Surgical Oncology 25 (2016) 190–199

Contents lists available at ScienceDirect

Surgical Oncology

journal homepage: www.elsevier.com/locate/suronc

Molecular markers of prognosis and therapeutic targets in metastatic colorectal cancer

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ARTICLE INFO

Article history:
Received 29 February 2016
Accepted 19 May 2016

Keywords:
Colorectal
Targeted therapy
Metastatic colorectal cancer
Molecular markers
Biologic therapy

ABSTRACT

Metastatic disease ultimately occurs in approximately 50–70% of patients presenting with colorectal cancer. In patients with advanced disease, there is significant variability in individual patient outcomes. To improve understanding of tumor behavior, markers such as *KRAS* and *BRAF* mutation status are increasingly utilized. Additionally, newer surrogates of tumor biology, such as telomerase activity and the prevalence of circulating tumor cells and circulating tumor DNA, have generated increasing interest due to clinical potential. While the extent to which these newer markers can predict outcome and guide therapy is yet to be determined, *KRAS* mutation status is currently used to guide systemic therapy in selected patients. Furthermore, advances in our understanding of various tumorigenic pathways (such as the mitogen activated protein kinase pathway) have enabled newer targeted agents, including BRAF inhibitors. Interestingly, although inhibition of BRAF in patients has not translated into improved outcomes, characterization of *BRAF* mutations led to an association with microsatellite instability. A unique histologic characteristic of certain tumors in patients with microsatellite instability is the infiltration by lymphocytes at the tumor-stromal interface. This feature highlights the biology of the tumor in its microenvironment and underlies the efficacy of the programmed-death inhibitor, pembrolizumab, in patients with microsatellite unstable metastatic colorectal cancer. With an increasing number of prognostic markers and therapeutic options in metastatic colorectal cancer, the multidisciplinary approach becomes critical for appropriate treatment decisions.

1. Introduction

Primary colorectal cancer (CRC) is one of the most common cancers in Western society with up to 50–70% of patients developing metastatic disease [1,2]. Overall survival (OS) for patients with unresectable metastatic colorectal cancer (mCRC) is poor, with a median survival of approximately 24–27 months and 5 year survival of 10–15% [3]. While surgical resection represents the best chance at cure, only a subset of patients is eligible for curative-intent surgery. In addition, among patients who undergo curative-intent surgical resection, median survival is 40–55 months; however, long-term 10-year survival is only about 15–25% when surgery is combined with multimodal systemic therapy [1,2]. In fact, even in the setting of a microscopically complete (R0) resection, approximately 50–75% of patients who undergo a curative-intent resection will experience disease recurrence by 5 years [4–6].

Given the high incidence of recurrence following resection, there has been an interest in the risk stratification of patients following surgery, as well as the selection of patients for adjuvant multimodal therapy. Risk stratification of patients with mCRC has historically been guided by evaluation of various clinical and pathologic features. For example, Fong and colleagues proposed the "Clinical Risk Score" (CRS) to stratify patients into low versus high risk groups (i.e. OS high CRS, 32 months vs. low CRS, 46 months; $p < 0.05$) [6]. More recently, radiographic and pathologic response to chemotherapy has been proposed as a more useful and clinically meaningful tool to assess risk of recurrence and stratify patients with regard to long-term survival [7–9]. For example, the Response

Abbreviations: mCRC, metastatic colorectal cancer; CRC, colorectal cancer; OS, overall survival; DFI, disease free interval; MSI, microsatellite instability; CTC, circulating tumor cells; ctDNA, circulating tumor DNA; MMR, mismatch repair; MSS, microsatellite stable; EGFR, epidermal growth factor receptor; VEGF, vacular endothelial growth factor; wt*KRAS*, wild type KRAS; PFS, progression free survival; RFS, recurrence free survival; CIN, chromosomal instability; DFS, disease free survival.

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http://dx.doi.org/10.1016/j.suronc.2016.05.018
0960-7404/

Evaluation Criteria in Solid Tumors (RECIST) guideline uses cross-sectional imaging to measure tumor size before and after chemotherapy in order to provide an estimate of response to therapy [10]. Additional radiographic features such as morphologic response criteria (i.e. decreased attenuation, increased homogeneity, and loss of enhancement at tumor-liver interface after treatment) have also been combined with the RECIST criteria in an effort to improve prediction of patient-specific long-term survival [11,12]. Post-treatment pathologic tumor response can provide important information regarding the efficacy of treatment and long-term prognosis; unfortunately, this information can only be obtained after surgical extirpation [8,13].

The combination of clinical, radiographic, and pathologic measures provides a basis for the characterization of prognosis among patients with resected mCRC. These factors remain fairly non-specific, however, and have a relatively limited capacity to direct personalized therapy. In fact, with increasing targeted therapeutic options, there is an increased interest in better characterizing and defining underlying mCRC tumor biology in an effort to individualize treatment. Specifically, indicators of tumor biology may be valuable to guide appropriate therapies and to provide accurate prognostic data for patients and providers. Furthermore, identification of molecular markers and specific molecular pathways that are involved in mCRC may allow providers to better target the use of novel therapeutics. We herein review the key molecular markers and molecular pathways involved in the treatment of patients with mCRC.

2. Molecular markers

2.1. Prognostic markers in metastatic colorectal cancer

Currently, CRC has relatively few established biomarkers to predict patient outcomes. Molecular markers include microsatellite instability (MSI), KRAS and BRAF [14,15]. More recently, other investigations have identified hTERT, circulating tumor cells (CTC), and circulating tumor DNA (ctDNA), as potential predictors of outcome [16–18]. Fewer studies have reported on PI-3 Kinase, thymidylate synthase, TP53, Ki67 and hypoxia-inducible factor−1 alpha; the association of these markers with outcomes are less well established, and therefore will not be discussed [19–21].

2.1.1. DNA microsatellite instability

Microsatellites consist of repetitive units within DNA. The integrity of these regions is maintained by the mismatch repair (MMR) system. When deficiencies in the MMR system occur, the resultant MSI predisposes to genomic instability and consequent tumor formation [22]. The inability to repair single nucleotide DNA mismatches can occur from germline mutations in specific genes of the MMR system (*MLH1*, *MSH2*, *MSH6*, *PMS2 or TACSTD1*) or can arise sporadically as a result of *MLH1* promoter hypermethylation (associated with CpG island methylation phenotype (CIMP)) [15]. Sporadic MSI tumors are more commonly encountered (10–20% of patients with CRC) than tumors arising from hereditary germline mutations (Lynch Syndrome: 0.8–5%) in CRC [22].

Genomic instability is divided into two genotypic groups, MSI-high (MSI-H) and MSI-low (MSI-L), based on immunohistochemical analysis of MMR protein expression or quantification of microsatellite markers in the tumor [23]. MSI-H is defined as instability in greater than 30% of microsatellite loci or absence of expression of any MMR proteins. Instability in less than 30% of loci (generally one marker in the standard 5 marker panel) is indicative of MSI-low (Table 1) [23]. MSI-H is present in 15–20% of CRC overall and has a higher prevalence in stage II versus stage III or IV CRC (approximately 20% v 12% v 4%, respectively) [23]. MSI-H tumors are more commonly located in the right colon and are histologically typified by poor differentiation, mucinous features and lymphocytic invasion. MSI-H CRCs are also associated with a decreased risk of distant recurrence, which translates into an improved long-term prognosis in stage II and stage III CRC compared with microsatellite stable (MSS) tumors [23,24]. The favorable prognosis in stage II and III disease is not present in stage IV disease, possibly related to the strong correlation with *BRAF* mutations [15,22]. In addition to the associated high *BRAF* mutation rate, further prognostic (and therapeutic) considerations for MSI-H mCRC include the infrequent occurrence of *KRAS* mutations [23].

The disparate tumor biology seen in stage IV disease compared with stage II/III disease is also supported by the varying efficacy of some chemotherapeutics. For example, although sporadic MSI-H tumors (stage II/III) tend to exhibit chemoresistance to 5-fluorouracil (5-FU), a recent retrospective analysis demonstrated preserved efficacy of 5-FU in MSI-H stage IV CRC [15]. Therefore, among patients with mCRC, 5-FU is still considered the mainstay of systemic chemotherapy regardless of MSI-H status [15,23]. Recent evidence also suggests an important role for immunotherapy in these patients (*discussed in 2.4.4 below*) [25].

2.1.2. KRAS

Perhaps a more robust and clinically useful biologic marker among patients with mCRC is *KRAS* mutational status. KRAS has been shown to be predictive of response to biologic therapy, and to correlate with long-term outcomes in patients with metastatic disease. *KRAS* is a membrane bound proto-oncogene that functions downstream of the epidermal growth factor receptor (EGFR);

Table 1
Colorectal cancer molecular subcategorization [23].

CRC type	Subcategory	Characteristics	Prevalence
Microsatellite instability	MSI-H: >30% of marker loci with instability (Bethesda panel of 5 markers or alternate panel) OR lack of MMR protein on IHC	Germline: *MLH1*, *MSH2*, *MSH6*, *PMS2* Sporadic: Hypermethylation *MLH1* Hypermutation profile but stable karyotype; strong correlation with BRAF mutations (40–45%) Right-sided lesions with poor diff, mucinous features and lymphocytic invasion Associated with CpG-Island methylation phenotype-high (CIMP-H)	5% 10%
Chromosomal instability	Includes both MSI-L (<30% of marker loci with instability) and MSS (No evidence instability) tumors:	Unstable karyotype, demonstrates chromosome gains and losses *KRAS*, *TP53*, *APC*, *PIK3CA*, *SMAD4*, *CTNNB1* mutations More commonly associated with CIMP-low or negative	80–85%

MSI = microsatellite instability, MMR = mismatch repair, IHC = immunohistochemistry, MSS = microsatellite stable.