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REVIEW ARTICLE

Site-specific use of molecular markers could be a promising prognostic tool in colorectal cancer

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Summary

Proximal and distal colorectal carcinomas (CRCs) are generally considered as genetically and clinicopathologically distinct disease entities. Tumor location has been proposed as an additional prognostic indicator and –more recently– as a factor with significant influence on the prognostic value of particular molecular markers and/or combination of markers (KRAS, MSI, APC/MSI), allowing the discrimination of specific disease subsets with considerably disparate outcome and the identification of high risk cases. This article examines the clinical importance of particular recent proposals on this specific issue. Their strengths and limitations, as well as issues requiring further elucidation and practical problems hampering their clinical implementation are briefly discussed. Moreover, some suggestions intending to improve research methodology on this specific theme and to render the clinical use of this novel approach more effective and feasible, are presented. Hopefully, the assessment of certain molecular markers in a site-specific fashion could be another step towards personalized management of CRC, improving and complementing the molecular classification of the disease.

Key words: *APC, colorectal cancer, KRAS, MSI, prognosis, tumor location*

Introduction

CRC is particularly heterogeneous, evolving through multiple tumorigenic pathways with disparate outcomes and treatment responses [1,2]. Recent molecular classifications, based on the status of particular biomarkers, including microsatellite instability (MSI), CpG island methylation phenotype (CIMP) and alterations of KRAS, BRAF and p53 genes, identified various CRC subtypes with promising prognostic or predictive significance [1,3-6]. However, the clinical implementation of these proposals is hampered by the existing discordance among the various classification systems (regarding the suggested molecular subtypes) and the co-existing methodological variability (disparate samples and techniques) [7]. A recent consensus classification, although providing more refined and accurate tumor distinction, leaves a considerable proportion of unclassified or miscellaneous cases [6]. Moreover, the complicated molecular combinations characterizing the majority of the identified tumor subtypes render their assessment financially and technically rather impractical for routine

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clinical use. Therefore, TNM staging remains the principal prognostic determinant and guide for therapy decision in CRC patients, except for targeted (anti-EGFR) treatment in metastatic disease which is driven by KRAS mutational status [2,8].

Summary and interpretation of recent relevant articles

In this context and intending to improve the effectiveness and facilitate the clinical implementation of particular markers, their use in a site-specific manner has been recently suggested, indicating significant associations of mutations KRAS, MSI and APC/MSI status with CRC outcome which were closely dependent on tumor anatomical location [9-11] (Table 1). This approach is based on the known state that proximal (right-sided) and distal (left-sided) CRCs are (for the most part) biologically different tumors, with distinct evolutionary pathways – MSI/CIMP for the former and chromosomal instability (CIN) for the latter – and disparate clinicopathological features (including outcome and response to treatment) [2,8].

A study by Sinicrope et al., conducted in a large cohort of stage III colon cancer cases participating in an adjuvant chemotherapy trial, reported significant differences in the prognostic value of KRAS and MSI according to tumor location (poorer outcome of KRAS mutant and MSI cases in the distal colon). Such site-specific prognostic value was not detected for BRAF mutation which was associated with significantly poorer outcome, albeit regardless of tumor location. They also found independent prognostic effect of tumor site (worse proximally) [9]. Interestingly, these authors have published a more recent work examining again the prognostic value of the aforementioned markers by tumor site, albeit in a specific subset of stage III chemotreated patients (i.e. the cases with recurrence) [10]. Results were largely similar to those of their prior study, revealing maintenance of the previously ascertained interactions, this time regarding survival after recurrence (Table 1).

Despite the considerable strengths (large samples, focus on a particular stage, prospective analysis), the existing limitations in both studies (exclusively chemotreated cases, strict eligibility criteria, absence of rectal cancers) hamper the generalization of the findings. Further investigation examining other stages (especially stage II), untreated and rectal cases is needed to complete the picture. Nontheless, these results seem to support the use of tumor site (combined with KRAS and MSI status) as stratification factor in stage III chemotreated colon cancer patients for the identification of high risk cases requiring (at least) appropriate adjustment of their surveillance strategy. The potential use of this stratification for further- more specific- modification of the management of both primary and recurrent stage III cases could be also examined taking into account that the benefit from particular chemotherapeutic regimens was found differing according to tumor location [8].

Jorissen et al. found that proximally located microsatellite stable (MSS) tumors, lacking APC mutation (APC wild-type) had poorer outcome – compared with tumors without these characteristics (i.e. the rest of proximal and all distal CRCs). In contrast, APC status had no effect on the observed favorable outcome of MSI tumors [11] (Table 1). They also ascertained that specific APC mutation genotypes, although differing by site (in line with previous findings) [12], had virtually no influence on prognosis. Therefore, the cause behind the

Table 1. Recent articles reporting site-specific prognostic value of molecular markers in CRC

Articles	*Cohort*	*Marker status*	*Prognosis by tumor site and marker status*
Sinicrope et al. [9]	Stage III (chemotreated primary cases) n=3018	KRAS mut. MSI BRAF mut	Worse for distal tumors Better for proximal tumors Worse (regardless of tumor site)
Sinicrope et al. [10]	Stage III (chemotreated recurrent cases) n=1395	KRAS mut. MSI BRAF mut	Worse distally* Better proximally* Worse (regardless of tumor site)
Jorissen et al. [11]	Stages I-IV** n=745	APC wild-type/MSS MSI (regardless of APC status)	Worse for proximal tumors with this molecular subtype compared to all other MSS proximal or distal tumors Favorable (for proximal tumors)***

CRC: colorectal cancer, KRAS mut: KRAS mutation, MSI: microsatellite instability, BRAF mut: BRAF mutation, MSS: microsatellite stable. * Location of the primary tumor [10] ** Both chemotreated and untreated cases were included. There was no information regarding tumor status (primary/recurrent) [11]. *** Analysis was restricted to proximal tumors due to the very small number of MSI distal tumors [11].

observed prognostic disparity is probably multifactorial and attributable to the cumulative adverse prognostic effect of the constitutive characteristics of the particular tumor subset. Indeed, worse outcomes have been reported for APC wild-type (vs APC mutant) [12], MSS (vs MSI) [1,2,9,13] and proximal (vs distal) [2,9] tumors, suggesting a considerably higher malignant propensity and aggressiveness for APC wild-type / MSS proximal CRCs.

Interestingly, the molecular features of the particular subtype (APC wild-type / MSS) were quite similar with the so called "type 2" molecular subtype (MSS / CIMP$^+$, BRAF mutated cases) described in previous and contemporary studies with molecular classifications of CRC [1,5]. Both subtypes included MSS cases, while the APC-wild type (APC-wt) tumors of the former largely correspond to the CIMP$^+$ carcinomas of the latter. Tumors lacking APC mutation, rarely develop via the canonical (conventional) CIN pathway, as APC mutation is considered a constitutive and initiative molecular event of this pathway [1,11]. Instead, they evolve through the CIMP epigenetic mechanism (more common proximally, especially when combined with BRAF mutation) [1,2]. Remarkable similarity was also noted in the reported frequencies of both subtypes (<10% of all CRCs) and the recorded reduction in survival (HR: 1.8–2.2) which was consistently the lowest among the various CRC subtypes [1,5,11].

These similarities highlight the existence of a distinct CRC subset with poor outcome, identified on the basis of specific molecular features (greatly overlapping between the particular classifications) [1,5,11] and tumor location. In this context, the Jorissen proposal appears as more easily applicable in clinical practice, requiring the assessment of only two markers (APC,MSI) and limited (strictly) to proximal carcinomas. The implementation of this proposal is furthermore facilitated by the observed correlation of the particular subtype with the specific characteristics of the sessile serrated neoplasia pathway [11], allowing the selective assessment of APC and MSI in tumors displaying particular clinicopathological features. Nonetheless, additional research is necessitated as several issues should be examined and elucidated before the clinical establishment of the particular approach (see below).

Suggestions for future clinical implementation

First, larger sample size is required, considering the rather small frequency of the APC wild-type / MSS / proximal tumor subtype (9,3%) [11] and the necessity for better testing of the observed correlation with survival in particular disease stages (especially II and III, i.e. the most prognostically important disease subsets). Also, only primary, sporadic, untreated (preoperatively) and unselected CRC cases should be included (such criteria were not well defined in the Jorissen's study).

Second, specific survival analysis should be also conducted in chemotherapy and targeted therapy treated CRC cases, to assess both the potential prognostic and predictive significance of the APC–wt / MSS subtype. Such focused analysis was not performed by Jorissen et al. [11]. Retrospective analysis should be then validated in prospective studies investigating properly powered representative cohorts. Multicenter trials may be necessary to provide these requirements [2,7].

Third, the potential use of immunohistochemical technique (simpler and cheaper than DNA sequencing) for the evaluation of APC mutation, should be examined. However, this application demands adequate standardization regarding several methodological issues (specific monoclonal antibodies, certain tumor area for sampling, particular cell area for staining and – most importantly – determination of the appropriate immunoreactivity thresholds for positivity) [13]. Notably, the immunohistochemical evaluation of mismatch repair (MMR) genes alteration – responsible for MSI – has been generally validated and broadly established [13] (indeed, it was applied in Sinicrope's studies) [9,10]. Therefore, the fulfilling of the aforementioned criteria for APC evaluation, would render the combined assessment of both markers in CRC more practical and financially viable.

Another issue is related to the generalization of the findings among various CRC age subsets which has been recently disputed, particularly for the early onset disease subgroup, in which distal APC mut/MSS (instead of proximal APC-wt/MSS) cases showed the worse survival [14]. However, this observation - based on a small CRC cohort - demands validation in larger samples.

Lastly, the impact of intratumoral heterogeneity on the clinical significance of CRC molecular subtyping should be considered, as divergent mutational status of certain biomarkers may exist between primary and metastatic or recurrent tumors or even among regions of the primary tumor, potentially influencing both outcome and treatment response through the development of more malignant and refractory cell populations [2,15]. Whether and how this heterogeneity is associated with tumor location is unknown. However, it is noteworthy that the aforementioned pattern

of site-specific impact of KRAS and MSI status on CRC outcome was found largely similar in patients with either primary or recurrent tumors in Sinicrope's studies [9,10]. Further research is warranted to elucidate this issue (especially regarding the APC-wt/MSS subtype).

Recent guidelines recommended molecular testing of CRCs for KRAS mutations (as negative predictors of benefit to targeted therapy) and for BRAF and MMR status (for prognostic stratification) [16]. There was no mention of site-specific use of these biomarkers. However, the particular recommendations were based on relevant literature mostly published before 2015 and not including the articles discussed in the current study. The particular novel data (along with any further relevant information) could be taken into account in the next scheduled revision of the guidelines (after 4 years) [16], provided that adequate evidence will be presented until then.

Conclusion

The proposed site-specific use of selected markers (KRAS, MSI, APC/MSI) for CRC cases, although requiring further improvement and validation, emerges as a promising prognostic indicator and a potential guide for the planning of future treatment strategies beyond TNM staging. Therefore, this approach should be included in the ongoing research attempts for clinically applicable molecular classification of CRC.

Conflict of interests

The authors declare no conflict of interests.

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