Molecular Diagnostics in Colorectal Cancer

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ABSTRACT

Colorectal cancer (CRC) presents in one of three patterns: sporadic colorectal cancer in those without a family history (65-85%); those with a family history (familial CRC) 10-25% of cases; inherited CRC accounting for less of 10% cases and presents as well-characterized cancer predisposition syndromes including Lynch syndrome (hereditary non-polyposis colorectal cancer/HNPCC) which comprises about 1-5% of all colorectal cancer, and multiple polyps CRC, which includes familial adenomatous polyposis (FAP,1%), rare CRC syndrome < 0.1 %).

Many efforts have been made to discover the genetic and molecular features of CRC, and there is more evidence that these features determine the prognosis and response to treatment. Colorectal cancer (CRC) is a heterogeneous disease, with three known major molecular groups. The most common is the chromosomal instability group, characterized by an accumulation of mutations in specific oncogens and tumor suppressor genes. The second is the microsatellite instability group, caused by the dysfunction of DNA mismatch repair genes leading to genetic hypermutability. The CpG island methylation phenotype (CIMP) is the third group, distinguished by hypermethylation. In this review we would like to provide an up-to-date overview of molecular genetic aspects of CRC that are currently important and should guide clinical practice in colorectal cancer in the diagnosis and selection of therapy.

Keywords: *sporadic colorectal cancer, molecular diagnostics, chromosomal instability (CIN), microsatellite instability (MSI), CpG island methylation phenotype (CIMP)*

ABSTRAK

Kanker kolorektal (KKR) muncul dalam bentuk satu dari tiga pola yang ada: kanker kolorektal sporadik pada mereka yang tidak memiliki riwayat keluarga (65-85%); mereka yang memiliki riwayat keluarga yang menderita kanker kolorektal 10-25% kasus; dan KKR inherited (inherited CRC) yang jumlahnya kurang dari 10% kasus, dikelompokkan sebagai sindrom predisposisi kanker, yaitu Lynch syndrome (herediter non-poliposis kanker kolorektal) yang terjadi sekitar 1-5% dari semua kanker kolorektal, dan kanker kolorektal polip multipel, yang mencakup familial adenomatosa poliposis (FAP, 1%), sindrom kanker kolorektal langka < 0,1%.

Banyak upaya telah dilakukan untuk menemukan bentuk genetik dan molekuler kanker kolorektal, dan ada lebih banyak bukti bahwa bentuk ini menentukan prognosis dan respon terhadap pengobatan. Kanker kolorektal adalah penyakit heterogen dengan tiga kelompok utama molekuler yang diketahui. Yang paling sering ditemukan adalah kelompok instabilitas kromosom, ditandai dengan akumulasi mutasi pada onkogen yang spesifik dan gen supresor tumor. Yang kedua adalah kelompok instabilitas mikrosatelit, yang disebabkan oleh disfungsi gen

perbaikan kesalahan DNA yang mengarah pada hipermutabilitas genetik. CpG island methylation phenotype (CIMP) adalah kelompok ketiga, ditandai dengan hipermetilasi. Dalam ulasan ini, akan diberikan gambaran terkini tentang aspek genetik molekuler dari CRC yang dianggap penting dan diharapkan dapat dijadikan pedoman pada praktik klinis kanker kolorektal dalam diagnosis dan pemilihan terapi.

Kata kunci: *kanker kolorektal sporadik, diagnostik molekuler, instabilitas kromosom, instabilitas mikrosatelit, CpG island methylation phenotype (CIMP)*

INTRODUCTION

Colorectal cancer (CRC) is the 3rd most frequent cancer in Western Countries, and the 2nd leading cause of cancer death in the United States. CRC is therefore considered a major health problem.[1] CRC is caused by the loss of genomic stability that drives the development of CRC by facilitating the acquirement of tumor-associated mutations. Several forms of genomic instability have been identified, including chromosomal instability (CIN), microsatellite instability (MSI), and epigenetic gene silencing.[2]

The molecular biological markers status of CRC patients are now considered the important factors to determine either the possibility of successful treatment for CRC (predictive factor) or life expectancy (prognostic factor). Clinical applications of genomic medicine and molecular diagnostics based on testing of tumor tissues are becoming a reality in clinical practice, with significant impact on personalized therapies for cancer patients. Advances in targeted therapies for CRC have recently emerged and are rapidly moving targets.[3] Our findings suggest that cancer locations (proximal, distal colon and rectum) differ in their associated molecular alterations in carcinogenesis.[4,5]

EPIDEMIOLOGY OF COLORECTAL CANCER

Colorectal cancer (CRC) is the third most commonly diagnosed cancer, with over 1.2 million new cases each year, and the second leading cause of cancer-related mortality, with approximately 600,000 deaths each year.[1] In Europe, some 447,000 new cases of CRC, and 215,000 deaths occurred in 2012. Incidence rates vary by ~20-fold. Highest incidences are in North America, Western Europe, Australia, New Zealand, and Japan, lowest in India and Northern Africa.

The population in Indonesia is more than 235,000,000 and the age standarized incidence rates per 100,000 for CRC by gender was 19.1 for male and 15.6 for female, and CRC displayed a tendency to occur in patients under the age of 40 with the proportion of 35.26%. The study of 760 patients who have been examined by colonoscopy at Pirngadi Hospital Medan revealed 197 (25.9%) of the patient had colorectal cancer, 16.8% of them were under 40 years old and most of CRC were located in the rectum (74.6%) than in colon (18.8% distal and 6.6% proximal colon).[6,7,8,9] CRC incidence rates are rapidly increasing due to the effect of many risk factors. The underlying causes of CRC are complex and heterogenous. Both environmental factors and genetic events contribute to CRC risk.

PATHOGENESIS OF COLORECTAL CANCER

Inherited and familial CRC derive, at least in part, from germline mutations. Familial CRC presents without precisely defined Mendelian inheritance patterns or genetic etiology. Sporadic CRC derives from somatic mutations, and is not associated with family history.[10] CRC is a heterogeneous complex of diseases. Molecular feature is important because it reflects the mechanisms of carcinogenesis. In molecular aspects, CRC develops via accumulation of genetic mutations. Progression to CRC is considered a step wise process, with an accumulation of various genetic and epigenetic alterations, leading to transformation from a normal cell to a premalignant tumor and finally to a malignant and potentially metastatic tumor.[11,12] Growing evidence suggests that epigenetic changes might even be higher than the genetic changes and are major determinant in the origin of the tumor and tumor heterogeneity.[10]

Risk Factors for CRC are divided into: (a) non-modifiable risk factors**:** age more than 40-50; personal or family history of CRC or adenomatous polyps; inflammatory bowel disease (IBD): ulcerative colitis and Crohn's Disease, increase risk 8-10 years after initial diagnosis; familial syndrome (FAP, HNPCC, MUTYH associated polyposis, Peutz-Jeghers, Juvenile polyposis); (b) modifiable risk factors: diet high in unsaturated fat and red meat or processed meat, high-fat/low fiber diet, physical inactivity, obesity, alcohol, environment, use of cigarettes and other tobacco products.[1]

In order to find new diagnostic and therapeutic solutions that could help reduce CRC related deaths, it is important to understand the etiological biological

nature of CRC. Understanding the molecular genesis of CRC is a fundamental step in the identification of molecular targets that might be useful in defining the prognosis of CRC patients and their therapy.[11,12, 13,14,15]

CLINICAL MANIFESTATION

Early colon cancer usually has no symptoms. Signs and symptoms typically occur only in advanced colon cancer. Approximately, 25% of CRC present with metastases at initial diagnosis. Patient complain of changes in bowel habits lasting more than a few days: diarrhea, constipation, or a feeling that the bowel does not empty completely, feeling or being bloated. Bleeding from the rectum, blood in the stool, cramping or gnawing stomach, cramps, weakness and fatigue, unexplained weight loss, constant tiredness, or unexplained anemia (iron deficiency). Clinical presentation related CRC locations, in proximal colon cancers: iron deficiency anemia, melena, hematochezia, obstruction (rare), fatigue, weakness. Distal colon cancer: hematochezia, abdominal pain, obstruction, overflow diarrhea, changes in bowel habit, left quadrant discomfort and weight loss, may develop. Bleeding from the rectum in rectal cancer.[6,7]

Colorectal tumors spread to other parts of the body by direct extension into adjacent structures and metastasis through the lymphatics and blood vessels. The favoured metastatic sites of colorectal cancer are lymph nodes, liver, lung and bones. Metastatic diseases (50% of CRC patients will develop metastases): right upper quadrant (RUQ) pain, ascites, weight loss, fatigue. Other symptoms: symptoms from local invasion (bladder, small bowel); may mimic diverticulitis; fever of unknown origin.[14]

GENERAL DIAGNOSTIC EVALUATION

General diagnostic evaluation are colonoscopy, chest x-ray, abdominal ultrasound or computerized tomography (CT) of chest abdomen and pelvis. Laboratory test for hepatic function panel, carcinoembryonic antigen (CEA). Kirsten rat sarcoma (KRAS) mutation status in patients with metastatic disease. MSI testing or IHC examination for MMR protein expressions for patients under 50 years old. Diagnostic evaluation for localized staging of rectal cancer: Transrectal ultrasound (TRUS): 80-95% accuracy of distinction between T1/2 vs. T3 tumors. Magnetic resonance imaging (MRI) is high degree of accuracy for prediction of circumferential resection margin, less operator dependent, allows for study of stenotic tumors and pelvic adenopathy. TRUS and MRI may provide complementary information. CT scan helpful for staging distant metastases, limited for local tumor and nodal staging.[14]

Table 1. Staging, prognosis and treatment of colorectal cancer[14]

Stage	T	N	M	5-y Survival (%)	Tx
I	T1-2	N0		93	Surgery alone
IIA	T3	N0		85	Surgery± adjuvant chemo*
IIB	T4	N0		72	Surgery± adjuvant chemo*
IIIA	T1-2	N1		83	Surgery± adjuvant
IIIB	T3-4	N1		64	chemo (5-FU/Cap +
IIIC	T1-4	N2		44	OX (MOSAIC NEJM 2004;350:2343
IV			M1	5	Chemoterapy ± surgery[b]

* 4% absolute benefit from adjuvant 5-FU (QUASAR Lancet 2007;370:2020) & may benefit pts w/presence of poor-risk features (lymphovascular invasion or LVI, perineural invasion or PNI, poorly differentiated histology, +LN, perforation, clinical obstruction, inadequate LN sampling [<12])

[b] Pts w/limited hepatic or pulm mets may benefit from metastasectomy either preceded or followed by chemotherapy

Table 2. Staging and treatment of rectal cancer[14]

Stage	T	N	M	Tx
I	cT1	cN0		Surgery alone (local excision)
I	cT2	cN0		Surgery alone (total mesorectal excision or TME)
II A	cT3	cN0		Neoadj chemoradiation w/5-FU or Cap followed by resection, followed by adjuvant chemotherapy (NEJM 2004;351:1731)
II B	cT4	cN0		
III A	CT1-2	cN1		
III B	CT3-4	cN1		
III C	cT1-4	cN2		
IV			M1	Chemotherapy ± surgery[b]

* T1-2 N0 should be based on assessment by TRUS or MRI. If pathologic staging indicates > T2 disease, positive LN, or high risk features, adjuvant chemotherapy & chemoradiotherapy are recommended in either order. High risk features include (+) margins, LVI, PNI, poorly differentiated tumors, or deep submucosal invasion

[b] Pts w/limited hepatic or pulm mets may benefit from metastasectomy either preceded or followed by chemotherapy

Molecular Testing of Colorectal Cancers for Targetted and conventional Therapy

Molecular testing of CRC from tumor tissues has important implications for the treatment selection in CRC patients. Fresh tissue obtained from colorectal tumors, either by colonoscopic biopsy or surgery. Tumor tissues immediately fixated by using 10% formaldehyde buffering solution and made into paraffin blocks or formalin- fixed and paraffin-embedded tissues (FFPET). It is used for the examination of : histology of the tumor, immunohistochemical test for the evaluation of the protein expression of mismatch repair (MMR) or MSI status and CIN; while those tumor tissue is also used for examining microsatellite instability by polymerase chain reaction (PCR).[5,16]

The treatment approaches by using the molecular test results of CRC tumor tissue will give information for the selection of individualized therapy, representing the principles of personalized tumor diagnostics and targeted therapy. The application of tissue molecular testing of CRC discussed here considers the CIN, deoxyribonucleic acid (DNA) mismatch repair status and MSI, and CIMP, and takes into consideration the mutational status of the EGFR signaling pathway to select targeted therapy.

Chromosomal Instability

Chromosomal Instability (CIN) is characterized by any chromosomal copy number or structure change. CIN is the commonest genomic instability that encompasses 80-85% of all CRC and adenoma. It is suggested that CIN induces carcinoma through the loss or mutation of tumor suppressor genes such as APC, TP 53, and also through activation of oncogenes such as KRAS. CRC caused by CIN usually have poor prognosis.[17,18,19]

In the RAS family, KRAS gene plays the most important role. The activation of RAS genes can promote cell survival and suppress apoptosis. Most KRAS mutations occur in codon 12 (70-80%) and codon 13 of exon 2. In clinical applications, KRAS mutation analysis is widely used as a prognostic and predictive biomarker for anti_EGFR monoclonal antibodies like cetuximab and panitumumab to predict the therapeutic effectiveness in CRC. KRAS mutations predicts lack of response to therapy with antibodies targeted to EGFR. No role of anti EGFR in KRAS mutant colorectal cancers. Apart from KRAS, recent clinical studies start to focus on v-raf murine sarcoma viral oncogene homologue B1 (BRAF) and neuroblastoma-ras (NRAS). Mutations in BRAF occur in approximately 12% of all CRCs patients and it is mutually exclusive of KRAS mutation. Investigation of BRAF mutations is also recommended when KRAS mutation are not found. NRAS is closely related to KRAS, and found in approximately in 3-5% of all CRC patients. It occurs in codon 61. NRAS mutations are mutually exclusive of KRAS mutations.

Adenomatous polyposis coli (APC) gene plays a crucial role in the Wnt/Wingless pathway. APC gene is the most important gatekeeper of colonic epithelial cell proliferation and it is responsible for controlling the underlying oncoprotein called β-catenin. The loss of function in APC gene may lead to the transition to adenoma from normal colonic mucosa due to the up-regulation of β-catenin. Somatic APC mutations are present in most sporadic colorectal adenomas and cancers. Similar to KRAS, APC mutations appear in the early stage of the progression from adenoma to carcinoma.[18,19]

Table 3. Molecular biology test in colorectal cancer[20] (modified)

Name/ method Target	Intended use	Detected property	Source material	Molecular Method	Use/ availability
KRAS	TD EGFR TT	KRAS Mutation	FFPET or SFT	Sequencing	Clinical routine
KRAS	TD EGFR TT	KRAS Mutation	FFPET or SFT	SnaPshot/Strip assay, COLD PCR, ARMS, PNA clamping, Digital PCR	Studies.
BRAF	Chemoth. Suscept.	BRAF mutations	FFPET or SFT	Sequencing, Real time PCR.	Clinical routine
BRAF	Chemoth. Suscept.	BRAF mutations	FFPET or SFT.	Digital PCR, COLD –PCR	Studies.
MSI status PCR	Chemoth. Suscept.	MSI status	FFPET or SFT	PCR	Clinical routine
MSI status IHC	Chemoth. Suscept.	MSI status	FFPET	IHC	Clinical routine
MMR status:	Chemoth. Suscept. (CTS)	MMR/ MSI	FFPET	IHC, PEN of MLH1, MSH2,PMS2 and MSH6.	Clinical/ studies
APC PEN	Chemoth. Suscept.	CIN	FFPET	IHC	Studies
MSI status miRNA	Chemoth. Suscept.	MSI status	FFPET or SFT	Oligonucleotide microarray	Studies
TP53 mutation	Screening	P53 mutation analysis	FFPET or SFT	Sequencing	Clinical routine
TP53 mutation	Screening	P53 mutation analysis	FFPET or SFT	Oligonucleotide microarray	Studies
CIMP	Probable screening/ staging	Methylation	FFPET or SFT	Methylation microarray	Studies
miRNA assay for blood/stool	Screening	miRNA expression level	Plasma/ Stool	Microarray	Studies

APC: Adenomatous polyposis coli, PEN: protein expression negative, FFPET: Formalin-fixed paraffin-embedded tissues, SFT: Snap frozen tissue; CTS: Chemo therapeutic susceptibility; TD EGFR TT: Therapeutic decision EGFR targeted therapy

Deoxyribonucleic acid (DNA) Mismatch Repair Defects and Microsatellite Instability

Microsatellite are short tandem repeats of nucleotides that occur throughout the genome. In cells with deficient mismatch repair, errors in DNA replication accumulate and are detectable in these regions, identified as microsatellite instability (MSI). Approximately 15% of all CRCs show underlying defects in DNA mismatch repair (dMMR) and the tumor tissues show microsatellite instability (MSI). In 3-5% of MSI-positive (MSI-high) CRC, patients harbor germline mutations related to the Lynch syndrome and the remaining 12% or so are sporadic CRC.[3]

MSI-positive status (MSI-H) correlated with the tumors being in the proximal colon and with improved survival.[3] This was soon followed by the identification of genes responsible for hereditary non-polyposis colorectal cancer (HNPCC) MSH2 and MLH1. Subsequently, MSI has been shown to play a role in sporadic CRC.[3] At least six different genes (MSH2, MLH1,PMS1, hPMS2, MSH6 and MLH3) encode the mismatch repair system. Assessment of MSI status can be done by immunohistochemistry to evaluate expression of DNA mismatch repair protein that show protein expression negative (PEN), or by PCR-based DNA testing for MSI to assess instability at microsatellite sequences.[5,16, 20] CRC patients with MSI-H status receiving fluorouracil (5-FU) showed no improvement in disease-free survival, and infact, treatment was associated with reduced overall survival [21]. Regimens with 5-FU alone should be avoided in these patients with stage II CRC who may be candidates for chemotherapy.

Microsatellite Instability

Microsatellite instability (MSI) accounts for 15% of CRC. It is characterized by an altered length of the gene with small deletions and insertions of short repetitive deoxyribonucleic acid (DNA) sequences (microsatellite) distributed throughout the genome. Single MSI within the whole genome may have no significant effects but accumulation of the mutations can result in frame shifts within gene coding sequences and the subsequent inactivation of the genes would give rise to the progression of the tumor. The underlying cause of MSI can be explained by two mechanism: 1) defective mismatch repair (MMR) system, in which both allells of a MMR gene (MLH1, MSH2, MSH6, and PMS2) are non-functional. This results in the loss of ability to repair DNA replication mismatches in the affected cells; 2) hypermethylation of promoter in MMR genes that suppress the expression of the genes like MLH1.[17,18,22]

Polymerase chain reaction (PCR) based methods and immunohistochemistry (IHC) can be used to detect MSI-H. IHC staining detects DNA MMR system protein such as MLH1 and MSH2. The loss in these markers is indicative of MSI. In our study differences in the characteristics of chromosome instability (CIN), MMR and MSI CRC found in the proximal, distal colon and rectum were desribed. The tissues with adenocarcinoma were examined by IHC and PCR. IHC was done to examine the APC protein expression negative (APC-PEN), MMR-PEN (MLH1,MSH2,MSH6 and PMS2). MSI by PCR based on 5 markers of BAT25, BAT26, D2S123, D5S346,D17S250, which were performed. MSI-H was considered if there were ≥ 2 of abnormal markers.[4,5] IHC staining is best performed when the tumor tissue specimens are fixed promptly and properly since the quality of staining would be affected. Furthermore, the size of the specimens is also a concern in staining. The MSI classification system is highly valuable in prognosis and therapy since standard chemotherapy using 5-fluorouracil is not effective in treating MSI-high tumors. Instead, irinotecan-containing regimens have shown improved responses and better prognosis for MSI-High tumors.[19,22]

Epigenetic Gene Silencing

It is mostly caused by DNA methylation. Cancers with high degree of methylation can be considered as CpG island phenotype (CIMP) positive, and CIMP encompasses 35-40% of sporadic CRC. DNA methylation is involved in normal cellular control of gene expression. The methylation patterns of these CpG sequences are gene-specific. Aberrant CpG hypermethylation can lead to silencing of tumor-suppressor genes in carcinogenesis since the expression of the genes is repressed. For example, p16, p14, MGMT, and hMLH1 are commonly silenced genes in CRC patients. In some cases, the presence of epigenetic silencing overlapps with MSI. Some sporadic CRC with microsatellite instability is caused by DNA methylation. For example, DNA methylation of MLH1 gene promoter blocks its expression and destroys the ability of MMR system.[17,18,23,14] CpG regions that are hypermethylated in CRC when compared to normal individuals are valuable for biomarkers development. The methylation of MLH1 associated silencing is widely used as prognostic and predictive markers for CRC.[15,17,24]

Targeting Epidermal Growth Factor Receptor (EGFR) Signalling Pathways in Colorectal Cancer

Aberrant activation of EGFR signaling pathways is frequent in CRC, and is primarily activating mutations of genes in these pathways MAPK and PI3K. The following are the proportions of cases harboring various mutations in EGFR pathways genes: MAPKinase pathway: KRAS (40-45%), NRAS (2.5%), BRAF (5-10%); PI3Kinase pathway: PIK3CA (15%), PTEN (10-20%), AKT (5%); combined mutations: KRAS/NRAS and PI3K (10%).

An interesting finding is that in CRC as in other tumors, RAS and RAF mutations are mutually exclusive. Therefore, together, BRAF and KRAS are mutated in about half of all CRC cases.[3,25,26,27] KRAS mutations are found in about 40-45% of all colorectal cancers and occur mostly at exon 2 [codon 12 (70-80%) or 13 (20-30%)], while there are rare mutations in codons 61 and 146. BRAF mutations occur most commonly at exon 15 with thymine to adenine transversion at nucleotide position 1796, which leads to the substitution of valine for glutamate (a substitution mutation termed V600E), and are found in about 5-10% of all colorectal cancers. Importantly the BRAF V600E mutation occurs in 4-12% of DNA MMR proficient tumors (microsatellite stable; MSS) and in 40-74% of MSI-H sporadic CRC (MLH1 deficient), but is not found in MLH1-deficient MSI-H CRC in HNPCC-associated CRC. Mutations in the PI3K axis are seen in about 20% of all CRC cases.

The role of EGFR pathway gene mutations in the clinical management of CRC has been extensively studied. In terms of prognosis, KRAS mutations do not confer a poor prognosis, however, BRAF mutations confer a significantly poorer prognosis, as a compared to wild-type BRAF tumors. More importantly, the EGFR pathway has become an important therapeutic target. Cetuximab and panitumumab are anti EGFR antibodies that target the extracellular domain of the receptor. They have been shown to improve progression-free, and in some cases, overall survival in metastatic colorectal cancer. A landmark paper by Karapetis et al published in 2008 showed that in patients with wild-type KRAS tumors, treatment with cetuximab as compared with supportive care alone significantly improved overall survival (median, 9.5 vs. 4.8 months). In contrast, in patients with mutated KRAS tumors, there was no significant difference between those who were treated with cetuximab and those who were not. Several study concluded that patients with a colorectal tumor bearing mutated KRAS did not benefit from cetuximab, where as patients with wild-type KRAS CRC did benefit from cetuximab therapy.

Patients with metastatic CRC who are candidates for anti EGFR antibody therapy should have their tumor tested for KRAS mutations. There is up to 40% response rate to anti EGFR therapy in wild-type CRC while the remainder 60% wild-type tumors will not respond, presumably due to other gene/protein alterations in the EGFR or other signaling pathways. The predictive role of BRAF mutational studies in CRC is still unclear. While BRAF activating mutations should act similar to KRAS in terms of predicting and prognosticating. [28,29,30,31]

Future Molecular Diagnostics in Colorectal Cancer and Colorectal Adenoma: miRNA Detection.

MicroRNAs (miRNAs) are small non-coding RNA that are usually 19-23 nucleotids in length. Due to their small sizes, miRNAs are more stable in blood and FFPE tissues than other nucleic acids such as DNA and RNA. MicroRNAs (miRNAs) are involved in post-transcriptional regulation of gene expression. Therefore, they are able to function as oncogenes or tumor suppressor genes, and dysregulation of miRNA would be associated to cancers. Recent studies showed that miRNAs circulate in a stable and cell free form in the bloodstream. Therefore, miRNAs that are specific to CRC in blood samples may be identified for the development of non-invasive prognostic and predictive markers of the disease.

miR-135a and miR-135b play important roles in the regulation of the Wnt/Wingles pathway by down-regulating APC gene expression. miR-17-3p and miR-92a have been found to be elevated in plasma and their levels decreased after removal of the cancer tissues. In the plasma of CRC patients, circulating miR-92 and miR-17 concentrations have been reported to be elevated in the preoperative samples and the concentrations were markedly reduced in the postoperative samples. Those results suggested that circulating miR-92 and miR-17 are potential non-invasive diagnostic markers for CRC. Apart from the miRNAs mentioned above, miR-211 is also believed to be a potential marker for the diagnosis and prognosis of CRC.[2,11,20]

CONCLUSION

This review aims to summarize the issues on the use of biomarkers for determination of prognosis and monitoring of response to therapy. KRAS mutational

analysis of CRC tumor tissues is recommended as the standard of care in patients who are candidates for targeted anti-EGFR antibody therapy. Some experts now recommend whole RAS sequencing. In large practice centers, the trend is to test all colorectal adenocarcinomas for KRAS codon 12-13 mutations, for BRAF V600E mutations, and for microsatellite instability, thus allowing for selection of patients for conventional therapy as well as targeted therapy. In the last decade, the median survival of CRC patients has increased significantly (~20%) with the introduction of new routine diagnostics and personalized therapies.

It is obvious that determination of molecular predictive factors analyzed in routine diagnostics before selection of chemotherapy is important for individualized treatment in colorectal cancer. The optimal treatment strategy for metastatic CRC should be discuss in team work. The availability of multiple therapies and the judicious use of surgery have improved outcomes for metastatic CRC. Several biomarkers associated with predictive and prognostic values are available.

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