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POINT OF VIEW

Molecular basis of colorectal cancer: Towards an individualized management?

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ABSTRACT

Colorectal cancer (CRC) has become a highly relevant condition nowadays. In this respect, advances in the understanding of its molecular basis are key for an adequate management. From the time when the adenoma-carcinoma sequence was formulated as a carcinogenesis model to this day, when, among other things, three major carcinogenic pathways have been identified, the CRC concept has evolved from that of a single disease to the notion that each CRC is a differentiated condition in itself. The suppressor or chromosome instability pathway, the mutator or microsatellite instability pathway, and the methylator or CpG island methylation pathway allow various phenotypes to be identified within CRC. Similarly, the presence of different changes in certain genes confers several behaviors on CRC from both the prognostic and responsive standpoints to specific therapies. However, this apparent complexity does help develop the clinical management of this disease through the identification of novel, more specific therapy targets, and also markers for various behaviors within the condition, which will most likely lead us to an individualized management for these patients.

Key words: Colorectal cancer; microsatellite instability; methylator phenotype; chromosome instability; mutator phenotype.

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INTRODUCTION

Colorectal cancer (CRC) is becoming certainly important. In Spain it is the most prevalent malignancy, and the second most common cause of death from cancer (1). With both genders in mind, over 25,000 new cases are diagnosed yearly in Spain, where around 13,000 people die because of this condition (2). Thus, its indisputable relevance definitely demands a deeper understanding of the disease, which has evolved ever since Fearon and Vogelstein proposed their colorectal carcinogenesis model in 1990 (3). Today, however, despite the fact that most CRCs are supposed to emerge from adenomas via the suppressor pathway, initiated by an *APC* gene mutation (the classical description of colorectal carcinogenesis via the adenoma-carcinoma sequence (3)), this pathway currently occurs in only 60% of cases (4), with the rest emerging through two alternative routes. This progressive though merely apparent complexity contributes to clinical management development in this disease via the identification of new, more specific therapy targets as well as markers determining different behaviors within the same condition. Now we may venture the view that the time when individualized treatment may be defined from specific molecular profiles is perhaps not too far into the future.

THE EVOLVING UNDERSTANDING OF MOLECULAR BASES: CATEGORIZING DIVERSITY

From a general perspective, tumor development consists of cumulative changes in the genome of involved cells. Two types exist: DNA sequence changes and epigenetic changes, which affect gene expression rather than genes themselves. Sequence changes include chromosomal region deletions, which involve a loss of genes po-

tentially related to cell cycle downregulation (tumor suppressor genes); gene mutations that may activate or inactivate various proteins; gene amplifications entailing an overexpression of specific genes; and even loss or gain of entire chromosomes. Among others, epigenetic changes include gene silencing —absent gene expression— because of CpG island hypermethylation in their promoters, as is the case with gene *MLH1*.

A key driver of development in the understanding of CRC molecular bases is its high incidence, with specific mortality rates approaching 33% in developed countries (5). As a result, the current concept of CRC no longer refers to a single disease but a heterogeneous group of diseases caused by a differentiated genetic/epigenetic substrate. Initially, each CRC would emerge and develop uniquely, in a way unlikely superimposable to that of another CRC (6). A CRC would eventually emerge from a guided progression through multiple steps leading to the transformation of a normal cell into a neoplastic cell, and even in the presence of a preferential sequence, what matters is cumulative mutational changes (from five to seven), which will confer a definite phenotype in the long run.

CRC classifications are many (tumor location, pathology), but CRCs are now increasingly classified into several phenotypes according to molecular profiles. Despite the above regarding the potential exclusivity of each CRC, from a molecular standpoint their classification is based on predominant cell events: chromosomal instability (CI), microsatellite instability (MSI), or CpG island methylator phenotype (MP) Similarly, according to the factor initiating such events (suppressor pathway for CI; mutator pathway for MSI, or methylator pathway for MP). However, other changes should also be considered, which may be useful in classifying CRC in order, for instance, to predict their response to targeted therapies (presence of mutated or native *KRAS* in the response to treatment with monoclonal antibodies against epidermal growth factor, such as *cetuximab*); to identify clinically useful biomarkers for prognosis or response to therapy; and to facilitate genetic counselling for hereditary CRC. A classification schematic based on major carcinogenic pathways is shown in Table 1, where other molecular features are also included.

Major carcinogenesis pathways

Chromosomal instability

Tumors with CI commonly exhibit karyotypal changes with chromosomal gains and losses (7), as well as translocations. Similarly, allelic losses are relatively usual and show allelic unbalance in multiple *loci* (including 5q, 8p, 17 p, and 18q). Aneuploidy is characteristic in this sort of tumors (8,9). Techniques such as DNA ploidy analysis or heterozygosity loss analysis using microsatellite markers (LOH, *Loss of Heterozygosity*) are used for identification. The factor that allegedly initiates this pathway is the loss of a suppressor gene such as gene *APC*. Hence the designation "suppressor pathway for colorectal carcinogenesis", since this is a suppressor gene, associated with cell cycle downregulation, even though, as mentioned above, oncogene copy number gains are also seen.

Causes leading to CI are also heterogeneous, and multiple candidates have been identified — genes coding for mitosis regulating proteins (BUBR1) (10), AURKA (Aurora Kinase A) amplification, a centromere-associated serine-threonine kinase (11), *APC* (12,13), and *TP53* (14), among others.

Another designation for this type of tumor, which differentiates them from those discussed below, is "microsatellite stability (MSS) tumors. CI tumors and MSI tumors are considered mutually excluding. This group of CRCs (CRCs with CI) include most sporadic CRC cases (near 85%), and also familial adenomatous polyposis (FAP) cases with germ-cell *APC* mutations as genetic background. However, as was mentioned above, group categories are not mutually exclusive; cases occur that share characteristics from some of the various groups. Thus, a proportion of cases with positive MP (60%) also express MSS.

Table I. Summary molecular classification of colorectal cancer. Modified from Cunningham D. et al. 2010 (4)

	CHROMOSOMAL INSTABILITY PATHWAY	MUTATOR PATHWAY	"SERRATED" PATHWAY	
	Hereditary & sporadic	*Hereditary*	*Hereditary*	*Sporadic*
Methylation status	Negative	Negative	High	
MSI	MSS	High MSI	High MSI	Low MSI
CI	++++	——	——	——
KRAS	++++	+/-	——	——
BRAF	——	——	++++	++++
MLH1	Normal	Mutated	Methylated	Partly methylated

MSI: microsatellite instability. MSS: microsatellite stability. CI: chromosomal instability.

Microsatellite instability

MSI refers to length changes in short, repeated nucleotide sequences (microsatellites) in tumor DNA as opposed to normal DNA (15). Equivalent terms include RER (replication errors) phenotype or mutator phenotype, and this is called the carcinogenic mutator pathway. MSI is actually a reflection of impairments in the DNA mismatch repair system, which serves to correct matching failures occurring during DNA replication under the control of several genes (*MLH1, MSH2, MSH6, PMS2*, among others) (Figure 1). Changes in this system lead to cumulative microsatellite impairments, which are spread throughout the genome (16).

Today, the term MSI tumors refers to tumors with high MSI (2 or more microsatellite markers according to Bethesda's panel) (17), which is present in 15% of CRCs (18) and may emerge in two distinct ways. First, as seen in Lynch syndrome, with germ-cell mutations in any of the genes related to the DNA repair system as the underlying mechanism. Secondly, and covering most sporadic cases, MSI may result from hypermethylation at the promoter regions of DNA repair system genes (most often in *MLH1*).

Genes involved in mutation development in coding repeated mononucleotide sequences (coding microsatellites) are found in various tumor suppressor genes, including type-2 transforming growth factor- receptor (*TGFBR2*) or gene *BAX* (19,20).

The presence of high MSI results in characteristic though non-exclusive phenotypal features, in these tumors. Thus, there is a higher frequency of tumors with mucinous differentiation, with signet ring cells, with "Crohn-like" lymphocyte responses, or with peritumoral lymphocyte infiltration and tumor necrosis, or a higher frequency of poorly differentiated tumors or tumors located in the right colon (4,21). As will be seen below, these tumors also exhibit typical features regarding their clinical prognosis or response to specific antineoplastic therapies. The fact that a CRC has one or more of the above characteristics should draw the clinician's attention towards an association with Lynch syndrome, which must be ruled out. However, as these characteristics are not exclusive of CRCs with MSI, an MSI analysis or —more readily— an immunohistochemical study of DNA repair system protein expression, which is absent when this system's genes are defective, must be performed prior to the genetic study.

On the other hand, and similarly to some cases with CI, this type of tumors exhibits overlapping with those emerging from another pathway, the MP pathway, and around 40% of tumors with MSI phenotype may be associated with the so-called "serrated" pathway of CRC.

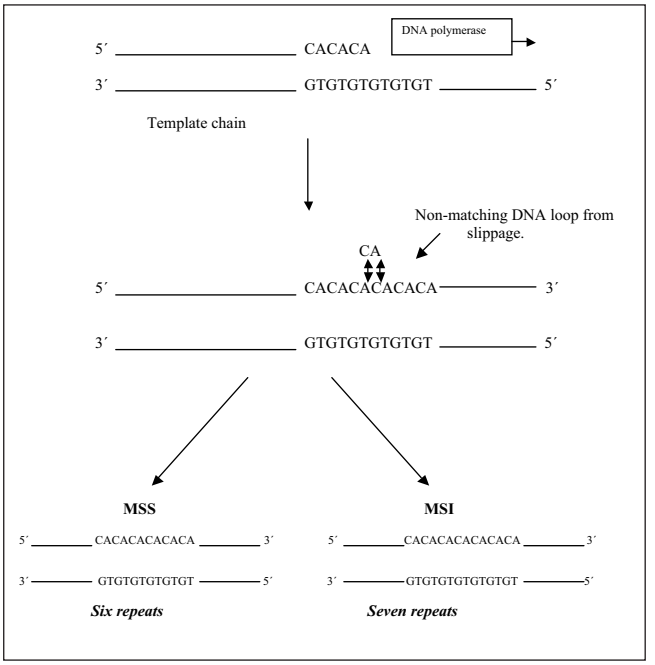


Fig. 1. A schematic of microsatellite instability and stability according to DNA error repair system status.

Serrated pathway or methylator phenotype

CpG islands are DNA regions making up approximately 40% of gene promoters in mammals. These regions contain high levels of cytosine-guanine pairs with phosphate bonds (hence the "p" in CpG). The CpG sites are unmethylated in expressed genes, hence CpG island methylation in gene promoters may inhibit gene expression and result in gene inactivation.

Transcriptional inactivation by CpG island methylation in tumor suppressor gene promoters is a major carcinogenesis mechanism also designated methylator phenotype. The mechanism by which various gene promoter regions are methylated has been seen to play a role in anout 35% of CRCs (22). This pathway, also called serrated pathway for colorectal carcinogenesis, would emerge from a precursor serrated lesion (whose histological characterization includes hyperplastic polyps, sessile serrated polyps, and serrated adenomas), and seemingly does not conform to standard mechanisms as seen in the classic adenoma-carcinoma sequence.

Selected differentiating features may be identified in this type of tumors. Thus, they seem to be more common in women, in tumors proximally located in the colon, and in poorly differentiated tumors; from a molecular point of view there is a higher presence of *BRAF* mutations, whereas *TP53* mutations display a lower rate (23-26).

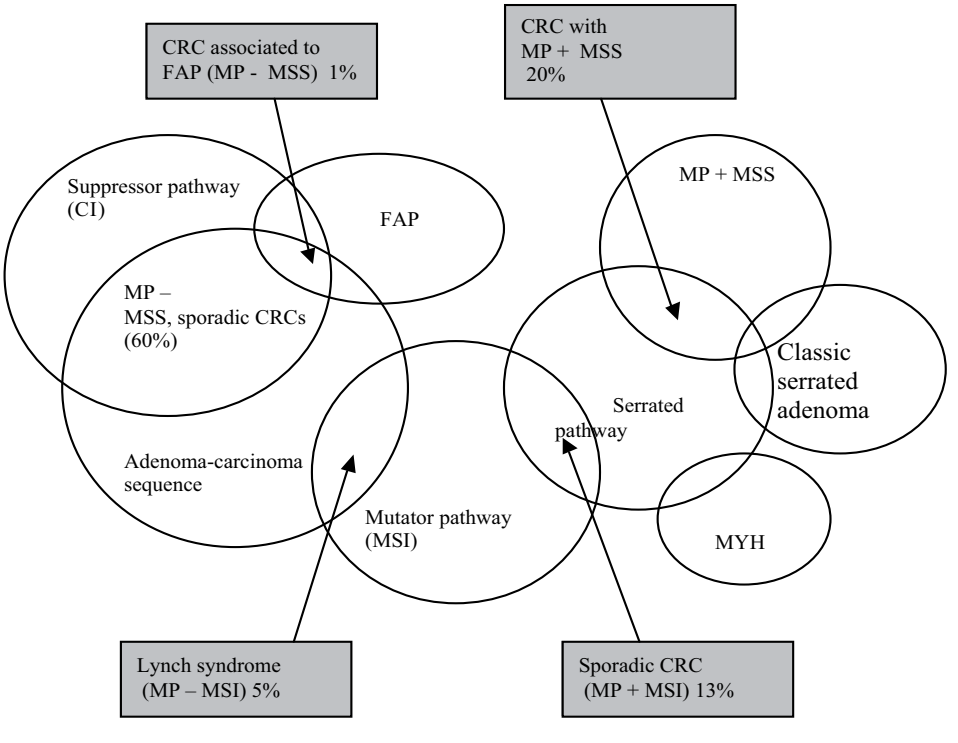
For most common CRCs emerging from this pathway the mechanism seems to be initiated by a mutation that activates gene *BRAF*, which results in an inhibition of physiological apoptosis in colonic epithelial cells. From this event serrated lesions may give rise to hyperplastic or sessile serrated polyps. These lesions are highly prone to CpG island methylation in promoter regions for multiple genes, and would therefore induce epigenetic silencing —indirect gene inactivation— in various genes, these genes being in principle selected at random. *MLH1* promoter methylation, most common in such cases, results in sporadic CRCs with MSI. This seems to be, however, a late event from which a rapid addition of mutations in other genes ensues, as is also the case in Lynch syndrome, with faster tumor progression.

CLASSIFICATION

As mentioned above, carcinogenesis pathways are mutually exclusive in some cases (as with MSI and CI) whereas some overlapping may be seen in other instances, as is the case with MP (Figure 2). Regarding the latter scenario, the MP pathway is revealing a further division of late according to methylation extent, which is categorized as high, low, or null MP depending on the percentage of methylated gene promoters (6), as was the case with MSI, which is also categorized in high MSI (often considered MSI), low MSI, and MSS. MP categories also emerged because of perceived phenotypal differences, and their association with selected genetic changes, particularly for high and low MP (Table 2). Regarding overlapping, a proportional relationship between MSI extent and MP level may be considered, with CRCs with high MSI having essentially a high MP, and CRCs with MSS being mainly associated with low and null MP.

With the same goal of categorizing the mosaic of molecular changes seen in colorectal carcinogenesis, in 2007 Jass JR. (4) formulated a CRC classification into five groups according to MSI and MP status, and found correlation with specific histological and molecular characteristics. More recently Ogino and Goel (2008) (6) modified this classification, and finally divided CRC up into six groups (Table 3) considering that the presence of MSS and low MSI only result in subtle differences, as is also the case with low and null MP.

Group 1. Presence of high MSI and high MP: Besides the characteristics shown in Table 3, there is specifically



CRC: colorectal carcinoma. FAP: familial adenomatous polyposis. MP: methylator phenotype. CI: chromosomal instability. MSS: microsatellite stability. MSI: microsatellite instability. MYH: MYH-associated polyposis.

Fig. 2. A representation of the various CRC groups according to carcinogenic pathways, as well as their overlapping (modified from Snover DC. 2010) (21).

MLH1 promoter methylation. This corresponds to sporadic CRCs with high MSI, which are associated with good prognosis.

Group 2. Presence of high MSI and low/null MP: This includes Lynch syndrome, but also sporadic cases. There is predisposition to right-colon CRC. Importantly, there is no propensity for poorly differentiated or signet ring cell tumors, hence the presence of such features as of themselves does not currently render Lynch syndrome more likely (21).

Group 3. Presence of low MSI/MSS and high MP: It is associated with poor prognosis, elderly women, and predominance in the right colon.

Group 4. Presence of low MSI and low MP: *MGMT* methylation has been observed in this group of tumors (27).

Group 5. Presence of MSS and low MP.

Group 6. Presence of low MSI/MSS and zero MP: Associated with predominant location in the distal colon (28).

Group proportions are for guidance only, and approximately illustrate the condition's overall distribution. The first four groups are those most uncommon — 10% for group 1; 5% for group 2; 5-10% for group 3; and 5% for group 4; the remaining two groups predominate at 30-35% and 40%, respectively (27,29).

Table II. A classification of the methylator phenotype in colorectal carcinoma and its characteristics.

Methylator phenotype	*Characteristics*
High	Location in proximal colon, elderly, females, BRAF +
Low	Males, KRAS
Null	Equal between genders, location in distal colon, CI, and non-mutated BRAF and KRAS.

CI: chromosomal instability.

CLINICAL USEFULNESS OF MOLECULAR MARKERS

These CRC classifications according to the various molecular pathways, as well as the recognition of their various changes, would be of little value should their implementation serve categorization or knowledge purposes. Molecular bases and genetic changes result in a specific phenotype that is often associated with varying tumor behaviors relevant for their prognosis (higher or lower survival rates), response to specific therapies, etc.

The most important development in recent years regarding metastatic CRC management was the identification of gene *KRAS* mutation status as a predictor of response or lack thereof to specific therapies targeting the epidermal growth factor receptor (EGFR). Nearly 40% of

Table III. Tumor classification according to microsatellite instability and methylator phenotype status. Modified from Ogino S. and Goel A, 2008 (5).

Group	*BRAF*	*CI*	*TP53*	*KRAS*	*Histopathology*
High MSI High MP	Mutated	Negative	Normal	Normal	Poorly differentiated CRC, with lymphocyte response and presence of signet ring cells and mucinous tumors.
High MSI Low/0 MP	Normal	Negative	Normal	Mutated	Lymphocyte response, mucinous features.
Low MSI/ MSS. High MP	Mutated	Negative	Normal	Normal	Presence of poorly differentiated CRCs, with signet ring cells.
Low MSI Low MP	Normal	Negative	Normal	Mutated	
MSS Low MP	Normal	Negative	Normal	Mutated	
Low MSI/ MSS 0 MP	Normal	Positive	Normal	Normal	

MSI: microsatellite instability. MSS: microsatellite stability. CI: chromosomal instability. MP: methylator phenotype. CRC: colorectal cancer.

patients with metastatic CRC show a somatic mutation in *KRAS*, this being a marker for lack of response to EGFR inhibitors such as cetuximab, which currently renders an analysis of mutations in said gene essential before therapy onset in these subjects. Similarly, the remaining 60% of subjects with wild-type, non-mutated *KRAS* have been seen to exhibit other molecular changes that again may determine a lack of response to anti-EGFR monoclonal antibodies. Such is the case with gene *BRAF*. This gene is impaired in 8-10% of metastatic CRCs, and the mutation excludes that of *KRAS* (5); a poorer prognosis associated with both total and disease-free survival has been seen in patients receiving these antibodies with a mutated *BRAF* (30), although further research is needed to confirm this link between *BRAF* mutations and resistance to treatment with anti-EGFR monoclonal antibodies.

High MSI has been in turn associated with a better prognosis, particularly in stage-II CRCs (31). Another important aspect of high MSI is the lack of response of these tumors to adjuvant fluorouracil (32), particularly in cases with DNA mismatch repair system changes that are typical of Lynch syndrome. Thus, MSI status is currently considered to decide which stage-II patients (20% of all stage-II patients) should not be treated with adjuvant fluorouracil (31).

Other studies are ongoing to assess other markers as potential predictors of prognosis or response to therapy, including loss of heterozygosity on the long arm of chromosome 18, thymidylate synthase, vascular endothelium growth factor (VEGF), and a number of interleukins.

CONCLUSION

As may be seen from the above, advances in the understanding of colorectal carcinogenesis have increasingly complicated the original formulation of the normal mucosa-adenoma-carcinoma sequence leading to today's individual identity for each CRC. Despite this, a number of categories have been established aiming not only at a simplification of the aforementioned complexity but also at a confirmed presence of various phenotypal profiles not only from a clinical or histopathological standpoint but also from a prognostic and therapeutic perspective, all of which restates the notion that CRCs have no uniform behavior. Therefore, these insights allow not only a recognition of each individual tumor's features but also the first steps towards a more than likely tailored approach to diagnosis and management in accordance with each CRC's molecular profile.

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