascertained and assayed from any tissue that may demonstrate the diagnostic or prognostic value, including from blood cells, epithelial cells via buccal swab, fresh or archival cancer tissue, as well as from cells shed into fecal material. For CRC, current examples of biomarkers for screening and surveillance include germline testing for suspected hereditary CRC syndromes, and stool DNA tests for screening average at-risk patients. Molecular biomarkers for CRC that may alter patient care and treatment include the presence or absence of microsatellite instability, the presence or absence of mutant *KRAS, BRAF* or *PIK3CA*, and the level of expression of *15-PGDH* in the colorectal mucosa. Molecularly targeted therapies and some general therapeutic approaches rely on biomarker information. Additional novel biomarkers are on the horizon that will undoubtedly further the approach to precision or individualized medicine.

Key Words: Colorectal cancer, Biomarker, Precision medicine, Molecularly targeted therapy, Microsatellite instability, KRAS, BRAF, PIK3CA, 15-PGDH, Familial colorectal cancer

INTRODUCTION

Colorectal cancer (CRC) develops as a consequence of intrinsic genetic changes that are influenced by local environmental factors.[1] Some genetic changes are exemplified from inherited germline mutations in which the CRC presents as an extreme case of the somatic mutation in sporadic colorectal cancer.[2] Among sporadic CRC patients, these genetic changes present as part of somatic *driver* mutations, usually 2 to 8 per individual CRC, that propel normal mucosa and benign precursor adenomas to malignancy, while others alterations are passenger mutations that may be multiple and appear to be happenstance during neoplastic progression.[3] In particular, the presence of driver gene mutations or epimutations may lend itself as a biomarker in which molecularly targeted therapy might intervene to modify the outcome of the patient, whereas passenger mutations might be helpful for diagnostic purposes to show the presence or absence of the neoplastic process in a particular patient.

A comprehensive molecular analysis of sporadic CRCs confirmed about 30 years of individual discoveries from which biomarkers might be drawn from.[1,4,5] CRCs can be grouped into *hypermutated*, ~15% of CRCs with the accumulation of a few driver and hundreds of passenger gene mutations,

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Abbreviations used: CRC: colorectal cancer; 15-PGDH: 15-hydroxyprostaglandin dehydrogenase; WT: wild-type; MMR: DNA mismatch repair; MSI: microsatellite instability; FIT: fecal immunochemical test; FAP: familial adenomatous polyposis; MAP: MYH-associated polyposis; PPAP: polymerase proofreading associated polyposis; FCCTX: familial colorectal cancer type X; NSAID: non-steroidal anti-inflammatory drug; VEGF: vasculare endothelial growth factor; EGFR: epithelial growth factor receptor; 5-FU: 5-fluorouracil' PGE2: prostaglandin E2;
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and *non-hypermutated*, ~85% of CRCs in which a few driver and only tens of passenger gene mutations but extensive copy number variation (aneuploidy) exists.[5] The somatic mutations and epimutations are different between hypermutated and non-hypermutated cancers. Hypermutated CRCs are driven by loss of DNA mismatch repair (MMR) through hypermethylation of *MLH1* or *POLE* inactivation, with most somatic mutations occurring principally in genes with coding microsatellite sequences generating microsatellite instability (MSI), in addition to *BRAF* mutation.[1,5] Non-hypermutated CRCs are driven by mutation and loss of APC and *TP53*, and consistently show mutation of *KRAS* and *PIK3CA*.[1,5] The combination of APC and TP53 inactivation appears to be a trigger for the extensive chromosomal instability and aneuploidy observed in non-hypermutated CRCs.[6] The consolidated knowledge from these extensive genetic and genomic analyses continues to inform the approach to biomarker utility, and its use into a clinically actionable change for improved patient care (Table 1).

BIOMARKERS FROM DRIVER AND PASSENGER MUTATIONS FOR CRC SCREENING

Fecal DNA Testing

Fecal DNA testing was developed based on knowledge of the genetics of the adenoma-to-carcinoma sequence in the colon, with the promise of being a noninvasive approach to CRC screening in the general, asymptomatic population over the age of 50 years.[2] Initial prototypes and versions included a plethora of genetic targets, including *APC, KRAS, TP53*, the microsatellite *BAT-26*, among several others, following a shotgun approach towards detection. Over time, fecal DNA tests have become refined in their targets, utilizing a mixture of driver and passenger gene alterations, including methylation of *NDRG4* and *BMP3, KRAS*, and with the inclusion of a fecal immunochemical test (FIT).[7] Most important is the ability of the fecal DNA test to detect adenomas and cancer in asymptomatic patients, and this has improved with each upgraded version of the test. The current version of the test (v3.0), approved by the U.S. Food and Drug Administration in 2014 with approved insurance covered by the U.S. Centers for Medicare and Medicaid Services, shows a 92.3% detection of CRC and a 42.4% detection of advanced adenomas (>9 mm, villous or malignant component), and a 42.4% detection of serrated polyps >1 cm, with a specificity of 86.6%. This compares to FIT alone, showing only 73.8% of CRC and 23.8% of advanced adenomas detected, 5.1% of serrated polyps >1cm detected, and a specificity of 94.9%.[7] Thus, fecal DNA testing greatly improves upon FIT testing alone, with a slight cost in specificity. Further refining the biomarkers utilized in fecal DNA testing will likely continue to improve detection of advanced adenomas in future versions of the test, and will likely be used more widely depending on the costs of the test as a noninvasive alternative for mass asymptomatic CRC screening.

Germline DNA Testing

Clinical suspicion that a familial CRC syndrome might be present generally comes from a strong family history for syndromic cancers, personal history of cancer(s), a young age

Table 1. Some current biomarker examples from colorectal cancer patients that modify the clinical approach to care. CRC=colorectal cancer

Biomarker	Clinical Utility
Germline mutation in various genes associated with hereditary CRC in white blood cell or buccal swab epithelial cell	Determines or confirms risk of cancer in patient/proband; information can extend to testing of related family members to determine their risk; sets up appropriate surveillance in at-risk patients and family members to extend lifespan
KRAS mutation; *BRAF* mutation in primary colorectal cancer specimen	Avoid use of EGFR inhibitors due to incessant signaling with mutation present in stage IV CRC patients
Methylation of *NDRG4* and *BMP3*, and KRAS mutation in NextGen Multitarget v3.0 Fecal DNA Test (stool)	Colorectal cancer screening in average risk patients every 3 years; if positive, perform colonoscopy
PIK3CA mutation in primary colorectal cancer specimen	Aspirin use can be effective with *PIK3CA* mutation present for secondary prevention
15-PGDH expression in normal colorectal mucosa	Aspirin can be effective to lower CRC risk with high levels of 15-PGDH
DNA mismatch repair protein expression (or microsatellite instability) in primary colorectal cancer specimen	Absence approximates microsatellite instability; could identify Lynch syndrome patient; predicts overall improved outcome with absence; absence predicts poor response to 5-fluorouracil chemotherapy

of onset of cancer in the proband or family, as well as the presence of clinical features of the syndrome.[2] After genetic counseling, appropriate genetic testing for mutated genes that may fit that syndrome would ensue in the most appropriate family member that can potential yield a detected mutation. Individual gene testing, that is, testing one or a related group of genes one at a time, was the norm. Cost reductions and advances in technology have allowed whole exome sequencing and whole genome sequencing to be offered for some tests.[2] Panels of genes that can simultaneously examine several genes for CRC risk are now offered commercially, and data suggests that this approach has high yield for unsuspected mutations in other genes,[8] and may be more cost effective compared to one gene examine at a time with some conditions.[9]

Essentially all germline mutations for familial CRC syndromes are driver mutations that cause the extreme presentation of the typical somatic mutation in a family or proband. Adenomatous polyposis syndromes follow the genetic mutational burden of hypermutated or non-hypermutated groups, like sporadic CRCs. Familial adenomatous polyposis (FAP), an autosomal dominant condition caused by germline mutation of the APC gene, and its near phenocopy, *MYH*-associated polyposis (MAP), an autosomal recessive condition caused by biallelic inheritance of two mutant MYH repair genes, are non-hypermutated conditions that show aneuploid CRCs.[2] The pattern of inheritance in the family pedigree is key to distinguishing FAP and MAP, as both can present with or show risk for oligopolyposis to extensive colonic polyposis, desmoids, duodenal and ampullary polyps and cancers. Conjoined testing of these two syndromes sometimes makes sense if clinically they are indistinguishable. However, the risks of cancer in related family members is different due to the inheritance pattern. Lynch syndrome, an autosomal dominant condition caused by germline mutation of one of several DNA mismatch repair (MMR) genes (*MLH1, MSH2, MSH6, PMS2, EPCAM*) and polymerase proofreading associated polyposis (PPAP), caused by germline mutation in the exonuclease domain of the polymerase genes *POLE* or *POLD1*, generate hypermutated CRCs. Lynch syndrome CRCs demonstrate MSI, whereas PPAP cancers do not,[5] and Lynch syndrome is much more common in the CRC population (~3%) compared to PPAP (a fraction of one percent, with only a handful of families described in the literature).[2] Both Lynch syndrome patients and some PPAP patients will develop CRCs as well as some endometrial and brain tumors for clinical spectrum overlap.[10] Most clinics specializing in hereditary CRC would test individuals for Lynch syndrome, particularly if any obtained cancer from the proband or blood relative shows MSI. Assessing for PPAP, due to it being a more rare condition, would only ensue if Lynch testing was negative. A phenocopy of Lynch syndrome, Lynch-like syndrome, has as the only difference from Lynch syndrome the lack of detectable germline mutation in a DNA MMR gene. Both conditions show MSI in their CRCs, and both present at younger age of cancer onset compared to sporadic patients. Lynch-like patients show two somatic hits within the CRCs.[11] Another Lynch syndrome phenocopy is the Familial CRC Type X syndrome (FCCTX), in which rare individual gene mutations define families without a common germline target.[12-15] Tumors of FCCTX patients do not show MSI and have intact DNA MMR function.[12]

Germline DNA testing for the rare hamartomatous polyposis syndromes also detect driver mutations, with the pattern of CRC formation from hamartomatous polyps likely being in the non-hypermutated grouping given no loss of major DNA MMR function or polymerase function. CRCs have been shown to arise from the hamartomatous polyps through adenoma transition.[16] Patients or families generally have a classic or partial observable phenotype associated with hamartomatous and/or hyperplastic polyps. These include *PTEN* germline mutations in the *PTEN* Hamartoma Syndrome (including Cowden's Disease, Lhermitte-Duclos, and Bannayan-Riley-Ruvulcaba syndromes); *SMAD4*, and *BMPR1A* germline mutations in juvenile polyposis syndrome with hereditary hemorrhagic telangiectasia overlap; *STK11* germline mutations in Peutz-Jeghers syndrome; and *GREM1* overexpression in Hereditary Mixed Polyposis syndrome.[2] There is no germline testing for serrated (hyperplastic) polyposis syndrome as generally there is no family history among probands, and no known association with any mutated genes to date.[2]

Overall, germline DNA testing is a biomarker that determines the clinical and surveillance approach to an affected patient and their family.

BIOMARKERS FROM DRIVER MUTATIONS FOR CRC PATIENT TREATMENT

The approach to CRC largely involves screening asymptomatic as well as susceptible populations. Chemoprevention and secondary prevention approaches to CRC involve the use of aspirin and NSAIDs, with the caveat for gastrointestinal side effects.[17] Treatment of CRC involves combinations of drugs tied to oral or parenteral forms of 5-fluorouracil (5-FU), and may include oxaliplatin and/or irinotecan for some stage II, all stage III, and some stage IV patients.[18,19] Molecularly targeted therapies have been added to treatment regimens for stage IV patients and include bevacizimab (targeting VEGF), and cetuzimab and panitumab (both targeting EGFR) and are effective in extending survival in combination with

the standard chemotherapy regimens.[18] Biomarkers from driver gene mutations have emerged that inform the practitioner regarding the utility of molecularly targeted therapies, as well as identifying the optimal patients for primary and secondary prevention.

Microsatellite Instability and Adjuvant 5-Fluorouracil Treatment

Microsatellite instability (MSI) is a biomarker for the absence of DNA MMR function.[1,20] This can be observed in patients with sporadic CRC (hypermethylation of *MLH1* and the presence of $BRAF^{V600E}$ mutation), Lynch syndrome patients (germline DNA MMR gene mutation), and Lynch-like syndrome patients (biallelic somatic DNA MMR gene mutation),[11] and CRCs are hypermutated. A surrogate for MSI is the absence of immunohistochemical detection of a DNA MMR protein from the CRC.[1,11,20]

The presence of MSI in CRC is associated with improved outcome when compared to same-staged CRC patients without MSI.[1,2,20,21] However, stage II and III patients whose CRCs manifest MSI do not appear to respond to 5-FU for improved survival like patients whose tumors do not show MSI.[22,23] Biochemical studies demonstrate that intact DNA MMR function is needed to recognize 5-FU that gets incorporated into DNA to trigger cell death of CRC cells, and this process is abrogated with MMR deficiency rendering CRC cells resistant to 5-FU.[24-27] Detection of the absence or presence of MSI has been rolled into commercialized prediction models to determine the consideration for 5-FU based chemotherapy for stage II patients.[28]

Mutant KRAS and BRAF and the Use of EGFR Inhibitors

About 55% of all CRCs show activating mutations in *KRAS*, *NRAS* or *BRAF* (with near exclusivity of found mutations between these oncogenic proteins), intracellular signaling components downstream of EGFR that incessantly signal through MAPK to turn on cellular proliferation.[5] In addition, about 7% of CRCs overexpress EGFR itself.[5] Use of the molecularly targeted EGFR inhibitors such as cetuximab and panitumab, two compounds approved for treatment for stage IV CRC patients, do not work effectively in patients carrying mutations in *KRAS* or *BRAF* due to the unhinging of EGFR regulation of these mutant downstream effectors. Hazard ratios for progression-free survival when an EGFR inhibitor is added to a 5-FU standard regimen was ~1.1 (showing no effectiveness compared to the standard regimen alone) in patients manifesting mutant *KRAS*, but was 0.7 (showing more effectiveness) in patients manifesting WT KRAS in their CRC.[29] Detection of the presence or absence of mutant KRAS has become the standard of care before considering EGFR inhibitor therapy.

Mutant PIK3CA and Aspirin Usage

Patients who have had CRC are surveyed more frequently because their risk for recurrence is greater than the general population. There is some evidence that secondary prevention via the regular use of aspirin, in addition to colonoscopic surveillance, might curtail recurrence risk. Aspirin inhibits the cyclooxygenase 2 enzyme (Cox-2), which downregulates WT PI3 Kinase, part of a mitogenic pathway that signals through AKT and mTOR, and is antagonized by PTEN. Activating mutations in a component of PI3 Kinase, *PIK3CA*, commences incessant oncogenic signaling. Thus, aspirin essentially mimics the normal antagonism of PTEN upon PI3 Kinase. Mutant *PIK3CA* has been shown to be a biomarker for aspirin effectiveness. Patients whose original CRCs show mutant *PIK3CA* have a lower probability for CRC-specific death with the regular use of aspirin compared to those patients who did not use aspirin.[30] There was no difference in the probability of death between aspirin takers and non-takers if their CRC showed WT *PIK3CA*.[30] Thus, the presence of mutant *PIK3CA* is a predictor for aspirin effectiveness as a secondary chemoprevention agent.

15-PDGH Expression and Aspirin Usage

15-hydroxyprostaglandin dehydrogenase (15-PGDH) is an enzyme that inactivates prostaglandin E2, a pro-proliferative prostaglandin generated by Cox-2 and action by microsomal PGE2 synthase-1. While Cox-2 levels are elevated in CRCs, generating more PGE2, 15-PGDH levels are diminished in CRCs, preventing the destruction of PGE2 and further enhancing its levels. The expression levels of *15-PGDH* from normal colorectal mucosa can predict the effectiveness of aspirin as a primary or secondary preventive agent. Compared to nonusers of aspirin, regular users of aspirin had no benefit from the aspirin if their *15-PGDH* levels were low (hazard ratio of 0.90, 95% CI 0.63 to 1.27, p=0.53).[31] However, regular aspirin users with high mucosal levels of *15-PGDH* demonstrated hazard ratios for CRC of 0.49 (95% CI 0.34 to 0.71, p=0.0002).[31] Thus, regular use of aspirin is associated with a lower risk for CRC in which the colorectal mucosa expresses high levels of *15-PGDH*. This biomarker can stratify who might benefit from aspirin, while avoiding the side effects of aspirin in the population who would not benefit from it.

CONCLUSIONS

Biomarkers are increasingly being used in the decision-

making and care of CRC patients. They can inform practitioners regarding patients who most benefit from a treatment approach, while avoiding unnecessary or non-beneficial effects in patients in which the biomarker indicates that such treatment won't be effective. Biomarkers can inform diagnostic approaches, such as screening and/or surveillance for CRC in patients at average or elevated risk. Biomarkers help discern individualized care, or precision medicine (Table 1). Future biomarkers that inform precision medicine for CRC patients will include: the detection and targeting of circulating tumor cells and RNAs in the blood, the development of individualized immunovaccines, and whole exome or whole genome sequencing to examine individual driver mutation(s) for each person's CRC.[2]

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