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REVIEW

Molecular approach to genetic and epigenetic pathogenesis of early-onset colorectal cancer

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Abstract

Colorectal cancer (CRC) is the third most frequent cancer type and the incidence of this disease is increasing gradually per year in individuals younger than 50 years old. The current knowledge is that early-onset CRC (EOCRC) cases are heterogeneous population that includes both hereditary and sporadic forms of the CRC. Although EOCRC cases have some distinguishing clinical and pathological features than elder age CRC, the molecular mechanism underlying the EOCRC is poorly clarified. Given the significance of CRC in the world of medicine, the present review will focus on the recent knowledge in the molecular basis of genetic and epigenetic mechanism of the hereditary forms of EOCRC, which includes Lynch syndrome, Familial CRC type X, Familial adenomatous polyposis, MutYH-associated polyposis, Juvenile polyposis syndrome, Peutz-Jeghers Syndrome and sporadic forms of EOCRC. Recent findings about molecular genetics and epigenetic basis of EOCRC gave rise to new alternative therapy protocols. Although exact diagnosis of these cases still remains complicated, the present review paves way for better predictions and contributes to more accurate diagnostic and therapeutic strategies into clinical approach.

Key words: Early-onset; Colorectal cancer; Epigenetic mechanism; Genetic mechanism; Clinical outcome

Core tip: Early-onset colorectal cancer (EOCRC) cases are heterogeneous population that include both hereditary and sporadic forms of the colorectal cancer (CRC). EOCRC cases have some distinguishing clinical and pathological features than elder age CRC. Recent findings about molecular genetics and epigenetic basis of EOCRC gave rise to new alternative therapy protocols. We herein discuss the latest findings about genetic and epigenetic features of EOCRC.

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INTRODUCTION

Colorectal cancer (CRC) is the third most frequent cancer type and despite improvements in diagnosis and treatment, this disease is the second leading cause of cancer death in developed countries[1]. The highest incidence of CRC is observed in Western Europe, North America and Australia in western populations. It is notable that although the rate of this disease is relatively lower in the communities of the sub-Saharan Africa, South America and Asia, the rate is gradually increasing depending on assimilating life-style and dietary habits of the western countries[2]. In more developed countries, screening programs for 50 years and elder people leads to early detection of CRC and opportunity for more satisfactory treatments; thus, death rates reduced approximately 2% per year[3-5]. However, CRC screening is not common for young adults (between 20-40 ages), the incidence of this disease is increasing gradually per year in individuals younger than 50 years[6]. The tumors of early-onset patients are more aggressive than elder cases[7,8].

Because of the advances in our understanding concerning the molecular mechanism of elder age CRC, we can describe the presenting phenotype depending on the molecular characteristics of the tumor in majority of the cases[9]. This vital knowledge contributes to the available studies in the literature for individual-specific and targeted therapies for CRC patients related to their drug responses. However, the molecular mechanism underlying the early-onset CRC (EOCRC) is poorly clarified in the relevant literature. Recent studies have revealed that EOCRC might evolve in a different pathway and the molecular basis of these cases may be unique for individuals[10]. Therefore, determining identifiable markers of this disease for early diagnoses is required to develop unique treatment protocols and increase the survival of the patients. However, to date, little knowledge has been gained about the molecular basis of young age. Given this gap to be highlighted, the aim of our review is to synthesize and evaluate the current literature regarding the genetic and epigenetic pathogenesis of EOCRC at molecular level.

MOLECULAR PATHOGENESIS OF EOCRC

In comparison to elder CRC, EOCRC cases have some distinguishing clinical and pathological features[11,12]. These tumors are pathologically recognized with low-grade tumor differentiation, mucinous component and high signet ring cells frequency[11,13]. Polyp development is contently observed during the follow-up period of EOCRC[10]. The majority of early-onset tumors occur in the distal colon and the rectum[14]. Previous studies underlined the significance of heritance as an indicator of EOCRC[11,12]. Supporting these views, early-onset and hereditary forms of CRC demonstrate similar well-known pathological features[11,13]. Nevertheless, the current knowledge is that EOCRC is a heterogeneous disease with both familial and sporadic cases. The molecular basis of this heterogeneity has not yet been fully clarified in the literature, however, the severe histopathological properties and a possible genetic feature of the tumor may predispose to expedited tumor growth in young age patients as reported[15,16].

HEREDITARY FORMS OF EOCRC

In the hereditary forms of CRC, the disease can be observed in one or more first and/or second degree relatives of the patient. Thus, familial CRC counts approximately 20% of all CRC patients[17]. With almost 3% observation rate, the most frequently occurring familial CRC is Lynch syndrome (LS)[18]. On the other hand, polyposis syndromes, such as familial adenomatous polyposis (FAP), MUTYH-associated polyposis (MAP), Juvenile polyposis syndrome (JPS) and Peutz-Jeghers syndrome (PJS), are less often observed familial colorectal syndromes[17].

LS

LS is frequently right-sided, an autosomal dominant cancer predisposition. The majority of these tumors are synchronous and metachronous. Extracolonic sites of patients, such as brain, ovary, endometrium, renal pelvis, ureter, stomach, small intestine and skin, are also among a high cancer risk[18]. LS is caused by various germline DNA mismatch repair (*MMR*) gene mutations[19-21]. Approximately 90% of the identified LS mutations are observed in *MLH1* or *MSH2* genes and approximately 10% of the LS mutations were identified in *MSH6* and *PMS2* genes[17,22]. The prevalence and characteristics of these mutations vary widely among populations. In 2010, we defined two frame-shift mutations (*MLH1* c.1843dupC and *MLH1* c.1743delG) and three missense mutations (*MLH*1 c.293G < C, *MLH1* c.954_955delinsTA and *MSH2* c.2210G < A) uniquely in Turkish LS cases[23]. In a study of Italian LS families, c.643_648 dupA, c.2156_2157 dupT, c.684_685 dupC and c.1701_1704 delT frameshift mutations andc.2206 G < T, that cause a truncating protein were first time determined in *MLH1* gene. Other truncating protein causing mutations, c.1089 G < T andc.2634-2 A < G, that results with a splice defect was originally reported in *MSH2* gene[24]. In Malaysia population, two novel mutations, c.3341_3342insC and c.3885_3891delTAAAAGC were characterized in *MSH6* and c.2395C > T mutation was defined in *PMS2* gene[25]. Recently, an unidentified mutation of *MLH1*, c.2044_2045del was linked to LS in a Caribbean Hispanic family[26].

Deficient MMR function of LS cases usually promotes microsatellite instability (MSI)[27,28]. MSI is characterized by length alterations within simple repeated sequences that are called microsatellites[28]. MSI is essential for deregulation of cell growth, differentiation and death[29]. MSI also plays roles in modulating the response of patients to various chemotherapeutic agents[27]. Losing the expression of MMR proteins *via* inactivation of MMR-

Table 1 MiRNA profile of lynch syndrome patients

MiRNA	Expression status	Function	Ref.
miR-155	Up	MMR deficiency	Valeri *et al*[46]
		MSI	Earle *et al*[48]
miR-26b	Down	MSS	Earle *et al*[48]
miR-31	Up	MSI	Earle *et al*[48]
miR-223	Up	MSI	Earle *et al*[48]
miR-486-5p	Down	MSI	Balaguer *et al*[47]
miR-622	Up	MSI	Balaguer *et al*[47]
miR-1238	Up	MSI	Balaguer *et al*[47]
miR-1362-5p	Down	MSI	Balaguer *et al*[47]
miR-132	Down	MMR deficiency	Kaur *et al*[49]
miR-345	Down	MMR deficiency	Kaur *et al*[49]

MSS: Microsatellite stabile; MSI: Microsatellite instability; MMR: Mismatch repair.

deficient crypt foci genes causes an MSI phenotype[30]. In these patients, the mutation rates of *ACVR2*, *TAF1B* and *ASTE1*, microsatellite-bearing target genes are higher than 80%[29-33]. Recent studies indicated that in MSI cases, frameshift mutations of apoptotic genes, such as *APAF1*, *BAX* and *FLASH,* lead to intratumoral heterogeneity[28]. The study of Markowitz *et al*[34] demonstrated the relation with DNA repair defects with a specific pathway of CRC progression and three different mutation of *TGFBR2* gene in 1995[34]. However, the latest study of de Miranda *et al*[35] showed the transcription and translation of *TGFBR2* with a 1 nucleotide deletion at its microsatellite sequence still produced a functional *TGFBR2* protein. This protein is required for phosphorylation of *SMAD2*, which is phosphorylated in most of the MSI CRC tissues[35].

The *MMR* gene modifications of LS occur by two hit usually point mutations or large rearrangements may give rise to the first hit. Accordingly, gene conversion or loss of the wild-type allele evokes the second hit[36]. However, recent observations demonstrated the high rate of promoter methylation occurrence as the second hit[37,38]. These findings emphasize the role of epigenetic events in formation of LS[37,38]. Indeed, depending on the studies, germ line hemiallelic methylation of *MLH1* and epimutations of *MSH2* lead to LS with insufficient MLH1 or MSH2 protein expression in mutation negative families[39-42]. Ligtenberg *et al*[43] state that germ line 3′ end deletions of *EPCAM* gene that is located upstream of *MSH2*, correlate with MSI and a loss of *MSH2* protein even though there was no identifiable mutation in *MSH2* gene[44]. Kuiper *et al*[44] found *EPCAM* deletions in approximately 2.3% of *MSH2*-deficient families. This study affirms the epigenetic transgenerational inheritance and the possibility of aberrant promoter methylation occurrence in neighbouring tumor suppressor genes by loosing of polyadenylation signals[28]. In addition, current evidence in the empirical studies supports the role of miRNAs that is responsible for translational rearrangement of proteins, in regulation of MMR genes expressions[45]. In comparison to sporadic MSI tumors, LS patients have a typical miRNA profile. Valeri *et al*[46] demonstrated the association of reduced expression of *MSH2*, *MLH1* and *MSH6* and induction of a mutator phenotype and MSI with over expression of miR-155 in LS. In another study, Balaguer *et al*[47] determined the up-regulation of miR-622 and miR-1238 in these patients. MSI status modulates the miRNA expression levels[48]. Earle *et al*[48] defined the increased expression of miR-155, miR-31, miR-223 and miR-26b in MSI tumors. In addition, Earle *et al*[48] linked over expression of miR-31 and miR-223 to LS. Not only miRNA regulates gene expression in an epigenetic way but also miRNA expressions may be regulated epigenetically. With containing a CpG island in the promoter region, most of the miRNAs are favorable for aberrant methylation which can give rise to dysregulation of miRNA[49,50]. Kaur *et al*[49] identified a correlation between miR-345 and miR-132 hypermethylation and MMR deficiency (Table 1).

Familial CRC type X

MMR germline mutations are observed in approximately 60% of the families, fulfilling clinical criteria for LS[51]. Although familial colorectal cancer type X (FCCTX) accomplish the same clinical criteria with LS, the morphological features, such as right-sided tumor location, poor differentiation, expansive growth pattern, tumor-infiltrating lymphocytes, peritumorous lymphocytes, Crohn-like reactions and lack of dirty necrosis, are not common in FCCTX as LS[52]. In addition, despite these, families demonstrate clinical features in which CRCs with MSI, FCCTX is not related to germline MMR gene mutations[51,53]. The age onset of FCCTX is relatively older than LS cases and this disease differ from LS with the tumorigenic pathways[54,55]. Basically, two individual molecular pathways involve in these families. One of these pathways is loosing of tumor suppressor gene loci genes, such as *TP53*, *APC*, *SMAD4* and *DCC*, somatic mutations of *APC* and *KRAS* and *MGMT* promoter methylation. At the second partway, there is no loosing of tumor suppressor gene loci genes and rarely presenting promoter methylation[56]. Therkildsen *et al*[57] linked to FCCTX tumors with gain of genetic material in two separate regions encompassing, 20q12-13.12 and 20q13.2-13.32. This study revealed that gain of material on chromosome 20q and loss on chromosome 18 differentiate FCCTX from LS. Findings of Dominguez-Valentin *et al*[58] showed that gaining mutations of *GNAS* gene which is located in 20q13.32 and encodes for the Gα-subunit may cause FCCTX *via* activation of the Wnt and ERK1/2 MAPK signalling pathways. Moreover, other 20q located genes, *CDH26*, *SRC* and *ASIP* that play role in proliferation and migration may have a potential to cause FCCTX[58]. Dominguez-Valentin *et al*[58] defined the up-regulation of *PTGER1* in these tumors which can cause tumor growth through altered prostaglandin E2 function[59,60]. Recently, an *SEMA4A* gene variant c.232G > A was determined in Austrian kindred with FCCTX. This study revealed that *SEMA4A* (V78M) lead to activation of MAPK/Erk and PI3K/Akt signaling. Moreover, *SEMA4A* mutations, c.1451G > C and c.977C > T and the single-nucleotide polymorphism c.2044C

Table 2 Molecular characterization of familial colorectal cancer type X patients

Molecular features		Ref.
Germline *MMR* gene mutations	-	Lindor *et al*[51] Klarskov *et al*[52] Sánchez-Tomé *et al*[53]
Tumor supressor gene loci loss		
APC mutations	77%	Francisco *et al*[56]
KRAS mutations	46%	Francisco *et al*[56]
MGMT methylation	36%	Francisco *et al*[56]
Chromosome gains	20q, 19 and 17	Therkildsen *et al*[57]
Chromosome loss	8p, 15, 18	Therkildsen *et al*[57]
Signaling by G protein coupled receptor (*GNAS, F2R, F2RL2, EDN1, EDNRA, GRM8, GNA2, GNG11, GNG12, HCRT, PTGER1, P2RY2, RAMP2, MC1R, TUBB3, VIP*)	up-regulated	Dominguez-Valentin *et al*[58]
SEMA4A variants		Schulz *et al*[61]

> T were determined as associated with the FCCTX phenotype[61]. Recent knowledge about the molecular characterization of FCCTX is summarized in Table 2.

FAP

FAP is an autosomal dominant cancer syndrome[62]. FAP is diagnosed with 100 or more adenomatous polyps in colon or rectum in patients with younger than 40 age[62]. Patients with FAP carry germline mutations of the adenomatous polyposis coli (*APC*) gene located on chromosome 5q21-q22[63]. APC protein is a large scaffolding protein which involves in Wnt signaling pathway. In this protein complex, APC leads to down regulation of b-catenin activity and play a central role in a destruction complex of Axin, GSK-3β and casein kinase 1. This complex directs a series of phosphorylation events on β-catenin that target it for ubiquitination and subsequent proteolysis[64]. In the absence of APC protein, b-catenin binds to several transcription factors of the TCF/LEF and initiates the altered expression of genes associated with proliferation, differentiation, migration and apoptosis. Moreover, the depletion of APC can lead to abnormal chromosome segregation and aberrant mitosis[65,66]. FAP occurs when there are mutations between codons 168-1580 and with severe disease between codons 1250-1464 of *APC* gene[67,68]. The majority of *APC* mutations are either frameshift or nonsense mutations resulting in a truncated protein[69]. The two most frequently described germline mutations are located at codon 1309 (c3927_3931delAAAGA) and codon 1061 (c.3183_87delACAAA)[70]. Although two-thirds of FAP patient disease is inherited, the rest of the cases have no family history and carry unique mutations. Almost all APC mutations results with a colonic phenotype but variable for extra-colonic manifestations, such as desmoid tumor, hepatoblastoma, thyroid carcinoma, medulloblastoma, a litany of benign lesions and brain tumors, particularly medulloblastomas[71-73]. Lamberti *et al*[74] found that *GSTT1* polymorphism showed an uncertain association with extra-intestinal manifestations in a study of 411 FAP patients. Recent studies demonstrated the enrichment of pyloric gland adenomas of the stomach, in addition to fundic gland polyps and foveolar-type adenomas in patients with FAP[75,76]. Hashimoto *et al*[75] analyzed the genetic alterations in these FAP-associated gastric lesions and they demonstrated that, as well as *APC* mutations, these cases had *GNAS* and *KRAS* mutations.

KRAS mutations have been observed in the early development of approximately 40% of colon cancers. Simultaneous *APC* depletion and *KRAS* mutation results with an augmentation in adenomas[76] and induce the spread of stem cell marker carrying cells within the tumor epithelium[77]. Phelps *et al*[78] stated that in FAP adenomas, intestinal differentiation is required two consecutive steps. In the first step, after APC loss, CtBP1 contributes to adenoma initiation and in the following step, KRAS activation and β-catenin nuclear localization promote adenoma progression to carcinomas. On the other hand, Obrador-Hevia *et al*[79] analysed somatic *APC* and *KRAS* mutations, beta-catenin immunostaining, and qRT-PCR of *APC*, *MYC*, *AXIN2* and *SFRP1* genes in sixty adenomas from six FAP patients with known pathogenic *APC* mutations. Based on this study, the Wnt pathway was constitutively activated in all APC-FAP tumors, with alterations occurring both upstream and downstream of APC. Thus, Obrador-Hevia *et al*[79] suggest that for Wnt signalling activation in APC-associated FAP adenomas, oncogenic *KRAS* is not essential.

FAP may also pursue a different way to Wnt signalling pathway alterations though epigenetic mechanisms. Although epigenetic alterations of Wnt signalling are an effective factor for FAP formation, APC mutations exist in almost all FAP patients. Romero-Giménez *et al*[80] evaluated the possible role of germline hypermethylation of the *APC* promoter in FAP families that were negative for *APC* mutations in 21 FAP families and they did not identify signs of abnormal promoter methylation, indicating that this form of epigenetic silencing is not a common cause of FAP. However, Kámory *et al*[81] observed promoter hypermethylation that causes somatic inactivation of *APC* in 21 sporadic cases (30%). In the study of Zhang *et al*[82] within FAP families, although methylation was not present in normal tissues, hypermethylation was determined in tumor tissues of one proband and her son. In addition, loss of heterozygosity was observed in another patient from the same FAP family. Segditsas *et al*[83] declared similar findings with Zhang *et al*[82] They detected *APC* promoter methylation in 27%-45% of colorectal tumors and cell lines but did not detect in normal colorectum. However, they substantially observed that methylation was independent of the *APC* mutations and was not associated with the CpG island methylator phenotype. Although methylation caused the loosing of 1A isoform mRNA and a reduction in total *APC* transcript levels, *APC* gene expression was retained from promoter 1B[83]. Moreover, a recent study of Pavicic demonstrated that promoter 1B deletions of *APC* are not very common[84]. Thus, all these studies imply that

Table 3 Genetic and epigenetic alterations of familial adenomatous polyposis patients besides adenomatous polyposis coli

		Alteration	Ref.
Gene			
	GNAS	Mutation	Hashimoto *et al*[75]
	MYC	Gene activation	Obrador-Hevia *et al*[79]
	AXIN2	Gene activation	Obrador-Hevia *et al*[79]
	SFRP1	Gene activation	Obrador-Hevia *et al*[79]
	GSTT1	Polymorphism	Lamberti *et al*[74]
	MGMT	Methylation	Wynter *et al*[86]
	p14ARF	Methylation	Wynter *et al*[86]
	p16INK4	Methylation	Wynter *et al*[86]
	IGSF4	Methylation	Berkhout *et al*[85]
	TIMP3	Methylation	Berkhout *et al*[85]
	ESR1	Methylation	Berkhout *et al*[85]
	CDH13	Methylation	Berkhout *et al*[85]
miRNA			
	miR-143	Down regulation	Kamatani *et al*[87]
	miR-145	Down regulation	Kamatani *et al*[87]
	miR-126	Down regulation	Yamaguchi *et al*[88]
	miR-20b	Down regulation	Yamaguchi *et al*[88]

even though *APC* promoter methylation occurs in early during colon neoplasia progression, it does not result in complete gene inactivation or act as a "second hit"[84] and promoter-specific alterations of *APC* rarely leads to mutation-negative FAP[84].

In addition to *APC*, hypermethylation of other genes are usually observed in both FAP-related and sporadic duodenal carcinomas[85]. Wynter *et al*[86] study showed that the methylation of *MGMT*, *p14ARF* and *p16INK4* genes promoter regions are frequently observed in both sporadic and familial adenomas. Berkhout *et al*[85] defined the high methylation rate of the *IGSF4*, *TIMP3*, *ESR1*, *APC* and *CDH13,* in both of these cases, however, in the same study, *PAX6* gene was determined as hypermethylated only in FAP-related carcinomas. Recently, the role of altered miRNA expression in Wnt signalling regulation and FAP development has also been evaluated. Lately, the studies indicated the decreased expression of miR-143, miR-145, miR-126 and miR-20b as an early event of colorectal carcinogenesis in FAP tumors[87,88]. Specifically, miR-126 and miR-20b play role in angiogenesis[88]. Thus, downregulation of these miRNAs is an important genetic event for the initiation step in colorectal tumor development[87]. Besides APC alterations, other genetic and epigenetic events determined in FAP patients were summarized in Table 3.

MAP

MAP is an autosomal recessive polyposis syndrome. Approximately 0.3%-1% of all CRCs is associated with MAP[89,90]. Cases with MAP typically present multiple colon adenomas, thus at the first glance, these cases may be diagnosed as FAP. However, because they also can have *MMR* gene mutations, it can reverberate to phenotype as LS[91]. Although existing of multiple colon adenomas, there is not any alteration in *APC* gene of these cases, but further analyses identified mutations in *MUTYH* gene which is a component of a base excision repair system and involves in protecting DNA from oxidative damage[92]. Farrington *et al*[93] reported that mutations of both *MUTYH* gene alleles increase the risk of endometrial tumors. These cases are rare and well known *MUTYH* mutations are linked to this disease are c.494A > G and c.1145G > A[94-96]. However, *MUTYH* mutations can vary with ethnicity[97]. c.536A > G and c.1187G > A in Caucasians, c.231 C > T and c.934-2A > C in Japan, c.1227_1228dup in Portugal, c.1376C > A in Finland were determined as the most frequent *MUTYH* mutations[98-100]. In the North-Eastern Italy, c.933+3A > C (IVS10 + 3A > C), accounts for nearly 1/5 of all *MUTYH* mutations[101]. In addition, because this mutation is also common in Germany, it is supposed to have a common origin in Western Europe[101]. MUTYH mutations that vary with ethnicity are summarized in Table 4.

Table 4 *MUTYH* mutations that vary with ethnicity

MUTYH mutation	Etnicity	Ref.
c.231 C > T	Japan	Miyaki *et al*[99]
c.934-2A > C	Japan	Miyaki *et al*[99]
c.1376C > A	Finland	Alhopuro *et al*[100]
c.933 + 3A > C	North-Eastern Italy, Germany	Pin *et al*[101]
c.536A > G	Caucasians	Yamaguchi *et al*[92]
c.1187 G > A	Caucasians	Yamaguchi *et al*[92]

Germline *MUTYH* mutations may also lead to the mutation of cancer-related genes, such as the *APC* and/or the *KRAS* genes, *via* G to T transversion[92]. In the study of Venesio *et al*[102]. mutated *MUTYH*-associated-polyposis adenomas exhibited only c.34G > T transversion in codon 12, or mutations in codon 13. They affirm that neither of these mutations was found in classical/attenuated familial polyposis adenomas.

JPS

JPS is a rare autosomal dominant disorder. JPS is diagnosed with numerous colon and rectum polyps or polyps with family history or juvenile polyps inside and outside of the intestine[103]. 20%-50% of JPS demonstrates familial pattern and the average disease onset of cases are 16 to 18[103]. JPS may coexist with Osler-Weber-Rendu syndrome [hereditary hemorrhagic telangiectasia (HHT)]. The most frequently encountered symptoms of HHT are Skin telangiectasia, epistaxis, intracranial haemorrhage, development of pulmonary arteriovenous fistulas, brain cavernous angioma and haemangioma[104]. Almost 60% of JPS cases demonstrate mutations in *SMADH4* and *BMPR1A* genes that are connected with TGF-β/BMP signal pathway[105].

To date, a number of mutations leading JPS and/or HHT have been described in *SMAD4* gene. These mutations include point mutations that are resulting with a stop codon or a change in the coded amino acid into another one, codons 361, 533 and 534 mutations, small deletions and insertions[103]. Specifically, Howe *et al*[106] determined the mutation, c.1244-1247delAGAC, in the hot spot of the *SMAD4* gene which leads to a

Table 5 Pathogenic germline mutations of juvenile polyposis syndrome

Mutation	Effect	Ref.
SMAD4		
c.1244-1247delAGAC	Hotspot mutation serious course of JPS with numerous cases of polyps, tumors located in the stomach and intestines	Howe *et al*[106]
BMPR1A		
c.230+452_333+441dup 1995	Frameshift mutation producing a truncated protein (p.D112NfsX2)	Yamaguchi *et al*[107]

JPS: Juvenile polyposis syndrome.

serious course of JPS with numerous cases of polyps and tumors located in the stomach and intestines. In addition, a considerable proportion of mutations in the *BMPR1A* gene are nucleotide changes generating a stop codon (nonsense) or leading to amino acid changes (missense). These mutations are distributed evenly in the entire gene sequence, intronic mutations (intron 1, 3, 4 and 5) and deletions between codon 224 and 359[103]. Yamaguchi *et al*[107] identified a *BMPR1A* mutation, which involves a duplication of coding exon 3 (c.230þ452_333þ441dup1995) that causes a frameshift mutation, producing a truncated protein (p.D112NfsX2) in a patient with JPS (Table 5).

In addition to mutations in *SMAD4* and *BMPR1A* genes, Juvenile polyps also was observed in Cowden, Bannayan-Zonana, and Gorlin syndromes. Cowden and Bannayan-Zonana syndromes is occurred by *PTEN* mutations and Gorlin syndrome develops *via* germline *PTCH* mutations. *PTEN* and *PTCH* mutations have been excluded as the causative mutations in almost all JPS patients[108-110].

To the best of our knowledge, there is a lack of knowledge about epigenetic regulation of JPS so far. However, recently, Ling *et al*[111] defined *SMAD4* as a miR-224 target as a metastasis factor, yet the relation of miR-224 and *SMAD4* expression in formation of juvenile polyps has not been clarified.

PJS

PJS is a rare (approximately 1 in 200000 observation rate) autosomal dominant disease[112,113]. PJS is characterized by occurrence of benign hamartomatous, Peutz-Jeghers-type polyps in the gastrointestinal tract in association with mucocutaneous pigmentation on the lips and oral mucosa[114]. PJS is diagnosed with presence of a hamartoma associated with two of the following three signs: Family history of PJS, mucocutaneous lentiginosis or polyposis of the small-bowel[115]. PJS patients face with abdominal symptoms during the first 10 years of life, almost 50% of patients experience the symptoms before the age of 20 years and they have an increased risk of developing gastrointestinal and extradigestive cancers[115-117]. Cancer development localized in small intestine, stomach, pancreas and colon in most of the cases[116]. In 93% of the affected individuals, there is a risk of developing complicating cancers between aged 15-64 years[116].

Percent of eighty to 90% of patients with PJS have family history[118] and according to genetic analysis, 40%-60% of these cases have germline STK11 (also known as LKB1) mutations as the major cause of this disease[112]. Because the downstream signalling pathway of STK11 has not been fully clarified, the knowledge about the mechanism of hamartomatous polyp formation and mucocutaneous pigmentation also insufficient at present. Studies demonstrated that induced *COX-2* gene expression has also been involved in the promotion of tumor formation from PJS polyps[119,120]. On the other hand, PJS cases with wild-type STK11 demonstrates multiple causative loci such as chromosome1p, a pericentric inversion in chromosome 6, a second PJS locus at 19q13.4 and a heterozygous germline mutation in the MYH11 gene[121-125]. Lately, Wang *et al*[121] performed sequence analysing in three Chinese individuals with PJS and identified 2 variants,OR4C45 c.767-768insAG and ZAN c.5767insG, which occur in PJS cases independently of STK11.

More than 145 different STK11 germline mutations have been reported in the literature result in a truncated premature protein or in transcriptional splicing errors[121,126-129] (Table 6). On the other hand, transcriptional silencing of this tumor suppressor gene by promoter hypermethylation has been shown as an alternative inactivation mechanism[130-133]. In addition to germline mutations, and promoter methylation, Wang *et al*[121] discovered four mutations in pre-microRNAs, MI0003131, MI0003530, MI0014206, and MI0005525, of which the corresponding mature miRNA, hsa-mir-492, hsa-mir-487b, hsa-mir-323b, hsa-mir-300 respectively.

SPORADIC FORMS OF EOCRC

The most well-defined hereditary form of CRC, LS, account for 2%-4% of total CRC and one-third of EOCRC cases[134,135]. FAP cases are observed in less than 1% frequency in total CRC cases. Thus, 70% of all CRC and the majority of EOCRC cases are introduced in sporadic form[136-138]. Sporadic EOCRCs are classified into two major groups. Chromosome unstable CRC (CIN) is characterized by gross chromosomal abnormalities and MSI[135]. Although MSI tumors behave less aggressively compared to CIN, CIN or MSI tumors do not always appear separately[139-141].

Sporadic EOCRC are morphologically characterized with poor cell differentiation, colloid component and lymphocytic stromal reaction[8,12,142]. Therefore, these cases are likely to be confused with LS patients. However, while MMR defects are observed *via* MSI pathway in LS, in sporadic cases MSI is not frequent. Studies to date imply that colorectal tumors characterized by MSI may be distinct from microsatellite stabile (MSS) tumors in many molecular aspects, such as an association with the methylator phenotype, a higher frequency of *BRAF* mutations and a lower frequency of *KRAS*, *APC*, and *TP53* mutations. Thus, MSI and MSS colon tumors

Table 6 *STK11* mutations associated with colorectal cancer caused by peutz-jeghers syndrome

STK11 mutation	Mutation type	Effect on protein	Ref.
c.511 G > A	Missense mutation	G171S	Dong *et al*[127]
c. 595 G > A	Missense mutation	E199K	Dong *et al*[127]
c.622 G > A	Missense mutation	D208N	Dong *et al*[127]
c.644 G > A	Missense mutation	G215D	Dong *et al*[127]
c.941 C > A	Missense mutation	P314H	Resta *et al*[128]
c.1062 C > G	Missense mutation	F354L	Dong *et al*[127]
c.1100 C > T	Missense mutation	T367M	Dong *et al*[127]
c.842delC	Frameshift mutation	truncates	Dong *et al*[127], Bartosova *et al*[129]
IVS2 + 1A > G	Intronic splice site mutation		Bartosova *et al*[129]
OR4C45 c.767-768insAG	Frameshift mutation	truncates	Wang *et al*[121]
ZAN c.5767insG	Frameshift mutation	truncates	Wang *et al*[121]

originate from different molecular backgrounds[143]. In sporadic cases, MMR deficiency occurs mainly through epigenetic inactivation of the *MLH1* gene through biallelic promoter methylation instead of MSI[136]. Both genetic and epigenetic inactivation of MMR genes result in a mutator phenotype, mutations in cancer related genes and CRC development[144]. Kirzin *et al*[137] identified *CTNNB1* as one of the most over-expressed genes in MSS-young patients compared to MSS-old patients and this leads to an over-activation of beta catenin in sporadic EOCRC. In addition, Fernandez-Rozadilla *et al*[145] determined a heterozygous deletion in the 10q22-q23 region involving *BMPR1A* gene of EOCRC cases with MMR proficiency. According to Luo *et al*[146] CDC42, TEX11, QKI, CAV1 and FN1 proteins are representative elements of EOCRC specific networks. Moreover, we defined *REG1A*, *CK20* and *MAP3K8* gene expressions strongly upregulated (more than twofold) in early-onset MSS CRC compared with MSI CRC tumors[147]. CK20 expression is observed in the majority of colorectal tumors[148,149], however, a limited number of studies have evaluated the relationship between *CK20* expression levels and MSI status[147]. In one study, it was suggested that reduced or absent *CK20* expression in CRC is associated with both sporadic and hereditary MSI[150]. In another study associated with EOCRC, *CK20* expression levels were also identified as relatively reduced in MSI tumors[10]. It was determined that *CK20* expression levels are inversely correlated with numbers of aberrant microsatellite locus[150]. We determined the upregulation of *CK20* expression levels in MSS tumors compared with MSI-low (MSI-L) and MSI-high (MSI-H) tumors[150]. According to McGregor *et al*[150] regulation of *CK20* gene expression involves molecular pathways that are altered by MSI-H. We defined 3.98-fold high *CK20* gene expression levels in MSS tumors with lymph nodes metastases than in MSI tumors with lymph nodes metastases[147]. In addition, 17.5-fold upregulation was identified in *CK20* expression levels in low-grade MSS tumors of patients with recurrence and distant metastases[147]. These results indicate that upregulation of *CK20* expression, specifically, is related to poor prognosis in patients with MSS tumors. Therefore, the results of our study indicate that *CK20* expression in MSS tumors allows for the determination of the biological characteristics of EOCRC tissues[147]. The encoded protein by *MAP3K8* gene is a member of the serine/threonine protein kinase family. In one of our study, *MAP3K8* expression in CRC was determined significantly elevated compared with normal mucosa[149]. In addition, we determined *MAP3K8* expression levels more than two fold upregulated in early-onset MSS CRC compared with MSI CRC tumors[147]. *MAP3K8* expression levels were significantly higher in the MSS tumors of patients with a short median survival. Thus, our observations revealed that upregulated *MAP3K8* expression was associated with a poor prognosis in patients with MSS tumors[147]. Human REG1A belongs to the superfamily of calcium-dependent lectins. In several previous studies, *REG1A* was found to be upregulated in CRC[151-153]. We also found that *REG1A* is upregulated in the tumors of early-onset sporadic CRC patients. Furthermore, 25.8-fold high *REG1A* gene expression levels were observed in MSS tumors with lymph nodes metastases. In addition, median survival and disease-free survival were significantly longer only for patients with MSI tumors with low *REG1A* expression compared with those with high expression of this gene. This result indicates that upregulated *REG1A* expression may be related to sporadic EOCRC tumor formation and characterization[147]. Additionally, a recent study from Sengupta *et al*[154] defined a relation with MSS CRC tumors and deletion in *RBFOX1* gene which encodes a highly conserved RNA-binding protein that regulates tissue-specific alternative splicing indicating important basic functions in development and differentiation in a British Bangladeshi MSS CRC population. This study showed that loss of RBFOX1 activity may lead to aberrations in the splicing of genes associated with CRC[154].

Different from MSS tumors, some sporadic EOCRC tumors belong to the MSI pathway[28]. Sporadic EOCRC with MSI is likely to arise from sessile serrated polyps through the serrated neoplastic pathway[155]. The *BRAF* gene, which plays an important role in the mitogen-activated protein kinase signalling pathway, is frequently mutated in these cases. *BRAF* V600E mutation is widely accepted as a prognostic factor of sporadic CRC with MSI and methylated *MLH1*[156]. Although the frequency of *BRAF* V600E mutation is high in MSI tumors, this mut-

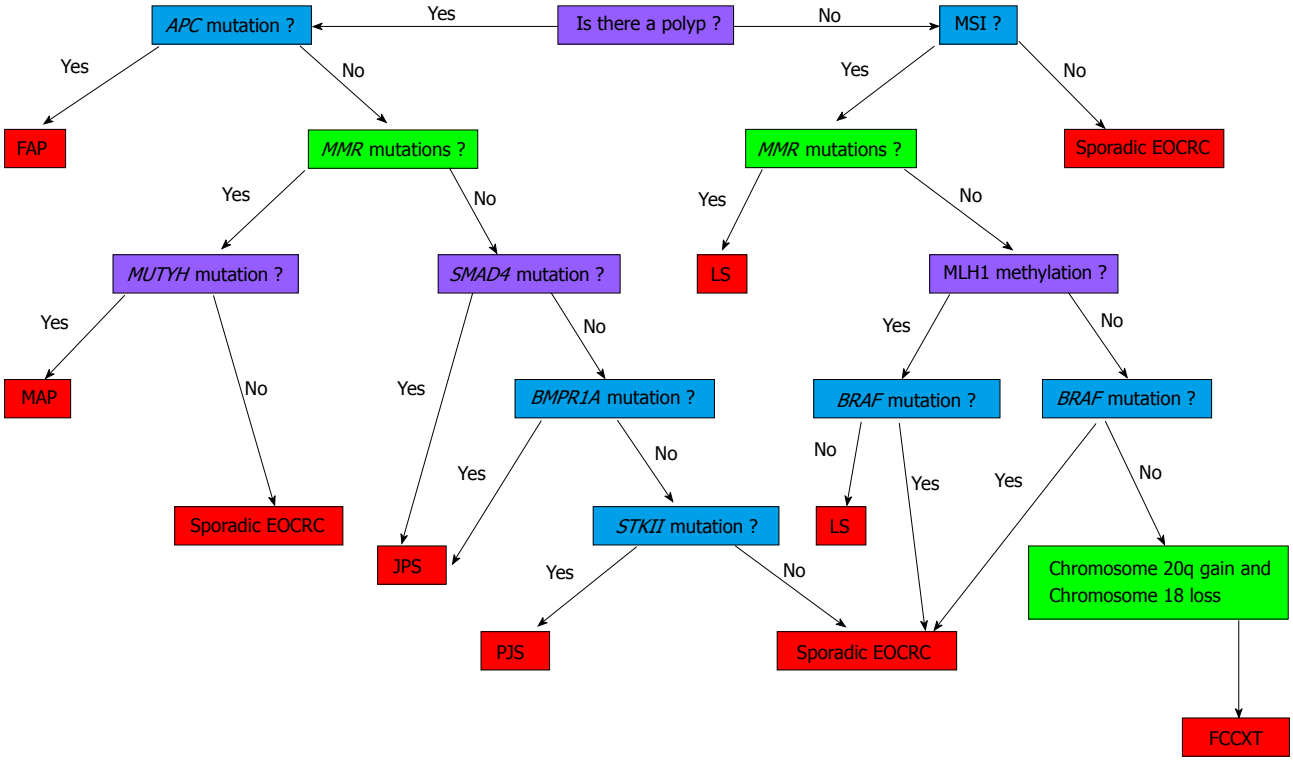

Figure 1 Genetic algorithm of early-onset colorectal cancer. MMR: Mismatch repair; EOCRC: Early-onset colorectal cancer; MSI: Microsatellite instability; APC: Adenomatous polyposis coli; FAP: Familial adenomatous polyposis; MAP: MUTYH-associated polyposis; LS: Lynch syndrome; JPS: Juvenile polyposis syndrome; PJS: Peutz-jeghers syndrome.

ation is not observed in LS cases, thus, this discrepancy between sporadic MSI cancer and LS might be used in a strategy for the detection of LS[156].

The different attitude of sporadic and hereditary forms of EOCRC may also be caused by epigenetic modifications, such as miRNA expressions and their methylation patterns[47,157]. Balaguer *et al*[47] demonstrated that miR-622, miR-362-5p and miR-486-5p could accurately classify the LS and sporadic MSI cases. The similarity of miRNA expression status of LS and sporadic MSI cases may be explained with occurrence of frameshift mutations in *TARBP2*, a miRNA processing gene, in both of these diseases[158]. Moreover, in one of our study, using miRNA polymerase chain reaction arrays, the expression profiles of 38 different miRNAs associated with CRC were evaluated in 40 sporadic Turkish EOCRC patients[157]. The expression of miR-106a was found to be upregulated, and miR-143 and miR-125b levels were found to be downregulated in sporadic EOCRC tissues compared with the normal tissues. In addition, 2.42-fold high expression level of miR-106a and 2.42-fold low expression level of miR-125b were observed in tumors with lymph node metastases compared with the normal colorectal mucosa samples[157]. On the other hand, epigenetic regulations of sporadic EOCRC tumors also differ between each other depend on MSI status. Earle *et al*[48] described the different expression profile of miR-223, miR-155, and miR-92 between MSI and MSS CRCs. So far, Kaur *et al*[49] have investigated the association of miR-132 methylation and sporadic MSI CRC tumors located in the proximal colon in a comparative study of Finnish and Australian population. In addition, different from MSS tumors, hypermethylation of miR-345 had a significant association with sporadic MSI in Finnish CRCs[49].

CLINICAL OUTCOME OF GENETIC AND EPIGENETIC FEATURES OF EOCRC

A major challenge in CRC therapy is drug resistance. The current knowledge of CRC genetics has increased the sufficiency of applied conventional cytotoxic chemotherapy and targeted therapy. Genetic screening of EOCRC patients for hereditary cancer syndrome is determinative not only for the rate of cancer risk of relatives but also for appropriate treatment. A pyrimidine analogue, 5-fluorouracil (5-FU) which is widely used in CRC therapy, involves in induction of DNA replication stress response in cells through inhibiting thymidylate synthase. However, studies showed that *APC* mutations reduces the sensitivity to 5-FU[159]. On the other hand, performance of MSI test is advisable for patients with strongly suspected on the basis of a known family history of colorectal and extracolonic cancers in the case of LS (Figure 1). Studies revealed that while adjuvant chemotherapy with a fluoropyrimidine does not have a beneficial effect on MSI cases and may even worsen the clinical picture, combination of oxaliplatin and infusional 5-FU/leucovorin regarded as more beneficial for these cases[160,161]. According to Violette *et al*[162] increased expression of Reg genes caused *in vitro* resistance to

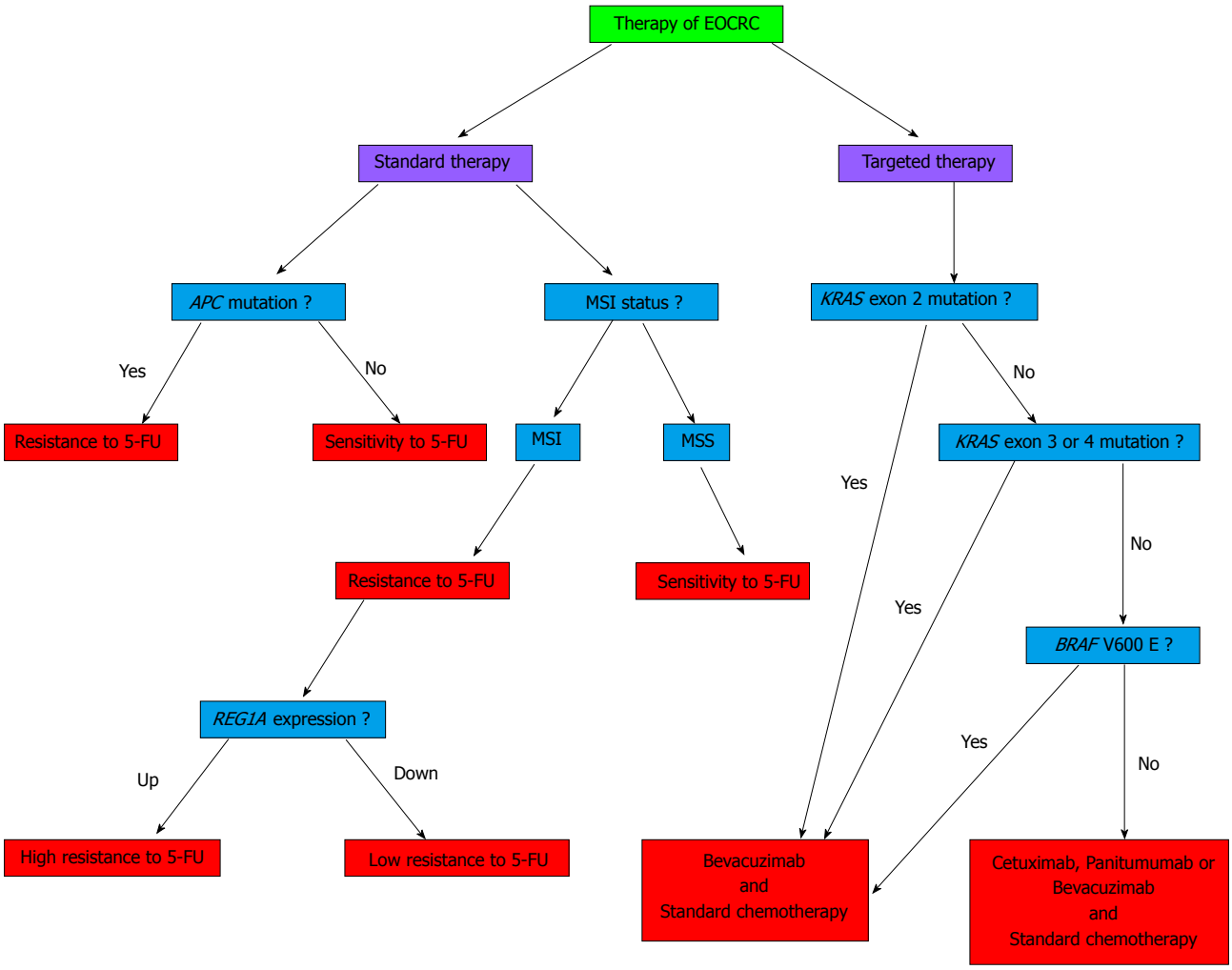

Figure 2 Therapy of early-onset colorectal cancer. EOCRC: Early-onset colorectal cancer; MSI: Microsatellite instability; MSS: Microsatellite stabile; 5-FU: 5-fluorouracil.

the 5-FU. Bishnupuri *et al*[163] observed a mitogenic effect of the Reg Ⅳ protein, with subsequent changes in the expression of genes associated with apoptosis and metastasis. The Reg proteins are previously unappreciated regulators of antiapoptotic proteins in early tumorigenesis and may contribute to increased resistance to apoptotic death during therapy[163]. As another mechanism of resistance to therapy, the result of our study of the poor prognosis of MSI tumors supports the hypothesis that high *REG1A* expression may contribute to increased resistance to apoptotic death during therapy in MSI tumors[147]. Because of the important role of REG1A in tumorigenesis and development of metastasis in MSI tumors, the use of *REG1A*-specific inhibitors in CRC patients have MSI that may represent a novel significant approach to the treatment of cancer. In addition, according to recent studies, alterations in epigenetic regulation of these genes may also lead to resistance to chemotherapeutic agents. For example, Deng *et al*[164] found out that reduced expression of miR-21 plays role in resistance to 5-FU therapy *via* targeting *MSH2*. However, miRNA studies have been performing in *in vitro* conditions and to prove the decisive importance of these markers further advanced studies required.

Nowadays, the application of targeted therapy for CRC has been increasing. The goals of these therapies are interrupting the survival and proliferation of cancer cells[165]. To date, United States Food and Drug Administration has approved several targeted drugs, such as cetuximab and panitumumab, the anti-EGFR antibodies that suppress the tumor angiogenesis and bevacizumab, an anti-VEGF antibody. Recently, different from bevacizumab, aflibercept and regorafenib have been used as new antiangiogenic agents[166-168]. Although EGFR is overexpressed in most of the CRC cases, because of the down-stream modifications of EGFR signalling pathway, patients demonstrated different response to this therapy[169]. Particularly, *KRAS* activating mutations in exon 2 avoid the sufficient therapy with EGFR inhibitors[170,171]. A small number of patients with wild type *KRAS* exon 2 were demonstrated to have mutations exons 3 and 4 that are also caused KRAS activation[172]. Activating mutations in the other genes that play role in downstream pathway of EGFR signalling, NRAS, BRAF, PIK3CA and PTEN are able to lead to resistance to anti-EGFR therapies[173]. Thus, to predict the success of anti-EGFR monoclonal antibody

therapy, examination of downstream mutations of EGFR signalling pathway should be required before receiving an EGFR inhibitor[170] (Figure 2). Second targeted signal pathway for CRC therapy is angiogenesis pathway. Bevacizumab is a monoclonal antibody that binds to VEGF-A preventing its interaction with VEGFR-2[174]. Regorafenib demonstrated a multikinase inhibitor activity against VEGFR-2, VEGFR-3, TIE-2, PDGFR, FGFR, RET, c-Kit and RAF/MEK/ERK pathway[175]. Aflibercept is a recombinant fusion protein and play a role in the inhibition of interactions between VEGF-A, VEGFB proteins and their specific receptors by acting as a trap receptor binding to VEGF-A and VEGFB[176]. Thus, the blockage of the genes that encoded these proteins enhances the success of the therapy.

CONCLUSION

Genetic predispositions have been identified in EOCRC clearly distinct from the other types of CRC. The current knowledge about the molecular and genetic basis of EOCRC provides information regarding prognosis of this disease and response to therapies. A proportion of EOCRCs are hereditary forms. Hence, cases should be evaluated for existing of a germline mutation in one of the several MMR genes for suspicion of LS, in the *APC* gene for suspicion of FAP, or in one of the genes associated with a more uncommon syndrome. Identification of a hereditary syndrome in individuals also provides predictive mutational testing for non-symptomatic relatives. They are found to be positive for the mutation can take precaution for reduction of the risk of cancer-associated morbidity and mortality in this way. In addition, a better understanding of the genetic mechanism of EOCRC is highly likely to lead to develop more beneficial targeted therapies. To date, specifically, studies on MSI CRC, such as LS, herald new diagnostic and therapeutic strategies into clinical approach. It is notable that further research remains to be conducted to more finely characterize the underlying mechanism of sporadic EOCRC, which could allow improved prevention, diagnosis, and treatment of these cases.

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