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Recent Advances in the Use of Molecular Biomarkers in Colorectal Cancer

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Abstract

Globally, colorectal cancer (CRC) is the third most common cancer in men and the second most common cancer in women, accounting for an estimated 1.4 million new cases and almost 700,000 deaths in 2012. Global incidences vary 10-fold, with the highest rates occurring in developed countries (e.g., Australia, New Zealand, Europe, the United States of America [USA]) and the lowest rates occurring in Africa and South-Central Asia. Within Europe in 2008, the highest incidence of colorectal cancer was in Hungary and Denmark. The lowest incidence was in Cyprus and Greece. In Ireland an annual average of 1445 colon cancer and 606 rectal cancers was registered between 2005 and 2009. Approximately 21% of patients with CRC have metastases at diagnosis, and nearly 50% have cancers that will eventually metastasize, accounting for the high mortality rate. Patients with early stage CRC often have no symptoms, which reinforces the importance of screening. This activity briefly describes the rationale for using molecular markers in the diagnosis and prognosis of CRC.

Keywords: Colorectal cancer; MSI-H; KRAS mutation; BRAF mutation; Anti-EGFR therapy

Introduction

Why use molecular markers?

Although CRC has historically been treated as one disease, the molecular profile of each tumour is unique and characterized by multiple genetic and epigenetic changes [1-6]. In recent years, these molecular profiles have been used to better understand disease biology and in some cases guide treatment decisions [2,5]. Some of these markers include [7,8].

1. Gene mutations (*KRAS, NRAS, BRAF, PIK3CA, APC, TP53, and SMAD4*)

2. Copy number alterations (*ERBB2 and MET*)

3. Changes in methylation status (*MLH1*)

4. Translocations

5. Impaired expression at the mRNA or protein levels

Numerous studies indicate that several major subtypes of CRC exist, and each subtype is associated with a characteristic molecular pathogenesis and natural history [9]. Because CRC tumours may have different genetic mutations and alterations driving their growth, targeted therapeutics are not equally active in all patients [10]. Classic examples are the anti-epidermal growth factor receptor (EGFR) monoclonal antibodies cetuximab and panitumumab; cetuximab is approved for the treatment of *KRAS* wild-type, EGFR-expressing metastatic CRC (mCRC), and panitumumab is approved for the treatment of *RAS* wild-type mCRC (defined as wild-type in both *KRAS* and *NRAS* as determined by a US Food and Drug Administration [FDA]-approved test for this use) [10-12]. In unselected patients, the clinical benefit from monotherapy with either drug is modest [10]. However, response rates nearly double when the population of potential responders is enriched by predictive biomarkers such as *KRAS* and *NRAS*. [10] Although more research is needed to determine a comprehensive and unified molecular classification of CRC that can be translated into clinical practice, molecular markers are already routinely used to determine prognosis and guide treatment decisions [6,10,12,13].

Prognosis and drug selection: Mutations in KRAS, *NRAS, BRAF and ERBB2*

For patients with mCRC, genomic profiling is critical to identifying appropriate therapies [6]. A recent analysis showed that the prevalence of *RAS* (*KRAS* or *NRAS*) mutations in mCRC is as high as 60%, with mutations in *KRAS* exon 2 being the most common (43%), followed by *KRAS* exon 4 (6.2%), *NRAS* exon 3 (4.2%), *KRAS* exon 3 (3.8%), *NRAS* exon 2 (2.9%), and *NRAS* exon 4 (0.3%) [6]. Several clinical trials in mCRC have shown that *RAS* mutation status predicts response to anti-EGFR therapy, in particular to cetuximab and panitumumab [8,14,15].

Although treatment with anti-EGFR agents—either as single agents or in combination with chemotherapy—offers improved outcomes in patients with RAS wild-type mCRC, nearly all patients eventually develop treatment resistance.[6] In addition, several other potential molecular alterations have been associated with primary or acquired anti-EGFR treatment resistance [10,16]. For example:

BRAF: *BRAF*-activating mutations occur in approximately 8% of patients with mCRC [16]. Despite recent improvements in survival for the general population of patients with mCRC, multiple studies show that patients with *BRAF*-mutant mCRC continue to have poor responses to most systemic therapies, including anti-EGFR therapies, and prognosis remains poor [10,17]. *BRAF*-mutated tumours are associated with female sex, advanced patient age, right-sided colon location, high-grade and mucinous histology, and peritoneal metastases [6,17].

ERBB2: Emerging data indicate that *ERBB2* (also known as "*HER2*") amplification is a marker of resistance to anti-EGFR therapy [8,10,18]. Several studies suggest that anti-*HER2* therapies, which are

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currently approved by the US FDA for the treatment of other tumour types (breast and gastric), are active in patients with *HER2*-amplified mCRC [19-22]. Referral to a clinical trial is encouraged for patients who fall into this category [13].

Genomic markers of acquired resistance

Researchers have used "liquid biopsies" to determine the genetic drivers of acquired EGFR antibody resistance [8,23-25]. Several studies have found that tumour-derived *KRAS* and *NRAS* mutations emerge under the selective pressure of anti-EGFR therapy [24-26]. Additionally, acquired EGFR mutations ("extracellular domain mutations") can affect cetuximab and panitumumab binding, leading to decreased effectiveness of these therapies [8,26]. Other potential genetic alterations associated with EGFR antibody resistance include, but are not limited to, mutations or amplifications in *BRAF, MAP2K1, ERBB2, MET*, and *KIT* [25,26].

Microsatellite instability (MSI)

A loss of DNA mismatch repair (MMR) occurs in approximately 15% of patients with CRC [27]. MMR deficiency leads to DNA replication errors, resulting in changes in the nucleotide length of microsatellites (regions of the genome with nucleotide repeats [eg, CACACA]) [28,29]. Over time, these cumulative replication errors lead to high levels of MSI (MSI-H) [28].

Because of its clinical importance, MSI testing is recommended in all patients with resected or metastatic CRC [13]. Indeed, among patients with resected stage II and stage III CRC, MSI-H disease is associated with favourable prognosis and predicts limited benefit from adjuvant 5-fluorouracil (5-FU) monotherapy [30,31]. Additionally, approximately one-quarter of MSI-H tumours occur as a result of a germline mutation (Lynch syndrome, also known as hereditary nonpolyposis CRC) [32]. Therefore, the recognition of MSI-H CRC is critical to identifying patients who are appropriate for genetic counselling and germline testing [33].

Immunotherapies are active in patients with MSI-H mCRC [34]. Based on its durable responses and long-term survival benefit, the anti–programmed cell death 1 receptor (PD-1) monoclonal antibody pembrolizumab was recently approved by the US FDA for use in adult and paediatric patients with unresectable or metastatic, MSI-H or MMR-deficient solid tumours that have progressed following prior treatment and who have no other satisfactory alternative treatment options, or in patients with CRC that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan [35,36]. Similarly, the anti–PD-1 therapy nivolumab was recently approved by the US FDA for use in adult and paediatric patients with MSI-H or MMR-deficient mCRC that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan; in addition, this agent has demonstrated significant clinical activity in patients with MSI-H mCRC in combination with the cytotoxic T-lymphocyte-associated protein 4 inhibitor ipilimumab (the combined use of nivolumab and ipilimumab in this context is currently off-label) [37,38]. To broaden the patient populations that may benefit from immunotherapy, novel strategies and treatment combinations are under development.

Discussion on Current Guidelines

Recently, representatives from the American Society for Clinical Pathology (ASCP), the College of American Pathologists (CAP), the Association for Molecular Pathology (AMP), and the American Society of Clinical Oncology (ASCO) collaborated to provide evidence-based recommendations and establish standard molecular biomarker testing strategies for CRC tissues to guide anti-EGFR and conventional chemotherapy regimens [16]. The guideline document contains 21 consensus statements and includes critical recommendations for the use of *RAS* and *BRAF* markers. For example, it covers key areas such as [16].

RAS mutational analysis (KRAS and NRAS exons 2, 3, and 4) for CRC patients considered for anti-EGFR therapy

It is now recommended that clinicians perform "extended" or "expanded" *RAS* mutational analysis (that includes *KRAS* exons 2, 3, and 4 and *NRAS* exons 2, 3, and 4) before the administration of anti-EGFR therapy. Together, *KRAS, NRAS,* and *BRAF* mutations have been reported to occur in more than one-half of all CRC cases. Cetuximab and panitumumab are monoclonal antibodies that bind to the extracellular domain of EGFR, blocking the binding of EGF and other EGFR ligands, thereby blocking EGFR signaling. Targeted anti-EGFR therapies increase progression-free and overall survival in patients with *KRAS* and *NRAS* wild-type mCRC but not in patients with *KRAS*- or *NRAS*-mutated mCRC.

BRAF V600 position mutational analysis for prognostic stratification in select CRC patients

It is important to know the *BRAF*c.1799 (p.V600) mutation status of a patient's CRC because patients with BRAF-mutated mCRC have worse outcomes than those without the mutation, and standard therapy often provides limited benefit. For such patients, data suggest that the use of FOLFOXIRI (folinic acid, 5-FU, irinotecan, and oxaliplatin) plus bevacizumab as first-line therapy may improve outcomes [39]. Recent data also suggest that novel therapeutic strategies (eg, cetuximab plus irinotecan plus vemurafenib [off-label]) may provide therapeutic benefit [40]. In addition, those with *BRAF*-mutated mCRC should be considered for clinical trial participation.

Laboratory approaches for the molecular testing of biomarkers (eg, selection of assays, type of specimens, timing of ordering, turnaround time)

Although earlier testing approaches focused on one or a few testing targets (eg, *BRAF* p.V600 mutations), current approaches are increasingly using multi-gene panels, such as targeted next-generation sequencing cancer panels that include hundreds of genes and amplicons known to be mutational hotspots in cancer. The aim of future research is to identify biomarkers that can provide a noninvasive and cost-effective diagnosis—the optimal panel of prognostic biomarkers—as well as predictive biomarkers for current and future treatment regimens.

Conclusion

Many recent studies have examined different aspects of CRC, expanding the understanding of the disease at the molecular level and promoting the development of new treatment regimens, especially those used in advanced CRC. The mutational testing of genetic biomarkers can provide clinically actionable information to support treatment decisions, including the use of targeted therapy with monoclonal antibodies.

Biomarker testing results can have prognostic value. For example, *BRAF* mutations are consistently associated with poor outcomes in mCRC patients, including those who relapse after adjuvant therapy, whereas patients with MSI-H tumors may have better prognosis.

Mutational testing can have predictive value for targeted therapy with monoclonal antibodies. For example, mutations in *KRAS* and *NRAS* exons 2, 3, or 4 predict lack of benefit with anti-EGFR therapies. Because *KRAS* and *NRAS* mutation status is critical for guiding therapy, all patients with mCRC should undergo testing. In addition, data suggest that MSI status predicts benefit from immunotherapy and can be used to guide treatment decisions.

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