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REVIEW

Differential colorectal carcinogenesis: Molecular basis and clinical relevance

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Abstract

Colorectal cancer (CCR) is one of the most frequent cancers in developed countries. It poses a major public health problem and there is renewed interest in understanding the basic principles of the molecular biology of colorectal cancer. It has been established that sporadic CCRs can arise from at least two different carcinogenic pathways. The traditional pathway, also called the suppressor or chromosomal instability pathway, follows the Fearon and Vogelstein model and shows mutation in classical oncogenes and tumour suppressor genes, such as *K-ras*, adenomatous polyposis coli, deleted in colorectal cancer, or *p53*. Alterations in the Wnt pathway are also very common in this type of tumour. The second main colorectal carcinogenesis pathway is the mutator pathway. This pathway is present in nearly 15% of all cases of sporadic colorectal cancer. It is characterized by the presence of mutations in the microsatellite sequences caused by a defect in the DNA mismatch repair genes, mostly in hMLH1 or hMSH2. These two pathways have clear molecular differences, which will be reviewed in this article, but they also present distinct histopathological features. More strikingly, their clinical behaviours are completely different, having the "mutator" tumours a better outcome than the "suppressor" tumours.

Key words: Colorectal cancer; Microsatellite instability; Clinical outcome

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers in developed countries. The American Cancer Society estimated that up to 153 760 new colorectal cancer cases were diagnosed in USA during 2007 (the fourth most common cancer for that period of time), with 52 180 associated deaths[1].

Ferlay *et al*[2] estimated that colorectal cancer was the second most common form of cancer in Europe during 2006 with 412,900 cancer diagnoses (12.9% of total cancers) and 207,400 deaths (ranking second position). These figures illustrate the clinical impact of colorectal cancer. Due to the worldwide scale of the problem, colorectal carcinogenesis is one of the most extensively studied types of cancers.

CRC is traditionally divided into sporadic and familial (hereditary) cases. Approximately, 75%-80% of colorectal tumours have a sporadic origin. Of all patients, a high proportion have one first to third-degree relative with CRC. It is quite clear that even in sporadic CRC cases, the descendants have a higher risk of suffering colorectal cancer. In this review, we will focus on sporadic CRC.

SPORADIC COLORECTAL CANCER: DIFFERENTIAL CARCINOGENESIS, DIFFERENT CLINICAL BEHAVIOUR

Currently, it is considered that there are two major pathways in colorectal carcinogenesis. One of them is called the "canonical" (adenoma-carcinoma sequence) or "suppressor" pathway and involves chromosomal instability (CIN)[3]. It is characterized by allelic losses on chromosome 5q (APC), 17p (p53), and 18q (DCC/SMAD4). The second pathway of colorectal carcinogenesis involves microsatellite instability (MSI), and is called the "mutator" pathway. The MSI pathway is present in approximately 15%-20% of sporadic CRCs[4].

Apart from their molecular differences, these two pathways present different clinical behaviours and distinct histopathological features, as will be discussed below.

SUPPRESSOR OR CANONICAL PATHWAY

The "canonical" pathway is present in 80%-85% of colorectal carcinomas and it is assumed to follow the Fearon and Vogelstein approach. It is accepted that in the majority of cases, carcinomas arise from pre-existing adenomas. Fearon and Vogelstein[5] proposed a model of colorectal carcinogenesis that correlates specific genetic events with evolving tissue morphology. As is shown in Figure 1, every step from the normal mucosa towards the carcinoma involves specific and well-defined genetic alterations. This linear model has evolved to a more complex, comprehensive, and mechanistic approach[6]. However, in spite of the impact of new knowledge on the Fearon and Vogelstein scheme, the model as such still stands[7]. Alterations in tumour suppressor genes, such as APC, p53, and DCC, and in oncogenes, such as *K-ras*, are characteristic of this model and of the suppressor pathway. CIN tumours are also characterized by a high frequency of allelic imbalance (most commonly involving chromosomal arms 5q, 8p, 17p, and 18q), chromosomal amplifications, and translocations[8].

APC

The adenomatous polyposis coli (*APC*, 5q21) gene contains 15 exons and it is mutated in 60% and 82% of colon and rectal cancers, respectively[9]. Its best-known role is in the Wnt pathway, where it is part of a multiprotein complex that joins β-catenin and causes its phosphorylation, subsequent ubiquitination, and destruction in the proteosome. This complex is mainly constituted by APC, axin, and GSK3β. If this complex is disrupted, by multiple causes, β-catenin is not directed towards degradation and is available to translocate to the nucleus and co-transactivate several genes[10]. The list of Wnt target genes is quite long, but it is important to note some cell cycle regulating genes (*cyclin D*, *c-Myc*), and some genes related to tumour progression (*MMP-7*, *MMP-26*). One of the main causes of disruption of the multiprotein complex is mutations in *APC*. These mutations interfere with binding to β-catenin and result in the Wnt pathways becoming constitutively active.

However, APC also plays Wnt-independent roles, whose alteration can also be related to carcinogenesis[11]. It participates in cytoskeletal regulation, as it has been shown to associate with microtubules and actin cytoskeleton, suggesting that one role for APC may be in regulating directed cell migration[12]. APC also has a role in mitosis. APC has been reported at kinetochores, where it might promote correct chromosomal alignment[13] and at centrosomes, where it could influence centrosome duplication[11]. It has been described that APC deficient cells cannot properly detect chromosomal abnormalities during anaphase[14]. Therefore, loss of APC might interfere with the correct regulation of mitosis and contribute to CIN[3]. Inactivation of APC has also been related to the promotion of tumorigenesis, through loss of cell adhesion[15]. It has been shown that a mutation in *Apc* in mice can decrease the level of E-cadherin at the cell membrane[16]

Thus, we can consider that mutations in APC are a frequent early event in the carcinogenesis of CCR and APC is related to carcinogenesis at different levels: its activity in the Wnt pathway, its relation with the cytoskeleton, its role in chromosome segregation and, finally, its role in adhesion.

K-ras

K-ras is a proto-oncogene located at 12p12.1 that encodes a 21-kDa GTP-binding protein. *K-ras* is frequently mutated during the very early stages of colorectal cancer development (35%-42% of colorectal cancers and advanced adenomas present mutations on this proto-oncogene)[17]. When it is bound to GTP, the ras protein is active. This protein is involved in many different processes. It activates a large number of transduction signal pathways, among them the mitogen-activated protein kinases (MAPK) pathway. Recently, it has been demonstrated that mutant *K-ras* promotes hyperplastic growth in the colonic epithelium (signalling through MEK) and suppresses differentiation in *APC*-mutant colon cancers[18]. It also regulates epithelial cell polarity. During the development of CRC, epithelial cells can loose their polarity and it has been described that

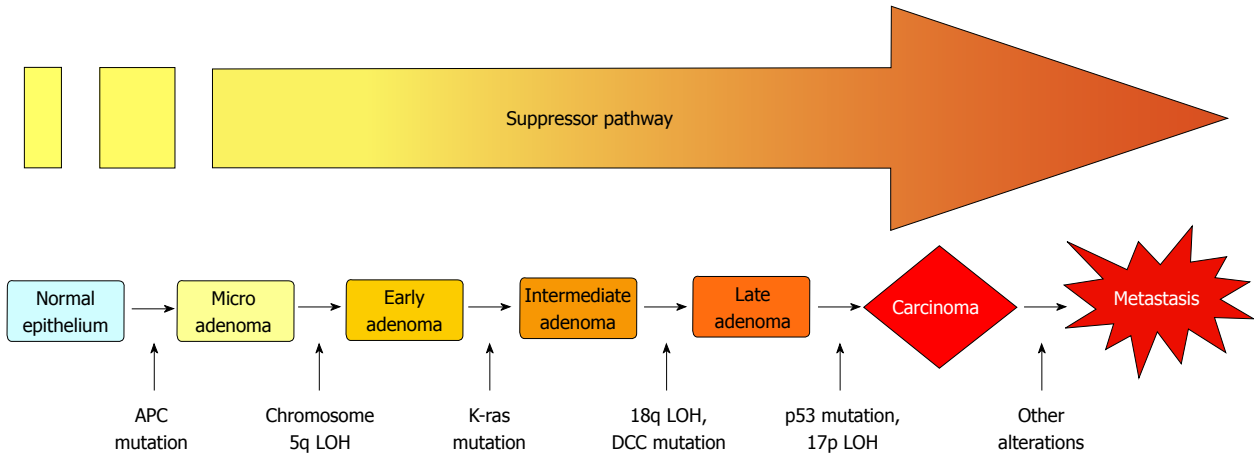

Figure 1 Molecular alterations in the suppressor pathway.

an acquired mutation in *K-ras* reduces adherens junction-mediated cell–cell contacts[19].

DCC

DCC (deleted in colorectal cancer) is located at 18q21.1 and has been proposed as a tumour suppressor gene. About 70% of colorectal cancers show allelic losses in *DCC*[20]; some cancers had somatic mutations of this gene, and its expression is often reduced in colorectal cancer tissues and cell lines[21]. The DCC protein is a transmembrane receptor of the Ig superfamily for netrins, factors involved in axon guidance in the developing nervous system. However, DCC has a role not only in axon guidance, but in intracellular signalling. Chen *et al*[22] demonstrated that the wild-type DCC, but not the mutant, induced apoptosis and activated caspase-3, and that DCC expression induces a rapid G2/M cell cycle arrest in some cell lines. DCC was also shown to activate Rac-1 when netrin-1 is present[23]; thus it is implicated in actin organization and cell motility. As reviewed by Mehlen and Fearon[21], transgenic mice expressing a constitutive form of Rac-1 in the intestine showed differentiation of the epithelium with accompanying alterations in their apical actin. Hence, DCC-mediated Rac-1 activation might be important for epithelium differentiation.

p53

p53 is encoded by the *TP53* gene located on 17p13.1. Its expression is abnormal in more than 50% of human tumours[24]. Mutation or loss of *p53* usually occurs at the time of the transition from adenoma to cancer in the Fearon and Vogelstein sequence. As reviewed by Worthley[3], the frequency of p53 abnormalities increases with the progression of the lesion. Thus alterations are found in 4%-26% of adenomas, 50% of adenomas with invasive foci, and in 50%-75% of CRCs[3,17]. P53 protein induces G1 cell-cycle arrest to facilitate DNA repair during replication of cells exposed to environmental or oncogenic stress[20]. When DNA damage is too great to be repaired, it can induce apoptosis and this is considered a major pathway whereby p53 exerts its tumour suppressor function[25].

MSI PATHWAY

Molecular alterations

The MSI, or mutator pathway, is present in approximately 15%-20% of sporadic CRCs. MSI tumours (also called Replication ERor, RER+) are characterized by a huge accumulation of mutations (mutation rates in these tumour cells are 100- to 1000-fold more common compared to normal cells[8]) in microsatellite sequences (High Microsatellite Instability, MSI-H). Microsatellites are short sequences repeated in tandem throughout the genome[26,27]. This accumulation of frameshift mutations is caused by a primary defect in the mismatch repair (MMR) genes (Figure 2). There are at least seven genes in the MMR system: *hMLH1*, *hMLH3*, *hMSH2*, *hMSH3*, *hMSH6*, *hPMS1* and *hPMS2*[28]. When MMR proteins are functional, errors made by DNA polymerase in microsatellite sequences during replication, are repaired. The acquisition of thousands of mutations characteristic of the MSI-H phenotype, requires the inactivation of the MMR genes[4]. Germline mutations, or epigenetic changes, in *hMLH1* (mainly silencing caused by methylation) and *hMSH2* are the most common cause of MSI-H in sporadic CRC (and in HNPCC, Hereditary Non Polyposis Colorectal Cancer). *hMSH6* mutations are less frequent and alterations of the other MMR genes are very rare[4]. These data enforce the idea that loss of hMLH1 and hMSH2 is associated with complete inactivation of MMR, whereas defects in other proteins cause only a partial MMR deficiency[28].

MSI-H sporadic colorectal cancers do not show big cytogenetic abnormalities and are usually not aneuploid[29]. This type of tumour presents reduced frequency, or absence, of mutation or allelic losses at the genes usually altered in the "suppressor" pathway, *APC*, *K-ras* and *p53*, and loss of heterozygosity at 5q, 17p, and 18q[30]. Instead, mutations are described in microsatellite sequences present in genes implicated in colorectal carcinogenesis, such as *TGFβRII*[31], *IGF2R*[32], *BAX*[33], *MSH3*[34], *MSH6*[34], *caspase 5*[35], *APC*[36], *β-catenin*[37], *Tcf-4*[38], *axin*[39], *MMP-3*[40], *E2F-4*[41], *BCL-10*[42], *cdx-2*[43], and *hRAD50*[44] (See Table 1 for further information). Additionally, a number of normally

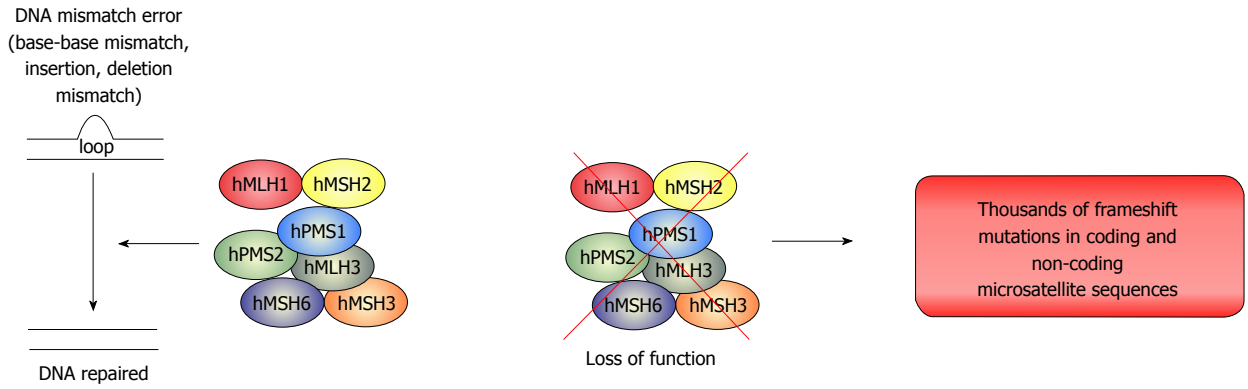

Figure 2 Origin of the high microsatellite instability phenotype.

functioning genes are silenced by methylation. Most sporadic MSI-H cancers show the CpG island methylator phenotype, characterised by widespread DNA hypermethylation[30].

Defining MSI-H tumours

International consensus criteria for classifying a tumour as MSI-H were established in 1998[45]. A panel of five microsatellite sequences was proposed for defining the MSI-H tumour groups. The recommended panel is composed of two mononucleotide repeats (*BAT26* and *A4725*) and three dinucleotide repeats (*D5S346*, *D2S123,* and *D17S250*). MSI-H tumours are defined as having instability in two or more markers, whereas MSI-L tumours are defined as having instability in one marker. Microsatellite stability (MSS) is defined by no instability at those five loci. It is also important to stress that instability is defined as a change of any length due to either insertion or deletion of repeating units, in a microsatellite within a tumour, when compared to normal tissue.

Clinical and histopathological characteristics

One of the most important and intriguing characteristics of individuals with MSI-H tumours is that they have distinct clinical and histopathological features. This is why it is so important to determine a patient's carcinogenic pathway. Samowitz *et al*[46] reported that MSI-H was more frequent in individuals with colorectal cancer diagnosed before the age of 55 or over the age of 70, than in those between 55 years and 70 years of age. However, these data have not been confirmed by other authors[47,48]. MSI-H tumours are located predominantly in the right-sided colon[48-52] and have generally been reported more frequently in women[46,53]. It has been proposed that the analysis of MSI in CRCs might be helpful in predicting the development of metachronous multiple colorectal carcinomas[54].

MSI-H colorectal tumours typically present with a greater depth of invasion but with a lower overall stage[49,50,52]. MSI has also been associated with the presence of local lymphocyte infiltration and low frequency of distant metastases[48,50,55]. In spite of its longer survival after surgical resection (see below, in MSI-H and prognosis), MSI-H carcinomas tend to be poorly differentiated[52]. Some studies have demonstrated that MSI-H occurs more frequently in mucinous-cell type tumours[53,56,57], but others have not found any difference in histological cell type[49]. The absence of dirty necrosis is also associated with MSI-H[50]. However, as reviewed in Raut[52], it is not yet possible to use a single pathological feature to diagnosis MSI-H. Greenson *et al*[50,58] have recently developed a model that permits pathologists to predict the likelihood of MSI using a combination of simple histological and clinical data (mucinous differentiation, lymphocyte infiltration, and dirty necrosis).

MSI-H and prognosis

Many authors have reported a better outcome for MSI-H tumours (whether sporadic or inherited) than those with MSI-L or MSS tumours[46,47,59-62], though others have not[63,64]. The prognostic advantage conferred by the presence of high instability has been shown to be most evident in stage Ⅱ and Ⅲ disease[62]. Individuals with distant metastases present (stage Ⅳ) showing MSI-H in the *TGF-βRⅡ* gene (transforming growth factor-β receptor Ⅱ) had improved prognosis as compared with those with native TGF-βRⅡ[46]. Moreover, MSI status is considered to be predictive of a favourable outcome, independent of tumour stage and of patient treatment[49]. Therefore, the MSI-H phenotype is associated with a good prognosis, independently of the molecular biology (germ line mutations or transcriptional silencing *via* hypermethylation) provoking it[52]. In the short term, therapeutic decisions might be taken in MSI-H patients considering this differential prognosis. For example, Benatti *et al*[62] demonstrated that 5-FU-based chemotherapy does not seem to provide survival benefits among patients with MSI-H tumours, so the use of 5-FU in patients with MSI-H tumours should be limited to avoid harmful side effects of unnecessary chemotherapeutic regimens

Classification of colorectal cancer by MSI status might also have prognostic value in patients undergoing curative surgery, as suggested by Banerjea[65]. MSI-H cancers display enhanced immunogenic properties and this might contribute to their better prognosis.

Table 1 Genes with microsatellite instability in their coding sequences. Adapted from reference [75].

Gene	Function	Coding repeat
ACTR II	Growth/differentiation factor receptor	(A) 8
AIM2	IFN inducible	(A) 10
AXIN-2	Wnt signaling	(A) 6*2, (G) 7, (C) 6
BAX	Proapoptotic factor	(G) 8
BCL-10	Proapoptotic factor	(A) 8
BLM	Response to DNA damage	(A) 9
Caspase-5	Proapoptotic factor	(A) 10
CDX2	Homeobox transcription factor	(G) 7
CHEK1	Response to DNA damage	(A) 9
FAS	Proapoptotic factor	(T) 7
GRB-14	Growth factor bound protein	(A) 9
hG4-1	Cell cycle	(A) 8
IGFIIR	Growth factor receptor	(G) 8
KIAAO977	Homologue to mouse cordon bleu	(T) 9
MBD-4	DNA glycosylase and methyl CpG binding protein	(A) 10
MLH3	MMR	(A) 9
MSH3	MMR	(A) 8
MSH6	MMR	(C) 8
NADH-UOB	NADH ubiquinone oxidoreductase	(T) 9
OGT	O-linked GlcNAc transferase	(T) 10
PTEN	Cell cycle	(A) 6*2
RAD-50	Response to DNA damage	(A) 9
RHAMM	Cell motility	(A) 9
RIZ	Cell cycle and apoptotic protein	(A) 8, (A) 9
SEC63	ER membrane protein	(A) 10, (A) 9
SLC23AI	Nucleobase transporter	(C) 9
TCF-4	Transcription factor (Wnt pathway)	(A) 9
TGFβR II	Growth factor receptor	(A) 10
WISP-3	Growth factor (Wnt pathway)	(A) 9

MSI-H *VS* MSI-L

As mentioned above, MSI-L tumours (also called mild mutator phenotype) are defined as having instability in one marker out of the five consensus microsatellite sequences (as defined by Boland in 1998[45]). However, not everybody defines MSI-L with the same criteria. The distinction between MSI-H and MSI-L depends on both the type and the number of microsatellites analyzed. For example, mononucleotide markers, such as *BAT26* and *BAT40*, are relatively specific for MSI-H cancers[8]. This is the reason why some groups use specific markers, such as *MYCL*, for defining MSI-L tumours.

MSI-L tumours have been considered by some authors to be halfway between MSI-H and MSS. However, MSI-L colorectal tumours do not show clear differences in their clinicopathological features when compared with the classical "suppressor" tumours[66]. Yearsley *et al*[57] found no difference between MSI-L *vs* MSS using clinical and histological parameters such as percentage of mucin, histological type, grade, and lymphoid host response. Moreover, its molecular characteristics are more similar to those from MSS than MSI-H tumours (reviewed in[8]). For example, it has been described that LOH at 1p32, 2p16, 7q31, 8p12-22, and 17q11 is more frequent in MSI-L than in MSI-H[66-68] and that *K-ras* mutations occur more frequently in MSI-L carcinomas than in MSI-H colorectal tumours, with no difference in frequency between MSS and MSS-L cancers, by some authors[66,68]. The rate of *K-ras* mutation is higher in the MSI-L group than in the stable cancers[69]. Analysis of mutations in MSI-H target genes revealed that they are absent in MSI-L tumours[4].

Some authors have even wondered about the real existence of the MSI-L group of tumours[70]. However, Jass and others defended the notion that MSI-L is a separate group of tumours, arguing that when a panel of sensitive markers is used, approximately 8% of sporadic colorectal cancers can be classified as MSI-L[69,71]. Others authors have demonstrated the existence of specific markers for MSI-L, such as *MYCL* and *D2S123*, which are mutated at a higher rate outside the MSI-H subset[66].

In conclusion, we can consider that MSI-L CRCs are indistinguishable from MSS using most clinicopathological parameters. However, these tumours can be validated as a distinct molecular phenotypic category, as they present molecular alterations different from MSI-H and MSS (reviewed in reference [4]).

SPORADIC MSI-H TUMOURS *VS* HNPCC

HNPCC (Lynch syndrome) constitutes approximately 2%-4% of all CRC cases[72]. The presence of MSI is also a hallmark of this type of hereditary cancer. However, the molecular mechanism causing MSI-H is different in sporadic CRC than in HNPCC. In sporadic CRCs, MSI-H is provoked mainly by epigenetic silencing (hypermethylation) on hMLH1, whereas in HNPCC is more frequent a germ line mutation in an MMR gene, followed by a "second hit".

Most of the molecular characteristics of sporadic MSI-H tumours and HNPCCs are similar. However, some small differences have been described recently. Oliveira *et al*[73] demonstrated the presence of distinct patterns of *K-ras* mutations in cancers according to *hMLH1* methylation status and germ line DNA MMR defects. *BRAF* mutations (a serine/threonine kinase involved in the RAS/RAF/MAPK pathway) in a specific hotspot site have been more frequently detected in sporadic MSI-H tumours than in HNPCCs[74].

There is no difference in overall survival amongst MSI-H patients with HNPCC and those with sporadic CRC[49].

CONCLUSION

Sporadic colorectal cancers can be classified in two clearly different subtypes, according to the molecular events that give rise to the tumour. The first one is the so-called canonical, CIN or suppressor pathway. It is the most frequent pathway and it is characterized by mutation or deletion of *K-ras*, *APC*, *DCC*, and *p53*, among others genes. The specific genetic events that occur during this pathway have clear correlations with evolving tissue morphology. The second pathway is the mutator or MSI-H pathway,

which is less frequent. Its main molecular characteristic is a huge accumulation of mutations in microsatellite sequences throughout the genome, caused by primary alteration in the MMR genes. As well as their important molecular differences, the existence of these two pathways is relevant for their different phenotypes. MSI-H tumours and CIN tumours have distinct clinical and histopathological features. Known molecular differences between the two groups of tumours are still not sufficient to fully explain why MSI-H tumours have a better outcome; its most intriguing characteristic. Recent studies are beginning to shed light on this differential clinical behaviour, but further work is required.

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