REVIEW

Multiple primary colorectal cancer: Individual or familial predisposition?

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Abstract

Colorectal carcinoma (CRC) is one of the most frequent cancers. Along the surface of the large bowel, several foci of CRC may appear simultaneously or over the time. The development of at least two different tumours has been defined as multiple primary CRC (MPCRC): When more than one tumour is diagnosed at the same time, it is known as synchronous CRC (SCRC), while when a second neoplasm is diagnosed some time after the resection and/or diagnosis of the first lesion, it is called metachronous CRC (MCRC). Multiple issues can promote the development of MPCRC, ranging from different personal factors, such as environmental exposure, to familial predisposition due to hereditary factors. However, most studies do not distinguish this dichotomy. High- and low-pentrance genetic variants are involved in MPCRC. An increased risk for MPCRC has been described in Lynch syndrome, familial adeno-matous polyposis, and serrated polyposis. Non-syn-dromic familial CRCs should also be considered as risk factors for MPCRC. Environmental factors can promote damage to colon mucosae that enable the concurrence of MPCRC. Epigenetics are thought to play a major role in the carcinogenesis of sporadic MPCRC. The methylation state of the DNA depends on multiple environmental factors (*e.g.*, smoking and eating foods cooked at high temperatures), and this can contribute to increasing the MPCRC rate. Certain clinical features may also suggest individual predisposition for MPCRC. Different etiopathogenic factors are suspected to be involved in SCRC and MCRC, and different familial *vs* individual factors may be implicated. MCRC seems to follow a familial pattern, whereas individual factors are more important in SCRC. Further studies must be carried out to know the molecular basis of risks for MPCRC in order to modify, if necessary, its clinical management, especially from a preventive point of view.

Core tip: Multiple primary colorectal cancer (MCRC),

both Synchronous and Metachronous tumours, is not deeply studied yet, and also has a great clinical impact. Both genetic and environmental factors may affect in the development of MCRC, collaborating in promoting different foci of dysplasia. In general terms, Metachronous forms are mainly related to family factors whereas Synchronous tumours are linked with individual factors. With the exception of cases of hereditary forms of colorectal carcinoma (CRC), the others appears without a well-known molecular basis, and maybe different from sporadic colorectal cancer. For all these reasons, we present a review focused on the state of the art of these particular forms of CRC.

INTRODUCTION

The colon is one of the localizations where carcinomas most frequently occur. The large bowel mucosa has a great extension. Thus, high- and low-penetrance genetic variants as well as environmental exposure all affect a large field, where several foci of colorectal cancer (CRC) may appear along the surface simultaneously or over time. The development of at least two different tumours has been defined as multiple primary CRC (MPCRC); when more than one tumour is diagnosed at the same time, this is known as synchronous CRC (SCRC), while when a second neoplasm is diagnosed some time after the resection and/or diagnosis of the first lesion, it is called metachronous CRC (MCRC). Initial studies did not distinguished between both concepts, and Moertel *et al*[1] were the first to describe in 1958 the currently most used criteria. MCRC was defined as a pathologically proven adenocarcinoma, separated from the line of anastomosis, different from recurrence, and diagnosed at a minimal interval of 6 mo after the initial CRC; CRCs diagnosed within 6 mo after the initial diagnosis were considered as SCRC[1].

MPCRCs make up 5%-10% of all CRCs. Estimations of the risk of developing MCRC vary widely in the literature, and range from 1.5% to 9%[2,3], depending on the time interval of the series. Recent series describe a risk of MCRC of 3.4%, 10 years after the first diagnosis[4]. On the other hand, large series of CRC estimate a prevalence of SCRC between 3.1% and 3.9%[5,6].

Multiple primary tumours usually arise on a common etiologic substrate, either genetic or environmental. Recurrence after endoscopic polypectomy is considered a risk factor for the development of multicentric CRC. Different adenoma features such as size, villous component, and number and location of polyps, may predict a high risk of metachronous lesions[7]. Nonetheless, recent findings in molecular colorectal carcinogenetics have provided evidence that chromosomal instability, microsatellite instability, and gene methylation are involved in various predisposing lesions or factors for SCRC and MCRC.

As mentioned before, multiple factors can promote the development of MPCRC ranging from different personal factors such as environmental exposure to familial predisposition due to inheritance. However, most studies do not distinguish this dichotomy. There are different entities that increase the risk of MPCRC. First, there are hereditary CRC syndromes (Lynch syndrome, Familial Adenomatous Polyposis), which present germ-line mutations and promote the development of several lesions overtime[8]. On the other hand, there are diseases and conditions that affect a large area of the colonic mucosa during specific periods of time and promote the formation of several foci of dysplasia, such as inflammatory bowel disease[9]. However, the origin of most of the cases of MPCRCs is still unclear; nowadays, well-defined factors only explain about 10% of SCRCs[8]. Perhaps the basis of most MPCRCs should be described as a situation in which one of the two main factors (genetic predisposition or environmental influence) predominates, or in which these factors are balanced.

There are two main points in which MPCRC stands out. Firstly, tumour multiplicity provides a good model to examine common molecular alterations and, more specifically, a potential field effect[10]. Secondly, and possibly more importantly, there is the possibility of prevention within this subset of CRC, *i.e.*, the existence of different prophylactic actions such as extensive surgery or chemopreventive treatment[11,12]. As is well known, the extension of surgical resection can be influenced by the presence, or at least the risk, of SCRC or MCRC. Moreover surveillance programs can be tailored if risk factors of MCRC are identified in order to reduce morbidity and even mortality[4,13].

Below we give an overview of the current of knowledge of both hereditary and environmental factors that influence SCRC and MCRC, and the importance of gaining more specific knowledge of these factors is adressed. In Table 1 publications are summarized that show the prevalence of and risk factors for MPCRC. In Table 2 publications are summarized that address the main molecular features analysed for MPCRC.

Familial predisposition

There is much evidence that some MPCCRs are linked with a hereditary pattern. Lynch syndrome (LS), also named hereditary nonpolyposis colon cancer (HNPCC), is the most common hereditary CRC syndrome. It has an autosomal dominant hereditary pattern, and it is defined by the presence of a germ-line mutation in one of the four DNA mismatch repair (*MMR*) genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*)[14]. MPCRC tends to appear

Table 1 Different studies about the prevalence of multiple primary colorectal carcinomaand the main clinical risk factors

Ref.	Study design	Prevalence of MPCRC No. of cases (% of global)	Risk factors for MPCRC
[4]	10283 CRC patients. Study of MCRC *vs* solitary CRC	135 (1.3)	Previous SCRC. OR = 3.4, 95%CI (1.9-5.9) Less frequent in rectum. OR = 0.3, 95%CI (0.1-0.6) Not associated with the development of MCRC: Sex, age, TNM stage, or grade of differentiation of the initial CRC
[2]	1298 CRC patients. Study of MPCRC features	53 (4) MPCRC 33 (2.5) SCRC 20 (1.5) MCRC	Lynch > sporadic (P < 0.001) MCRC (5.8% *vs* 1.3%) SCRC (5.8% *vs* 2.4%)
[8]	1793 CRC patients. Study of SCRC features	102 (3.6) SCRC	Frequencies of predisposing disease in SCRC patients: 5% FAP (5) 6% SP (6) 2% UC (2)
[9]	1537 CRC patients 69 FAP 780 UC 685 *de novo* CRC		Prevalence of SCRC in special populations: 21% of CRC in FAP 18% of CRC in UC 2.5% of sporadic CRC
[37]	382 CRC patients Study of SCRC *vs* solitary CRC	28 (7.3%) SCRC 208 (54.5%) synchronous adenomas	Male gender: OR = 1.97, 95%CI (1.13-3.45) Age ⩾ 59 yr: OR = 2.57, 95%CI (1.54-4.29) History of adenomas: OR= 3.04, 95%CI: 1.04-8.85 Obstructive tumours: OR = 0.48, 95%CI: 0.27-0.85
[32]	15562 CRC cases. SCRC *vs* solitary CRC	596 (3.8%) SCRC	Male gender: OR = 1.41, 95%CI (1.19 -1.68) Adenomas present: OR = 2.02, 95%CI (1.69-2.41) Aged over 75: OR = 1.31, 95%CI (1.08 -1.59)
[12]	1522 CRC patients. Study of SCRC *vs* solitary CRC	27 (1.8%) SCRC	Male gender SCRC (70%); solitary CRC (56%), P = 0.001. Personal history of adenoma SCRC (4%); solitary CRC (1%), P = 0.001 Right sided tumour location SCRC (32%); solitary CRC (25%), P = 0.003
[57]	382 patients with CRC. Study of MCRC features	28 (7.3%)	Statistical differences: Older than 59 years OR = 2.57, 95%CI (1.54-4.29) History of adenoma OR = 3.04, 95%CI (1.04-8.85) Obstructive CRC OR = 0.48, 95%CI (0.27-0.85) Alcohol univariate analysis P = 0.006, no significance in multivariate analysis No statistical significance: Personal history of other tumours History of cancer in first-degree family members Revised Bethesda criteria (at least one criterion) BMI Predominant symptom Predominant localitation
[13]	18782 CRC cases MPCRC features	134 (0.71%) SCRC 300 (1.60%) MCRC	SCRC Men: OR = 1.45; 95%CI: 1.02-2.06 Age older than 65: OR = 1.50, 95%CI (1.02-2.21) Located in proximal colon: OR = 1.70, 95%CI (1.20-2.41) Risk of CRC of first-degree relatives of SCRC patients (OR= 1.86; 95%CI: 1.37-2.53) MCRC: (OR = 2.34; 95%CI: 1.62–3.36) Solitary CRC (OR = 1.75; 95%CI: 1.63-1.88)

CRC: Colorectal cancer; MCRC: Metachronous CRC; SCRC: Synchronous CRC; SSAs: Sessile serrated adenomas; UC: Ulcerative colitis; SP: Serrated polyposis; BMI: Body mass index; HR: Hazard ratio; OR: Odds ratio.

more frequently among patients with LS compared with patients with sporadic CRC.

Win *et al*[15] investigated the MCRC risk in a retrospective cohort of 79 patients with previous rectal adenocarcinoma and with germline *MMR* gene mutations. Twenty-seven per cent of MMR mutation carriers were diagnosed with MCRC. Cumulative risk of MCRC was 19% (95%CI: 9%-31%) at 10 years, 47% (95%CI: 31%-68%) at 20 years, and 69% (95%CI: 45%-89%) at 30 years after surgical resection. In spite of colonoscopy surveillance, 22% of MCRC cases were diagnosed at stage II, and 6% at stage III. Fante *et al*[2] investigated a subset of 1298 registered patients with CRC, and 53 patients (4.1%) were identified with MPCRC. The frequency in LS patients rose to 11.5% (5.8% SCRC, 5.8% MCRC), whereas the frequency in sporadic CRC was 3.6%, the most commonly reported frequency in most studies[4]. Differences were greater when only metachronous lesions were compared; these were four times more frequent in LS cases (5.8%

Table 2 Summary of studies about the prevalence of multiple primary colorectal carcinoma and the main molecular features of synchronous and metachronous colorectal carcinoma

Ref.	Study design	Prevalence of MPCRC (% of global)	Risk factors for MPCRC	Carcinogenetic pathways
[10]	Solitary (29) MPCRC (12) Study of MPCRC features		No differences: Age Gender Body mass index Tumour location History of CRC of MSI	CIMP-high 17.2% solitary *vs* 66.7% MPCRC $P = 0.004$
[36]	57 MPCRC Comparison of methylation status of solitary CRC *vs* MPCRC 57			Higher methylation for p14 MGMT in MPCRC $P < 0.05$ Correlations: MINT1 (r = 0.8) p16 (r = 0.8), MLH1 (r = 0.9) MGMT (r = 0.6) at the same site (MSI-H)
[16]	4760 CRC patients Study of SCRC *vs* solitary CRC	58 (1.2%) SCRC: 42 (72%) sporadic 4 (7%) UC 8(14%)Lynch 1 (2%) FAP 3 (5%) SP	Older patients ($P = 0.001$) Right colon ($P = 0.0003$) Synchronous polyps ($P = 0.0001$) Classical adenoma 47% *vs* 12% SSAs 16% *vs* 0%	36% *vs* 12%; ($P = 0.0005$) 92% if SSA precursor
[17]	2884 patients SCRC *vs* solitary CRC	77 (2.7%) SCRC	21 (27%) had a family history of Lynch	54 (32%) MSI-H (> in women and elderly) congruence (MSS/MSI) Yes: 67 patients (87%) No: 10 patients (13%)
[30]	2884 CRC Study of MPCRC methylation state in SCRC *vs* MCRC	33 (1.1%) MCRC 77 (2.6%) SCRC	MSI-H MCRC were younger (64 *vs* 76 years, $P =0.01$)	MSI-H tumors in 12 (36%) MCRC 29 (38%) SCRCP Promoter methylation 50% MCRC 83% SCRCP $P = 0.03$
[35]	2,068 CRC patients SCRC *vs* solitary CRC	47 (2.3%) SCRC	Mean age 68.9 *vs* 65.5 ($P =0.016$) No difference: Family history of CRC BMI	MSI-high ($P = 0.037$). > BRAF ($P = 0.0041$) > CIMP-high ($P = 0.013$) Correlation pairs LINE-1 (r = 0.82; $P = 0.0072$) CpG islands ($P < 0.0001$)

CRC: Colorectal cancer; MCRC: Metachronous CRC; SCRC: Synchronous CRC; SSAs: Sessile serrated adenomas; UC: Ulcerative colitis; SP: Serrated polyposis; BMI: Body mass index; HR: Hazard ratio; OR: Odds ratio; MSI: Microsatellite instability; FAP: Familial adenomatous polyposis.

vs 1.3%; $P < 0.001$)[2]. Hu *et al*[16] reported similar findings: in a study that included 54 SCRC cases, more than 14% were in the context of LS. Moreover, 27.6% of patients with SCRC had first-degree relatives with different LS-related cancers[17].

Being affected by multiple primary neoplasms is a criterion for screening for LS according to the revised Bethesda guidelines[18]. MPCRC is a very important parameter in PREMM126, a computer model to estimate the overall cumulative probability of having a mismatch repair gene mutation. Males under 78 years old with MPCRC have at least a 5% cumulative risk of an MMR gene mutation, calculated by the PREMM126 model, independent of information regarding their relatives. Five per cent is the cut-off recommended for referral for genetic evaluation and/or for considering molecular testing of tumour samples for microsatellite instability (MSI) or immunohistochemistry[19]. As is well known, identifying LS is essential in order to intensify screening colonoscopy.

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder showing mutations in the *adenomatous polyposis coli* (*APC*) gene. It is characterized by the presence of hundreds to thousands of adenomas that can become malignant. Hu *et al*[16] reported that 2% of patients with SCRC suffer from FAP. However, the frequency of SCRC identified in that series seems to be greater than the current findings in global series of CRC, where FAP represents less than 1% of all CRCs. The common surgical management of FAP includes prophylactic resection of the entire colon. When a more conservative approach is taken (for example when the rectum is preserved, or in attenuated forms of FAP), strict surveillance should be done in order to avoid the development of MCRC[20].

Different studies have suggested an association of MPCRC with the serrated pathway, as a consequence of a field defect arising in the mucosa of patients with large

sessile serrated adenoma (SSA), resulting in increased risk for SCRC[16]. Serrated polyposis syndrome refers to a condition characterized by multiple, large and proximal hyperplastic polyps[21]. Although there are still many doubts about the significance and the pathogenesis of serrated polyposis, the main consensus criteria for the diagnosis of serrated polyposis include the possibility of a familial pattern. According to the World Health Organization[22], serrated polyposis is diagnosed if any of the following criteria is met: (1) at least five serrated polyps proximal to the sigmoid colon, two or more of which have a diameter of greater than 10 mm; (2) any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis; and (3) more than 20 serrated polyps of any size but distributed throughout the colon.

A study of 58 patients with SCRC identified 13 patients whose tumours were derived from SSA (SSA-associated SCRC): Three of them (23%) (SSA-associated SCRC) met criteria for serrated polyposis[16]. Moreover, a family history of CRC in patients with serrated polyposis syndrome has been reported in different studies, ranging from 33% to 59% of patients[21,23,24]. Nevertheless, no germline mutation associated with serrated polyposis syndrome has yet been identified. The main carcinogenetic pathway related with serrated adenocarcinoma is the DNA methylation pathway CpG Island Methylation Phenotype (CIMP). CIMP is often related with environmental exposure, and therefore some authors hypothesize about the possibility of an inherited abnormality of epigenetic regulation[25]. Such an abnormality would lead into the accumulation of somatic methylation events in tumour suppressor genes and would synergize with somatic oncogenic activation of *BRAF*, resulting in the development of premalignant serrated lesions. Other investigations found a weak association with mutations in *MUTYH* or *MBD4* genes, especially when adenomas and serrated polyps are simultaneously present[23]. The presence of conventional adenomas in serrated polyposis is also associated with an increased risk of CRC[26].

Excluding the high-penetrance inherited CRC syndromes, around 10% of CRC patients have a family history of the disease. These non-syndromic familial CRCs have been defined as "apparently sporadic forms of the disease that occur in families more often than expected by chance"[27,28]. Samadder *et al*[13] found an increased risk of CRC in first-degree relatives of patients with MPCRC, whereas a relative of a sporadic CRC patient has an increased risk of 1.75 (OR = 1.75, 95%CI: 1.63-1.88) compared with no affected relatives: first-degree relatives of SCRC patients had an about 1.9-fold increased risk of CRC (OR = 1.86, 95%CI: 1.37-2.53), and first-degree relatives of MCRC patients had a 2.5-fold increased risk (OR = 2.34, 95%CI: 1.62-3.36). The differences in risk between solitary CRC and MCRC were statistically significant (OR = 1.41, 95%CI: 1.05-1.91)[13].

When talking about familial predisposition in MPCRC, differences between SCRC and MCRC should be taken into account. Whereas MCRC can be facilitated by some inherited predisposition, with a continuous possibility during the entire lifespan of a person to develop a carcinoma, SCRC appears more likely to be the result of damage through some environmental factor during a specific period of time that enables the concurrence of two tumours at the same time. SCRC tends to be diagnosed at an advanced age compared with sporadic CRC, whereas MCRC tends to appear at an earlier age. This also supports the predominantly familial pattern of MCRC[13]. We have explained above how the risk of having an MCRC in LS is higher than that of having an SCRC[2]. The pathological features of MCRC also suggest a hereditary pattern. It is known that mucinous adenocarcinoma is a typical histologic feature of LS-associated CRC: In a large series of 102 patients with SCRC and 56 patients with MCRC, no differences in the incidence of mucinous carcinomas between SCRC and solitary CRC were found; nevertheless they are more common in metachronous forms[29].

Another factor supporting the hypothesis of the weaker relationship of SCRC with hereditary forms is the higher relation with sporadic MSI forms. Compared with solitary CRC, where about 10%-20% of patients show high MSI, the two types of MPCRC show more than 30% of MSI tumours. In some series, MSI was present in 36% of MCRC cases and in 38% of SCRC cases[30]. Up to 81% per cent of those SCRC cases lose MLH1 protein expression because of promoter hypermethylation; thus, the frequency in SCRC is twice as high as in MCRC (81% *vs* 41%). Both multiple forms associated with MLH1 promoter hypermethylation were more likely to be diagnosed at an older age and showed less frequently CRC in a first-degree relatives[30]. On the other hand, the high prevalence of MSI tumours also explains the most frequent distribution of MPCRC: both SCRC and MCRC used to be located in the proximal colon[31].

Individual predisposition

The screening guidelines for LS list the presence of SCRC as a risk factor. However, LS-associated SCRC accounts for a minority of all SCRC cases. Hu *et al*[16] described LS only in the 38% of SCRC cases. Thus, apart from the already known hereditary forms of CRC, there is an important proportion of MPCRC without a clear basis of inheritance. Some clinical features may suggest individual predisposition for MPCRC. The risk of sporadic CRC increases with age, and therefore an increased prevalence of multiple tumours would be expected in the elderly population. As mentioned before, SCRCs are identified in older patients compared with solitary CRC (median age 70 years *vs* 60 years; *P* = 0.001)[16], and other studies support these findings[5,13]. This association between SCRC and age can be explained by cumulative environmental damage,

because hereditary patterns usually lead to an early onset of the disease. Nevertheless, other studies did not find any differences regarding to the age at presentation[8,32].

As we described above, MSI is associated with SCRC. Although it is frequent in LS, it is not exclusive of it, and MSI is identified in about 10%-15% of sporadic CCR cases[33]. Several small tumour-based studies have found that MSI and abnormal methylation (CIMP-High and *BRAF* mutations) were more frequent in sporadic SCRC and MCRC[13]. Overall chromosomal instability, MSI, and CIMP are implicated in developing various predisposing lesions for MPCRC. In a molecular study of SCRC, Lam *et al*[8] found a 60% positive status for chromosomal instability; 10% was MSI and CIMP-0, whereas 30% was MSI and CIMP positive. Hu *et al*[16] also reported similar findings: They described a signinficantly increased rate of MSI, up to 36% (21/58) *vs* 12% (13/109) in solitary tumours (*P* = 0.0005), and they also found a difference of 20% in the prevalence of precursor SSA: 22% (13/58) in SCRCs *vs* 2% in solitary CRCs (2/109)) (*P* = 0.0001). Along these same lines, some authors point out that the pathway by which MPCRC shows MSI differs from the classical mutations of LS[34]. Epigenetics are thought to play a major role in the carcinogenesis of sporadic MPCRC. Between 31% and 62% of SCRC tumours have lost of MLH1 protein expression because of the hypermethylation of the promotor[16,30]. SSAs are the most common lesions of the methylation pathway, as Hu *et al*[16] showed that most (13/21, 62%) of the MSI SCRCs were associated with precursor SSA lesions and were apparently sporadic, with concurrent loss of MLH1 and PMS2 expression, and positive for the *BRAF* V600E mutation. They also found that 22% of SCRCs developed from an SSA, as opposed to only the 2% of solitary CRCs (*P* = 0.0001). In agreement with these findings, Gonzalo *et al*[10] found a close association between MPCRC and CIMP, identifying 102 CpG sites that showed significant hypermethylation in multiple tumours compared with solitary CRCs: 66.6% of MPCRCs were CIMP-high, whereas 5 of 29 (17.2%) solitary tumours showed CIMP (*P* = 0.004). Nosho *et al*[35] also described differences in methylation-related features in MPCRC: They found alterations in six of the eight CIMP methylation panel markers, with higher methylation levels of the long interspersed nuclear element 1 (LINE-1) and a higher frequency of *BRAF* mutations. Konishi *et al*[36] analysed the methylation status of a limited number of makers in 57 MPCRCs and 69 solitary CRCs, and found that the methylation status of *p14* and *MGMT* was significantly higher in multiple tumours; in addition, they described concordant methylation within tumour pairs at the same colonic site. Nosho *et al*[35] also found CIMP correlation between tumours at the same colonic site, and no correlation was found for pairs of tumours located in discordant locations. In summary, as the methylation state depends on multiple environmental factors (smo-

king, eating foods cooked at high temperatures), it may contribute to increasing the rate of MPCRC.

There are several studies that correlate environmental exposure with increased risk of MPCRC. Borda *et al*[37] studied possible risk factors for developing this entity, and they proposed alcohol intake as a risk factor for MCRC and SCRC. The relation between CRC and alcohol intake has been described before, even with moderate intake (about 20-40 g/d)[38]. Moreover, a cumulative alcohol intake of more than 9800 g, calculated as weekly average alcohol intake multiplied by years of drinking, has been described as a risk factor for SCRC[39]. Another study showed that risk of MCRC was not associated with gender, age at diagnosis, country of recruitment, cigarette smoking status, maximum dimension of primary tumour, and histological grade of the rectal cancer[15]. This study associated the risk of MCRC exclusively with the higher stage at diagnosis of the tumour (HR = 6.14, 95%CI: 1.21–13.14; *P* = 0.03) and a prior diagnosis of SCRC (HR = 11.54, 95%CI: 10.6-12.5; *P* = 0.04). Tobacco smoke contains a variety of genotoxic substances, including polycyclic aromatic hydrocarbons, nitrosamines, and heterocyclic and aromatic amines. Compounds in cigarette smoke activate the aromatic hydrocarbon receptor which can lead to DNA methylation[40]. Samowitz *et al*[41] described how heavy smoking could be related with CIMP and *BRAF* mutation, but no differences were found in MSI rate, although other authors did find an association[42].

SCRC is more frequently associated with adenomas than solitary CRC. Nowadays, precursor entities such as multiple serrated sessile polyposis are more commonly related with SCRC[43]. Tobacco can lead to an MPCRC because an increased number of polyps, either adenomas or serrated polyps, has been found in patients with direct exposure to tobacco[44]. However, a special pathogenic role has been described for serrated polyposis[21]. The tendency for these lesions to be multiple, when there is an association with smoking, and the frequency of *BRAF* mutation and CIMP, point to a defect that may result from interactions between environment with a low penetrance genetic predisposition[25].

As we have seen, tobacco and alcohol consumption have been adressed in several studies, and both are considered risk factors for CRC. When Borda *et al*[37] investigated both habits in relation with SCRC they found that both SCRC and tobacco were associated with male gender, but they did not find a statistically significant difference using multivariate analysis[37]. A study by Piñol *et al*[12] supports the observation that SCRC is independently associated with gender (*P* < 0.001). Another risk factor studied by Borda *et al*[37] was body mass index (BMI), and they found that a BMI of less than 21 was a protective factor for MPCRC.

Inflammatory bowel disease (IBD) can increase the risk of CRC. The prevalence of CRC in ulcerative colitis (UC) is 3.7%, increasing to 5.4% in those cases

with pancolitis[45]. Continuous inflammation induces molecular damage and causes a hypermethylation state of the colon mucosa that can promote carcinogenesis. Colonoscopy surveillance may be carried out in order to diagnose the precancerous state of dysplasia. Chromosomal instability and MSI pathways also have a major role in UC-associated CRC. However, there are differences in the instance and frequency of these alterations. *APC* loss of function is less frequent and usually occurs later in the UC-associated dysplasia-carcinoma sequence than in the classical adenoma-carcinoma sequence. By contrast in patients with UC, *p53* mutations occur earlier and are often detected even in mucosa without dysplasia or still indefinite for it. Issa et al studied the CpG methylation status of four genes (*ER*, *MYOD*, *p16* exon 1, and *CSPG2*) in patients with UC[46]. The methylation status was higher in high-grade dysplasia patients both in apparent normal mucosa and in high-grade dysplasia areas, suggesting that methylation precedes dysplasia; increased methylation is also widespread in the mucosa of high-risk patients. Several polymorphisms of genes related with the inflammatory response may increase this CpG methylation state[47]. SCRCs have been frequently found in the colons of patients who had total colectomy for low-grade dysplasia[9]. Therefore it is not coincidental that MPCRC often appears in the context of IBD: SCRCs are present in 18% of UC-related CRCs[9]. In a retrospective study of 64 patients with Crohn´s disease and CRC, Maser *et al*[48] found MCRC in 39% of patients 7 years after segmental of subtotal resection, which increased and at a rate of 0.5% per year after 8 to 10 years. Duration and extension of colitis are the two major features associated with increased risk of CRC[49]. From a review of the literature, Eaden *et al*[45] derived incidence rates of CRC in UC patients which corresponded to cumulative probabilities of 2% by 10 years, 8% by 20 years, and 18% by 30 years. These data suggest an accumulated methylation over time, provoking a field defect that reflects acquired predisposition to CRC and that might promote MPCRC as well[46].

Simultaneous individual and familial predisposition
An important proportion of MPCRC should be described as a whole, in which hereditary component cannot be distinguished from acquired alterations. Although SCRC and MCRC are different entities, they are linked. Patients with MCRC received a diagnosis of SCRC at the time of the initial CRC more often than patients with a solitary CRC (11.1% *vs* 3.1%; *P* = 0.001)[4]. Other studies described the increased risk of developing a MCRC after a SCRC with a hazard ratio 11.54 (HR = 11.54, 95 %CI: 10.6-12.5; *P* = 0.04)[15]. Synchronous adenomas represent a multifocal disease affecting the colonic mucosa. In spite of resection of all the synchronous adenomas, another lesion may appear from a damaged mucosa and develop into a MCRC[50]. The susceptibility to develop two primary neoplasms, simultaneously or

consecutively, in the large bowel can be explained in different but not exclusive ways. Firstly, inheritance can mediate cumulative molecular defects that lead to dysplasia at different sites of the colon. On the other hand, one defined enviromental exposure may be implicated in the genesis of synchronous tumours, and continued exposure could result in another consecutive neoplasm. Nowadays, apart from hereditary forms such as LS or FAP, in most cases we do not know what the genetic inherited predisposition for MPCRC is. Whereas MCRC could be mediated by a constitutional factor that generates different polyps over time with a continuous possibility of developing a carcinoma, the carcinogenetic process begins at the same time in two different places of the mucosa for SCRC. This situation arises because of the exposure of a large area of mucosa to the same ethiology factors for a time sufficient to initiate carcinogenesis. Regardless of individual or hereditary causes, once the carcinogenic process has begun it can continue generating new lesions in the remaining colon. The risk for MCRC after diagnosis of the first CRC is higher than the prevalence of single CRC in a sex- and age-matched population, and the risk is higher in the first years following diagnosis, up to 61% in the first three years[4]. The risk is even higher when SCRC was previously identified, so that a close follow up must be carried out.

As we mentioned above, different etiopathogenic factors are suspected to be involved for synchronous and metachronous CRC, and different familial *vs* individual factors may be implicated (Figure 1). MCRC seems to follow a familial pattern, whereas individual factors are more important in SCRC[43].

Since SCRC and MCRC can appear in the same individual, in some occasions both familial and individual conditions concur. A study performed by Greenstein *et al*[9] reported that SCRC accounted for 2.5% of "de novo" CRC, and SCRC occurred in 18% of UC patients, and in 21% of patients with FAP[9]. Familial predisposition seems to be important in this development. The methylation state of LINE-1 appears concordant between SCRCs in the same individual, indistinctly of congruent or incongruent localization of the tumours[35]. Moreover, the methylation state of *LINE-1* was also high in normal mucosa of these cases[35]. These findings suggest a similar substrate of the mucosa in relation with environmental exposure, where probably heritable factors modify the phenotype, by mutation of *BRAF*, hypermethylation of promoters of *MMR* genes (*MLH1*), or chromosome instability. Both genetic and environmental factors may influence serrated neoplasia as well to develop into CRC, which in these cases acquires MSI status. A polymorphism in the *MLH1* promoter (-93 G>A) gives an increased risk for loss of *MLH1* function in serrated polyps. This polymorphism does not increased the number of serrated polyps, but appears to promote their malignancy[40]. Moreover, smoking promotes the development of MSI in serrated polyps[25].
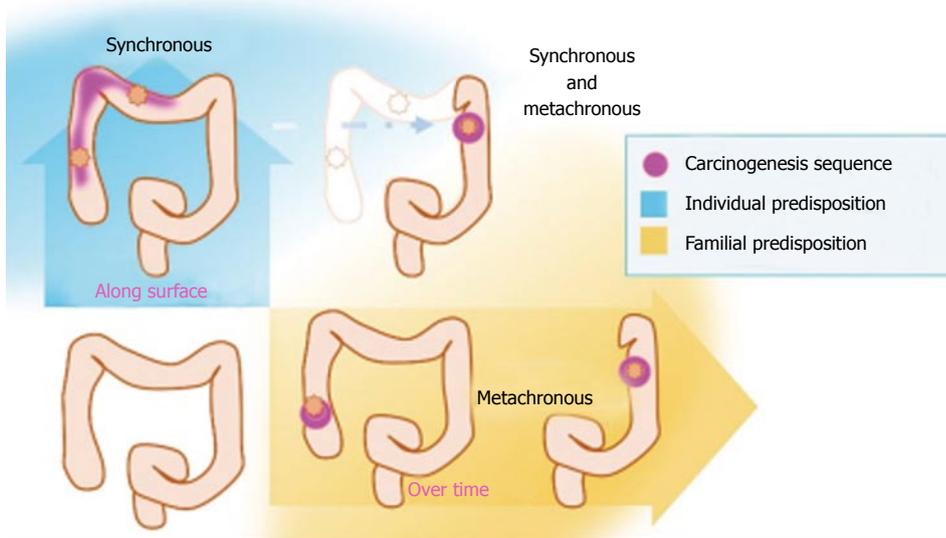
Figure 1 Both familial and individual factors may influence the genesis of multiple primary colorectal cancer. Genetic predisposition can promote the carcinogenesis sequence over time, whereas enviromental factors promote formation of different tumours along the colon surface at the same time.

HETEROGENEITY IN CRC

Finally, according to this feature of CRC, SCRC and MCRC may be defined as clinical entities and sometimes may not reflect the condition of the tumor at the molecular level. Genetic heterogeneity plays a fundamental role in this case. Sometimes MPCRCs show similar clinicopathological features, as shown by Huan *et al*[51], who explored a large cohort with 5346 patients with synchronous, metachronous and solitary CRC, finding similar clinicopathological features between SCRC and MCRC. Moreover, analysing primary CRCs and their metastatic sites[52], checking for mutations KRAS, NRAS, BRAF, PIK3CA, and TP53 genes in 615 patients found for most cases the same mutations between both link.

But in most cases, we have to take on account the proposed role of genetic heterogeneity in individual predisposition to CRC described by Galvan *et al*[53]. Either a single genetic defects or the polygenic conditions produce a cancer-prone condition in the human normal tissue; individual risk of cancer might be further modulated by environmental factors, leading to somatic mutations and ultimately to cancer. This is very striking when talking about the demethylation grade from normal mucosa of healthy people, single cancer patient and multiple CCR, emerging an increased rate of demethylation among normal mucosa of older patients, and in normal mucosa of patients with multiple CRC risk from younger patients, suggesting an inherited predisposition for the apparent field cancerization effect of somatic demethylation[54]. This influence of environmental conditions among hereditary predisposition is clearly shown by Rosty *et al*[55]. They found that the majority of CRCs arising in individuals with serrated polyposis (SP) do not harbor molecular hallmarks of serrated pathway CRCs but show a diverse range of molecular profiles. This suggests that CRC in SP patients may develop from non-serrated polyps through either a derivative of the traditional adenoma pathway. SP could therefore, potentially, be considered a disorder associated with a hypermature or inappropriately aged colonic mucosa, possibly secondary to an alteration in DNA methylation, with a resultant propensity to the development of early onset multiple serrated polyps and conventional adenomas.

Finally, another aspect thar supports the heterogeous condition of CRC is described by Zauber *et al*[56] who evaluated by a set of 6 different markers (LOH for APC, DCC, and mutations of KRAS, BRAF, MSI and methylation of *MMR* genes) 50 patients with SCRC and 5 MCRCs. They found that genetic changes may vary considerably, particularly when the tumors are found in different colon segments. Frequent differences in the molecular findings are also seen between SCRCs sharing the identical microenvironment of the same colon segment. These findings support the hypothesis that MPCRC may follow different pathways of carcinogenesis in the same patient.

CONCLUSION

MPCRC is a rare event but its prevalence is not negligible, and it has a great clinical impact. Both genetic and environmental factors may affect in the development of MCRC, collaborating in promoting different foci of dysplasia. If lesions progress at the same time, SCRCs arise, whereas MCRCs appear if they develop at different time. In general terms, MCRCs are mainly related to family factors whereas SCRC are linked with individual factors. Nevertheless, in some cases both entities arise in the same individual. Due to the heterogeneity of current studies, conclusive information on the molecular basis of MPCRC is scant, with the exception of cases of

hereditary forms of CRC. For this reason it is difficult to draw definitive conclusions at this time. New studies focusing on the carcinogenic mechanism must be done in order to better understand the molecular basis of MPCRC.

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