The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy (Review)

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Abstract. Colorectal cancer (CRC) results from the progressive accumulation of multiple genetic and epigenetic aberrations within cells. The progression from colorectal adenoma to carcinoma is caused by three major pathways: Microsatellite instability, chromosomal instability and CpG island methylator phenotype. A growing body of scientific evidences suggests that CRC is a heterogeneous disease, and genetic characteristics of the tumors determine their prognostic outcome and response to targeted therapies. Early diagnosis and effective targeted therapies based on a current knowledge of the molecular characteristics of CRC are essential to the successful treatment of CRC. Therefore, the present review summarized the current understanding of the molecular characteristics of CRC, and discussed its implications for diagnosis and targeted therapy.

Contents

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1. Introduction

Despite improvements in early detection and treatment method in recent years, colorectal cancer (CRC) remains the third most frequent and the fourth leading cause of cancer-associated mortalities worldwide (1,2). Approximately 65% of CRC cases are sporadic with no family history or apparent genetic predisposition (3). The remaining cases are familial, arising from moderately penetrant inherited susceptibility, possibly interacting with environmental factors (3,4).

CRC, like numerous other solid tumors, is a heterogeneous disease in which different subtypes may be distinguished by their specific clinical and/or molecular features. The majority of sporadic CRCs (~85%) exhibit chromosomal instability (CIN), with changes in chromosome number and structure (5-8). These changes include gains or losses of chromosomal segments, chromosomal rearrangements, and loss of heterozygosity (LOH), which results in gene copy number variations (CNVs) (5-8). These alterations affect the expression of tumor-associated genes, and/or genes that regulate cell proliferation or cell cycle checkpoints, which, in turn, may activate pathways essential for CRC initiation and progression (9,10). The remaining sporadic cases (~15%) have high-frequency microsatellite instability (MSI) phenotypes. However, hereditary CRC has two well-described forms: Familial adenomatous polyposis (FAP) (<1%) patients inherit a mutated copy of the adenomatous polyposis (*APC*) gene, whereas hereditary non-polyposis colorectal cancer (HNPPC, or Lynch syndrome) (1-3%) is characterized by MSI, a consequence of a defective DNA mismatch repair (MMR) system (11). The other forms of hereditary CRC include a rare syndrome called hamartomatous polyposis syndrome (<1%) and the common inherited cases caused by less penetrant inherited mutations (32%) (3).

Sequential acquisition of genetic and epigenetic alterations is well defined, and confirmed to drive the initiation and progression of adenomas to carcinomas in sporadic and inherited forms of CRC (12-14). Generally, CRC formation begins with the transformation of a normal colorectal epithelium to a benign adenoma, and then progresses through the stepwise accumulation of multiple genetic and epigenetic aberrations, subsequently leading to invasive and metastatic tumors (12-14). This process may take years to decades to escape the multiple regulatory layers of the cells and to fully

develop (Fig. 1) (13,15). There are three major pathways associated with CRC pathogenesis, namely: CIN, MSI and CpG island methylator phenotype (CIMP) (16).

The extent to which cancer has spread at the time of diagnosis is described as its stage. Currently, CRC staging is primarily based on the tumor-nodes-metastasis (TNM) system proposed by the American Joint Committee on Cancer (17). The survival rate of patients with CRC largely depends on the stage at which tumor is first diagnosed and varies between stages. For example, the 5-year-survival rate for patients with stage I colon cancer is 93.2%, which drops to 8.1% for patients with stage IV (17). Although TNM is currently the most common CRC staging system, and an important basis to determine the treatment method and assessing prognosis, it is not a reliable tool for prediction and prognosis. Particularly, CRC patients with similar histopathology may have completely different progression and outcome depending on their genetic and epigenetic background (18). Thus, understanding the molecular pathways underlying the initiation and development of CRC is essential to identify novel molecular biomarkers for diagnosis and prognosis, thereby improving the outcome. The present review summarized the current knowledge of the genetic and epigenetic integrity, the consequences of the DNA MMR machinery associated with CRC, and the role of molecular characterization in early diagnosis and in the treatment of CRC.

2. Molecular basis of CRC

CIN pathway. The average rate of genomic mutation in normal human cells is estimated to be ~$2.5x10^{-8}$ mutations/nucleotide/generation (19,20). However, this rate is higher in cancer cells due to the sequential accumulation of multiple mutations during cell divisions forming a so-called 'mutator phenotype' (21). Accordingly, mutations in MMR genes, genes that regulate cell cycle checkpoints, and/or cellular responses may elevate mutation rates to the level commonly observed in human tumors (21). The 'mutator phenotype' may have various manifestations, including point mutations, CIN, MSI, CIMP and LOH (21).

CIN appears to be the most common type of genetic instability in CRC, observed in 85% of adenoma-carcinoma transitions (5-7). CIN refers to a high rate of gains or losses of whole, or large portions of chromosomes. This leads to karyotypic variability from cell to cell that consequently forms an aneuploidy, sub-karyotypic amplification, chromosomal rearrangement, and a high frequency of LOH at tumor suppressor gene loci (5,6). In addition, CIN tumors are recognized by the accumulation of mutations in specific oncogenes, including KRAS proto-oncogene GTPase (*KRAS*) and B-Raf proto-oncogene serine/threonine kinase (*BRAF*), and tumor suppressor genes, such as *APC* and tumor protein p53 (*TP53*), thereby contributing to CRC tumorigenesis (6,10). The multistep genetic model of colorectal carcinogenesis proposed by Fearon and Vogelstein is now widely accepted, and used as a paradigm for solid tumor progression (12). According to this model, inactivation of *APC* occurs as the first event, followed by oncogenic *KRAS* mutations in the adenomatous stage, and eventually, deletion of chromosome 18q and inactivation of the tumor-suppressor gene *TP53* on chromosome 17p occur during the transition to malignancy (Fig. 1) (12,22-25).

Array-based comparative genomic hybridization and single nucleotide polymorphism techniques have enabled scientists to effectively determine CNVs in the entire human genome with higher resolution. Although the allelic loss of all chromosomal arms has been detected in certain tumors, its frequency varies considerably, and only a few of them are highly recurrent in CRC, including losses at chromosomal arms 1p, 5q, 8p, 17p, 18p, 18q, 20p and 22q (26-31). A high-frequency allelic loss at a specific chromosomal region denotes the presence of a candidate tumor-suppressor gene, including *APC* on chromosome 5q, *TP53* on chromosome 17p, DCC netrin 1 receptor (*DCC*), SMAD family member (*SMAD2* and *SMAD4*) on chromosome 18q (31). In contrast, a gain of chromosomal material suggests the presence of the potential oncogenes or genes that favor cell growth or survival. In CRC, gains at chromosome 7, and chromosomal arms 1q, 8q, 12q, 13q and 20q have been repeatedly reported by different research groups (26-31). It was reasoned that these chromosomal changes are associated with a gain and loss of function of tumor-associated genes offering mutated cells growth and survival advantages, leading to progressive conversion of normal cells into cancer cells (32,33). However, the gains/losses of chromosomal materials generally span a large region and comprise a large number of genes making identification of target genes challenging.

In the field of stem cell research, genetic analysis of human embryonic stem cell (hESC) lines, a pluripotent cell type that shares numerous characteristics with cancer cells, has also revealed multiple CNVs, and few of them are also recurrent, including losses of chromosomal band 18q21qter, and whole or partial gains of chromosomes 1, 12, 17 and 20 (34,35). Notably, 20q11.21 amplification was identified in >20% of the screened hESC lines (36). Previously, BCL2 like 1 (*BCL2L1*), which is located in the smallest common chromosomal region of gain and regulates the mitochondrial apoptotic pathway, has been confirmed as the key-driver gene of this amplification (37,38). Accordingly, the overexpression of Bcl-xL, an anti-apoptotic isoform of *BCL2L1* has offered cells a survival advantage by preventing apoptosis (37,38). Overexpression of this gene may also be responsible for the gain of 20q in various human cancer types (39).

Losses of 18q. Allelic loss at chromosome 18q is detected in ~70% of primary CRC in the late carcinogenic process (29,31,40,41), and is considered as a poor prognosis marker for survival in patients with CRC (42,43). The high frequency of allelic deletions involving chromosome 18q suggests the presence of candidate tumor-suppressor genes whose inactivation may serve a significant role in CRC, including *DCC*, *SMAD2* and *SMAD4* (12,25,44). *DCC*, located in the chromosome band 18q21.2, encoding a component of the neutrin-1 receptor, was proposed as a putative tumor-suppressor gene (45). However, much of the reported data on the loss and inactivation of *DCC* is circumstantial and fails to provide conclusive evidence that *DCC* functions as a tumor-suppressor gene (46). Furthermore, to the best of our knowledge, there is no evidence that germline mutations of *DCC* serve a role in heritable cancer; and few somatic mutations in *DCC* have been reported in CRC (46). The presence

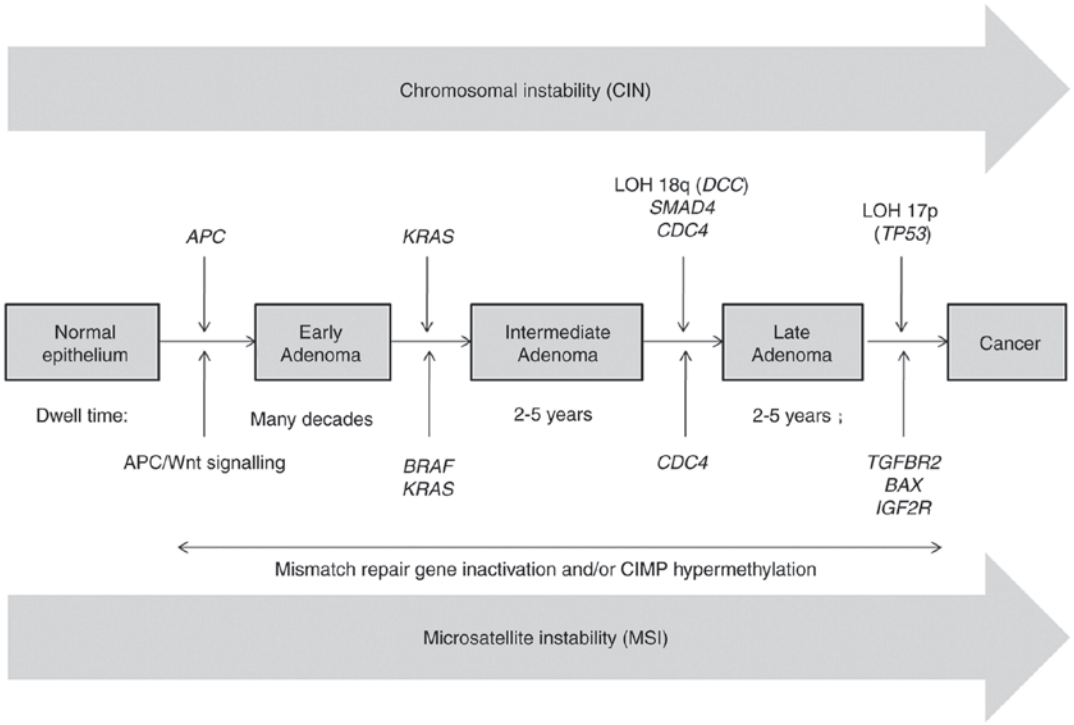

Figure 1. Colorectal adenoma-carcinoma sequence. The *APC* mutation is the first step transforming normal colorectal epithelium to adenoma. The adenoma-carcinoma sequence is caused by three major pathways: CIN, MSI and CIMP. CIN, Chromosomal instability; MSI, microsatellite instability; CIMP, CpG island methylator phenotype; *APC*, adenomatous polyposis; *KRAS*, KRAS proto-oncogene GTPase; *BRAF*, B-Raf proto-oncogene serine/threonine kinase; *TP53*, tumor protein 53; LOH, loss of heterozygosity; HNPPC, hereditary non-polyposis colorectal cancer; *MLH1*, mutL homolog 1; *MSH2*, mutS homolog 2; *DCC*, DCC netrin 1 receptor; *TGFBR*, transforming growth factor-β receptor; *BAX*, BCL2 associated X apoptosis regulator; *IGF2R*, insulin like growth factor 2 receptor; *CDC4*, cell division control protein 4.

of two other well-established tumor suppressor genes, *SMAD2* and *SMAD4* in the region of loss also challenges the function of *DCC* as a tumor-suppressor gene (47,48). In fact, *SMAD2* and *SMAD4* genes are localized in 18q21.1, the common region of loss of 18q in CRC (25). These *SMAD* genes encode downstream signal transducers for transforming growth factor-β (*TGF-β*), and their alterations may confer resistance to *TGF-β* and contribute to tumorigenesis (49). *SMAD4* was identified to be inactivated in ~60% of pancreatic cancer (50). However, the frequency of *SMAD4* and *SMAD2* somatic mutations is relatively low in CRC (51-53). Nevertheless, smaller regions of loss, which exclude *SMAD2* and *SMAD4*, have been reported in head and neck squamous cancer (54). In addition, their gene expression is retained in CRC with LOH of 18q (46). Taken together, these observations suggest that *SMAD2* and *SMAD4* are unlikely to constitute the major chromosome 18q target for inactivation in CRC, and that other tumor suppressor genes besides the *DCC* and *SMAD* genes may be the target for chromosome 18q loss.

APC/β-catenin. Activation of the Wnt signaling pathway via mutation of the *APC*, a multi-functional tumor-suppressor gene on 5q22.2, is essential and the earliest event in the development of CRC (55). APC protein is a key component of the β-catenin destruction complex involved in the degradation and suppression of the Wnt/β-catenin signaling pathway (56). Mutant *APC* disrupts the formation of the destruction complex leading to stabilization and accumulation of β-catenin protein in the cytoplasm. Accumulated β-catenin protein is translocated to the cell nucleus where it forms complexes with *TCF/LEF*, and induces overactivation of Wnt downstream effectors that, in turn, promote the proliferation, migration, invasion and metastasis of cancerous cells (57). The same outcome is also observed with mutations in β-catenin (58) and *AXIN2* (57), but to a lesser extent. Notably, mutations in *AXIN2* have been reported in CRC with MSI only (59).

APC mutations or allelic losses have been identified in ~90% of patients with CRC (60). Germline mutations in *APC* are responsible for FAP (15), while somatic mutations and/or allelic deletions of *APC* are described in sporadic CRC (61). The *APC* gene may also be epigenetically inactivated through promoter hypermethylation that has been identified in 18% of primary colorectal carcinoma and adenoma cases (62).

TP53. *TP53* is a tumor-suppressor gene located on the short arm of chromosome 17, which is commonly lost in colorectal carcinoma (40). *TP53* has been defined as the 'guardian of the genome' because it encodes a transcription factor that regulates the transcription of hundreds of genes involved in different processes, including DNA repair, cell cycle arrest, senescence, apoptosis and metabolism in response to a variety of the stress signals (63). Upon DNA damage, for example, *TP53* induces cell cycle arrest at the G_1 or G_2 phase, or triggers apoptosis when the damage is too severe and irreparable (64). Loss of *TP53* function, therefore, contributes to the propagation of damaged DNA to daughter cells.

TP53 alteration is the hallmark of human tumors, and the status of *TP53* mutation is associated with the progression and

outcome of sporadic CRC (65). Particularly, *TP53* loss of function has been reported in 50-75% of CRC cases, much higher compared with that in adenoma, indicating its role in the transition from an adenoma to carcinoma (66,67). To date, the majority of the *TP53* mutations reported in CRC are missense mutations that substitute AT for GC (68). Liu and Bodmer (69) have analyzed *TP53* mutations and their expression in 56 CRC cell lines, and reported a relatively high frequency of *TP53* mutations (76.8%), in which missense mutations accounted for 47.83% and point mutations that are transitions at CpG sites accounted for 37.5%. These mutations render an inactive protein with an abnormally long half-life that is detectable by immunohistochemistry (70).

KRAS. The *KRAS* gene belongs to the *RAS* gene family involved in signaling pathways that regulate cellular proliferation, differentiation or survival. *KRAS* is a membrane-bound GTP/GDP-binding protein with intrinsic GTPase activity and is expressed in the majority of human cells. The switch between its active GTP-bound state and the inactive GDP-bound state is regulated by GTPase-activating proteins and guanine nucleotide exchange factors (71). The *KRAS* mutations impair the intrinsic GTPase activity of *KRAS*, causing the accumulation of the *KRAS* proteins at the GTP-bound active state, eventually resulting in the constitutive activation of the downstream proliferative signaling pathways (72).

Oncogenic mutations in the *RAS* gene have been identified in ~30% of all human tumors (73), in which mutations in *KRAS* accounted for ~85%, NRAS proto-oncogene GTPase (*NRAS)* for ~15%, and HRas proto-oncogene GTPase *(HRAS)* for <1% (74-76). The high frequency of *KRAS* mutations and its appearance at a relatively early stage in tumor progression suggest a causative role of *KRAS* in human tumorigenesis. Several studies have reported an association between *KRAS* mutations, and poor prognosis of CRC (77,78), and lung (79,80) and liver (81) metastasis. In contrast, several other studies reported that *KRAS* mutations were strong independent predictors of survival in patients with CRC (80-82). These contradictory findings may be explained by the differences in the distribution of specific *KRAS* mutations, stage at diagnosis or other characteristics. *KRAS* mutations have emerged as an important predictive marker of resistance to anti-epidermal growth factor receptors (*EGFR*) agents, including panitumumab and cetuximab (83-86).

Activating *KRAS* mutations have been identified in 35-45% of CRC cases (40,80,87-89), and primarily occur in codon 12 and 13 (75,89). The most frequent changes observed in these codons are the substitution of glycine for aspartate (p.G12D, p.G13D) (90). The mutation rates of *NRAS*, in contrast, are lower (1-3%) and activating mutations of *HRAS* has not been detected in CRC (40,91,92). Previously, pyrosequencing of *KRAS*, *BRAF* and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α revealed that 53.8% of patients exhibit a *KRAS* mutation in codons 12 or 13, of which 57.9% were c.38G>A (pG13D), and 22.2% were c35G>T (p.G12V) mutations (93).

MSI. Another type of genomic instability is MSI, a typical characteristic of cancerous cells, occurring in 15-20% of sporadic CRC and in >95% of HNPPC. Microsatellites are repetitive DNA sequences consisting of tandem repeats, usually between one to five base pairs. Patients with MSI phenotype exhibit a high frequency of replication errors, particularly in repetitive DNA sequences, primarily due to the slippage of the DNA polymerase (94). The progressive insertion/deletions of nucleotides within the microsatellite sequences result in the appearance of longer or shorter alleles compared with those detected in the normal cells of the same individual (95,96).

To access the MSI status of a cancer, a standard panel of five microsatellite markers, including two mononucleotide (BAT26 and BAT25) and three dinucleotide (D2S123, D5S346, and D17S250) repeats, has been recommended according to the Bethesda Guidelines (97). Tumors are then classified based on the number of microsatellites exhibiting instability. Particularly, tumors are classified as MSI high (MSI-H) when ≥30% of the markers exhibit instability; those with <30% markers exhibiting instability are defined as MSI low, and those with no apparent instability are microsatellite stable (MSS) (97,98).

It is now accepted that MSI is associated with post-replicative DNA MMR deficiency, primarily involving mutL homolog 1 (*MLH1*) and mutS homolog 2 (*MSH2*) (94,99-101). Impairment of MMR genes can occur by either mutational inactivation or by epigenetic inactivation through CpG island methylation of the promoter of the genes. Loss or insufficiency of MMR activity leads to replication errors with an increased mutation rate and a higher potential for malignancy. In MSI-H gastric cancer, for example, hypermethylation of *MLH1* promoter is responsible for the development of >50% of cases, whereas mutations in *MLH1* and *MSH2* account for ~15% of cases (102,103).

Small insertions/deletions may create frame-shift mutations within repetitive tracts present in the coding region of essential tumor-suppressor or tumor-associated genes, resulting in an inactive protein and contributing to tumorigenesis in cancers with MSI-H (104). Using a large-scale genomic screen of coding region microsatellites, Mori *et al* (105) identified nine loci that were mutated in >20% of tumors, namely: Transforming growth factor-β receptor (*TGFBR2*) (79.1%), BCL2 associated X apoptosis regulator (*BAX*) (37.5%), human mutS homolog 3 (26.2%), activin A receptor, type II (58.1%), SEC63 homolog protein translocation regulator (48.8%), absent in melanoma 2 (47.6%), NADH-ubiquinone oxidoreductase (27.9%), cordon-bleu WH2 repeat protein like 1 (23.8%) and proliferation-associated 2G4/ErbB3-binding protein 1 (20.9%). *TGFBR2*, encoding a kinase receptor involved in transduction of the *TGFB1/2/3* signal from the cell surface to the cytoplasm to inhibit cellular proliferation, is the most commonly affected gene. Particularly, instability in the poly-adenine tract of this gene has been detected in ~85% of MSI-H colorectal tumors, rendering an inactive receptor and thus eliminating the growth-suppressive effects of *TGFB1* (106). Another commonly mutated gene in CRC is *BAX*, a pro-apoptotic gene belonging to the *BCL2* family. Frame-shift mutations within the poly-guanine sequence have been detected in 50% of MSI-H colorectal tumors, causing silencing of this gene and suppressing apoptosis (107). These alterations in the gene functions represent a possible mechanism for MSI carcinogenesis.

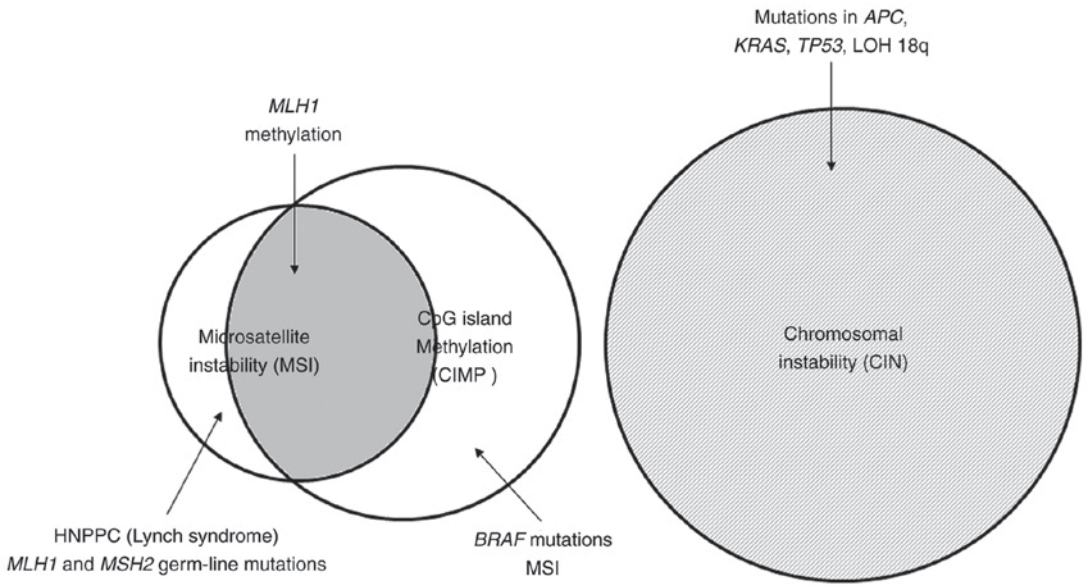

Figure 2. Overview of the genetic and epigenetic instability pathways that drive colorectal cancer onset and development. CIN, Chromosomal instability; MSI, microsatellite instability; CIMP, CpG island methylator phenotype; *APC*, adenomatous polyposis; *KRAS*, KRAS proto-oncogene GTPase; *BRAF*, B-Raf proto-oncogene serine/threonine kinase; *TP53*, tumor protein 53; LOH, loss of heterozygosity; HNPPC, hereditary non-polyposis colorectal cancer; *MLH1*, mutL homolog 1; *MSH2*, mutS homolog 2.

CIMP or aberrant DNA methylation. Transcription inactivation by DNA hypermethylation at promoter CpG islands of tumor-suppressor genes, causing gene silencing, is now recognized as an important mechanism in human carcinogenesis (108-111). The CpG island methylator phenotype has been identified in 30-35% colorectal adenoma cases, and is considered as an early event and a characteristic for the serrated pathway of colorectal tumorigenesis (108,112,113). However, the quantitative DNA methylation study performed by Ogino *et al* (114) reported that CIMP accounts for 17% of CRC, which is less frequent compared with previously reported and that clinical features of CIMP are similar to those of MSI-associated CRC (114). Notably, sporadic MSI colorectal tumors are almost exclusively associated with CIMP-associated methylation of *MLH1* leading to inactivation of this gene (107,115). In contrast, the familial MSI cases (Lynch syndrome) are generally caused by germline mutations in the MMR genes, primarily including *MLH1* and *MSH2*, and accounts for <5% of all CRC cases (Fig. 2) (107,116).

The CIMP status of CRC is currently assessed by a panel of methylation markers categorizing CRC as exhibiting or not exhibiting DNA methylation on the basis of certain thresholds (114,115,117,118). $CIMP^+$ colorectal tumors appear to have a distinct profile, including associations with the proximal colon, poor differentiation, MSI status, *BRAF* mutation and wild-type *KRAS* (113-115,119-121). Particularly, the frequency of *BRAF* mutations in $CIMP^+$ tumors is significantly higher compared with their $CIMP^-$ counterparts (114,115). Shen *et al* (122) analyzed the genetic and epigenetic alterations in 97 primary CRC samples, and demonstrated that CIMP-high tumors are associated with MSI status (80%) and *BRAF* mutation (53%); CIMP-low tumors are associated with *KRAS* mutations (92%); and $CIMP^-$ tumors typically have a high rate of p53 mutations (71%) (122). Furthermore, CIMP status has also been indicated to be negatively associated with 18q LOH status in colorectal tumors (117). Particularly, CIMP-0 was associated with 18q LOH-positive tumors and vice versa (117).

3. Clinical implication of the molecular genetics of CRC

The prognosis and therapeutic options for patients with CRC are associated with the stage at which they are first diagnosed. While early stage CRC is often cured with surgery alone, more advanced or metastatic CRC generally require additional adjuvant chemotherapy or targeted therapy, either alone or as a combined treatment. Early detection of CRC thus becomes important to reduce the incidence and mortality of the disease. Furthermore, due to their heterogeneity, the benefits from adjuvant chemotherapy for stage II and III CRC patients may vary largely. Thus, identifying molecular prognostic markers that are capable of recognizing patients with CRC more likely to recur or benefit from adjuvant chemotherapy may improve the prognosis and assist in the selection of appropriate therapy, and subsequently the general outcomes.

It is now widely known that certain alterations at the molecular level favor CRC onset, progression and metastasis (60). Several known mutations are considered to be associated with a poorer patient outcome and/or failure of response to a certain therapy (18). Patients with inactive *TP53* mutations, for example, are at an increased risk of mortality compared with their counterparts, but this mutation does not appear to affect the outcome of chemotherapy (123). However, the presence of somatic *KRAS* mutations has been considered as a predictor of resistance to anti-EGFR therapy (83-86,124). Thus, *KRAS* mutation status is currently used in clinical settings to predict the therapeutic effectiveness of CRC prior to chemotherapy to avoid any undesired effects and medical costs (125). *APC* is another commonly affected gene whose mutations generally appear in the early stage of CRC development (55,60). Notably, the risk of CRC for a patient with FAP, which begins with a

germline mutation in one allele of the *APC* gene is ~100% by the age of 40 years (6,7). Therefore, *APC* mutations are being considered as good diagnostic markers for identifying individuals at risk of CRC.

The majority (~75%) of CRC with MSI are sporadic cases caused by the loss of DNA MMR activity due to methylation of the promoter of the *MLH1* gene, while the other 25% of cases are classified as Lynch syndrome caused by germline mutations in the MMR genes (Fig. 2). Generally, MSI is detected earlier in life in patients with Lynch syndrome (<50 years old) as compared with the sporadic cases (>65 years old) (126). Particularly, CRC with MSI are more likely to occur in the proximal colon (126). Evidence has suggested that MSI is a favorable prognostic biomarker for CRC (127-129). However, its predictive role for the response to chemotherapeutic agents, including 5-fluorouracil (5-FU) is conflicting. Several studies demonstrated a lack of benefit of 5-FU-based adjuvant chemotherapy in patients with CRC with MSI tumors (130-133), while others reported the beneficial effects (127,134). Des Guetz *et al* (135) performed a meta-analysis involving 3,690 patients from seven different studies, and reported that chemotherapy had a beneficial effect among MSS, but not MSI-H patients (135). In addition, the more improved survival rate of MSI-H patients was due to a better prognosis rather than the benefit of chemotherapy (135). These findings suggested that MSI may be considered as a predictive marker of chemoresistance and that patients with CRC with MSI may be spared from adjuvant treatment. The MSI status among patients with CRC, thus, is highly valuable in prognosis and therapy of CRC, and should be thoroughly evaluated by performing polymerase chain reaction analysis using the Bethesda panel and/or immunohistochemistry staining for DNA MMR proteins, including MLH1 and MSH2, in order to contribute to treatment decision-making regarding chemotherapy administration.

Several groups have used gene expression profiling to classify CRC, and to identify genes associated with prognosis and prediction of disease outcome. De Sousa *et al* (136) used an unsupervised classification strategy involving >1,100 individuals with colon cancer and defined three main colon cancer subtypes. Two subtypes are associated with two well-characterized subsets of colon cancer, namely the CIN and the MSI group. The third subtype was largely MSS and overlaps partly with the CIMP group, and is associated with poor prognosis and resistance to anti-EGFR therapy (136). Using a similar approach, Sadanandam *et al* (137) defined six clinically relevant CRC subtypes by associating their gene expression profiles with corresponding clinical response to cetuximab. Patients with stem-like subtype and inflammatory subtype tumors, with poor and intermediate disease-free survival, exhibited an improved response to the combination chemotherapy regimen FOLFIRI (5-FU with irinotecan) in adjuvant or metastatic settings, whereas transit-amplifying- and goblet-like-subtype tumors, with markedly better prognosis, did not appear to benefit from these treatments. However, cetuximab-sensitive transit-amplifying and cetuximab-resistant transit-amplifying subtypes may be efficiently treated with cetuximab or a cMET inhibitor, respectively, in the metastatic setting (137). Although there are significant associations between MSI status and specific subtypes, the transcriptional signatures-based subtypes allow better refinement and provide insights for the development of subtype-specific therapies, which, in turn, may contribute to the more effective management of this disease.

4. Conclusion and future perspectives

Despite the great advancement in CRC research, the role of the molecular characterization in diagnostic tests and therapeutic decisions remains limited due to the fact that the function of the majority of mutations remains unclear and rarely provides any valuable diagnostic information. Further research is required to develop more easily applicable molecular tests for early detection of CRC, which is essential to improving the prognosis and treatment efficiency. Furthermore, it is essential to identify novel therapeutic targets as the majority of CRC cases are insensitive to EGFR inhibitor therapy.

Recent studies have provided a better understanding of CRC and assist in the development of novel treatment regimens. Particularly, the implementation of targeted next-generation sequencing (NGS) in clinical settings allows a reliable identification of the most common mutations, and is able to guide therapeutic decisions for patients with CRC based on personalized medicine (138). NGS is currently the most important technology for early diagnosis and prognosis, as well as identification of novel predictive biomarkers for available treatments with targeted therapy and immunotherapy for patients with CRC (138). Specifically, the combination of CRISPR/Cas9 technology and immunotherapy would significantly improve patient care by reducing side effects (139,140).

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Authors' contributions

HTN conceptualized the article, critically discussed the findings and co-wrote the article. HQD co-wrote the article. Both authors revised the article and approved the final version.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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