
بسم الله الرحمن الرحيم

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

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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.

Despite improvements in early detection and treatment method in recent years, CRC remains the third most frequent and the fourth leading cause of cancer-associated mortalities worldwide.

Approximately 65% of CRC cases are sporadic with no family history or apparent genetic predisposition.

The remaining cases are familial, arising from moderately penetrant inherited susceptibility, possibly interacting with environmental factors.

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.

These changes include gains or losses of chromosomal segments, chromosomal rearrangements, and LOH, which results in gene CNVs.

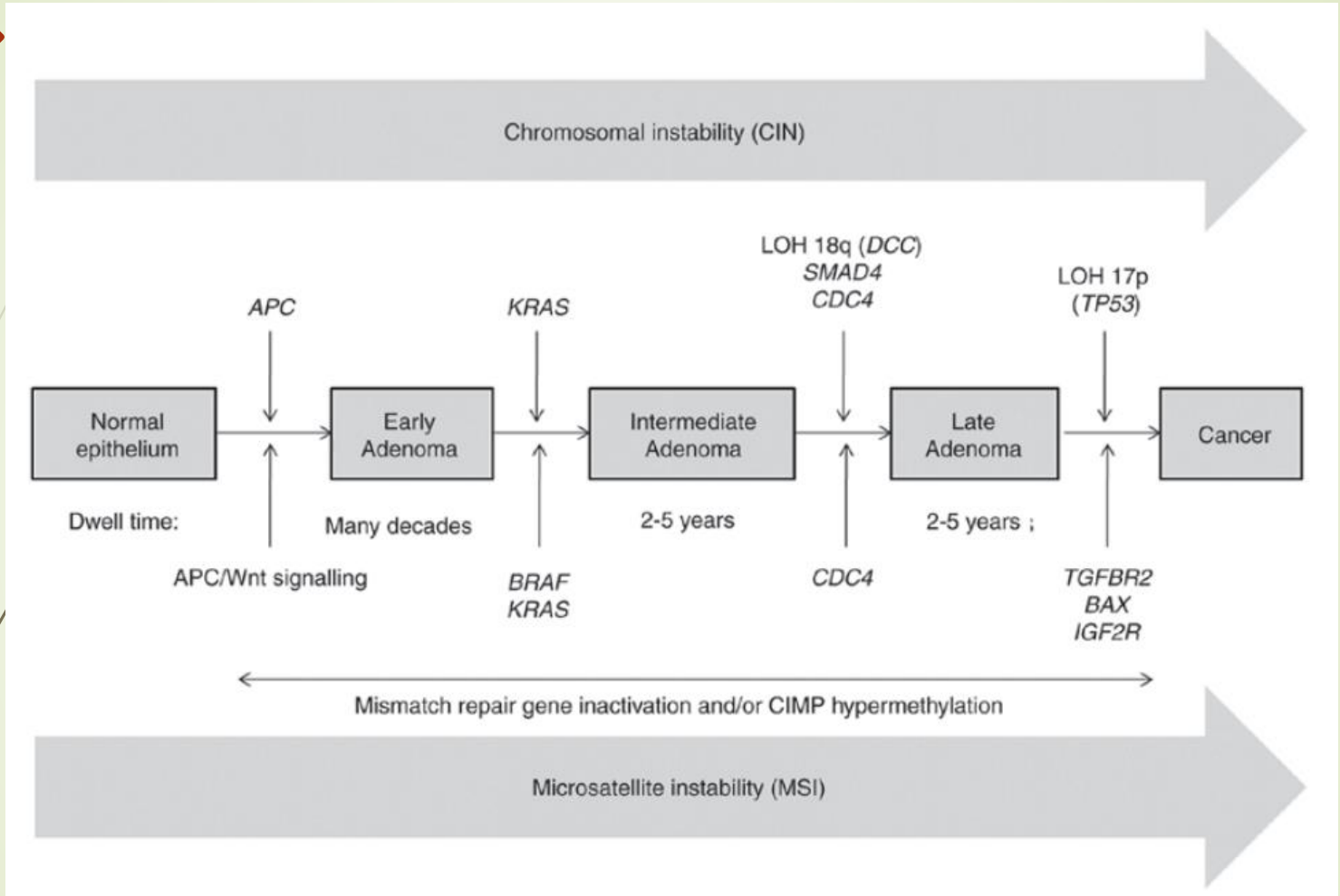
These alterations affect the expression of tumor-associated genes, and/or genes that regulate cell proliferation or cell cycle checkpoints, which, in turn, may activate pathways essential for CRC initiation and progression. The remaining sporadic cases (~15%) have high-frequency microsatellite instability (MSI) phenotype.

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 (HNPCC, or Lynch syndrome) (1-3%) is characterized by MSI, a consequence of a defective MMR system.

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%).

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. 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.

This process may take years to decades to escape the multiple regulatory layers of the cells and to fully develop (Fig. 1). There are three major pathways associated with CRC pathogenesis, namely: CIN, MSI and CpG island methylator phenotype (CIMP).


Chromosomal instability (CIN)
APC
KRAS
LOH 18q (DCC)
SMAD4
CDC4
LOH 17p
(TP53)
Normal epithelium
Early Adenoma
Intermediate Adenoma
Late Adenoma
Cancer
Dwell time:
Many decades
2-5 years
2-5 years ;
APC/Wnt signalling
BRAF
KRAS
CDC4
TGFBR2
BAX
IGF2R
Mismatch repair gene inactivation and/or CIMP hypermethylation
Microsatellite instability (MSI)

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. 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. 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.

Molecular basis of CRC

CIN pathway:

The average rate of genomic mutation in normal human cells is ~2.5x10-8 mutations/nucleotide/generation. 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’. Mutations in MMR genes, may elevate mutation rates to the level commonly observed in human tumors. The ‘mutator phenotype’ may have various manifestations, including point mutations, CIN, MSI, CIMP and LOH.

CIN appears to be the most common type of genetic instability in CRC, observed in 85% of adenoma-carcinoma transitions. 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.

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. 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.

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).

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.

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.

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.

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.

Losses of 18q:

Allelic loss at chromosome 18q is detected in ~70% of primary CRC in the late carcinogenic process, and is considered as a poor prognosis marker for survival in patients with CRC. 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.

In fact, SMAD2 and SMAD4 genes are localized in 18q21.1, the common region of loss of 18q in CRC. 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.

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. 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.

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.

APC mutations or allelic losses have been identified in ~90% of patients with CRC. Germline mutations in APC are responsible for FAP (15), while somatic mutations and/or allelic deletions of APC are described in sporadic CRC.

The APC gene may also be epigenetically inactivated through promoter hypermethylation that has been identified in 18% of primary colorectal carcinoma and adenoma cases.

TP53:

TP53 is a tumor-suppressor gene located on the short arm of chromosome 17, which is commonly lost in colorectal carcinoma. 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.

Upon DNA damage, TP53 induces cell cycle arrest at the G1 or G2 phase, or triggers apoptosis when the damage is too severe and irreparable.

TP53 alteration is the hallmark of human tumors, and the status of TP53 mutation is associated with the progression and outcome of sporadic CRC. 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.

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 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.

Oncogenic mutations in the RAS gene have been identified in ~30% of all human tumors, in which mutations in KRAS accounted for ~85%, NRAS for ~15%, and HRAS for <1%.

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, and lung and liver metastasis.

In contrast, several other studies reported that KRAS mutations were strong independent predictors of survival in patients with CRC. 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.

Activating KRAS mutations have been identified in 35-45% of CRC cases, and primarily occur in codon 12 and 13. The most frequent changes observed in these codons are the substitution of glycine for aspartate (p.G12D, p.G13D).

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.

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.

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 no apparent instability are microsatellite stable (MSS).

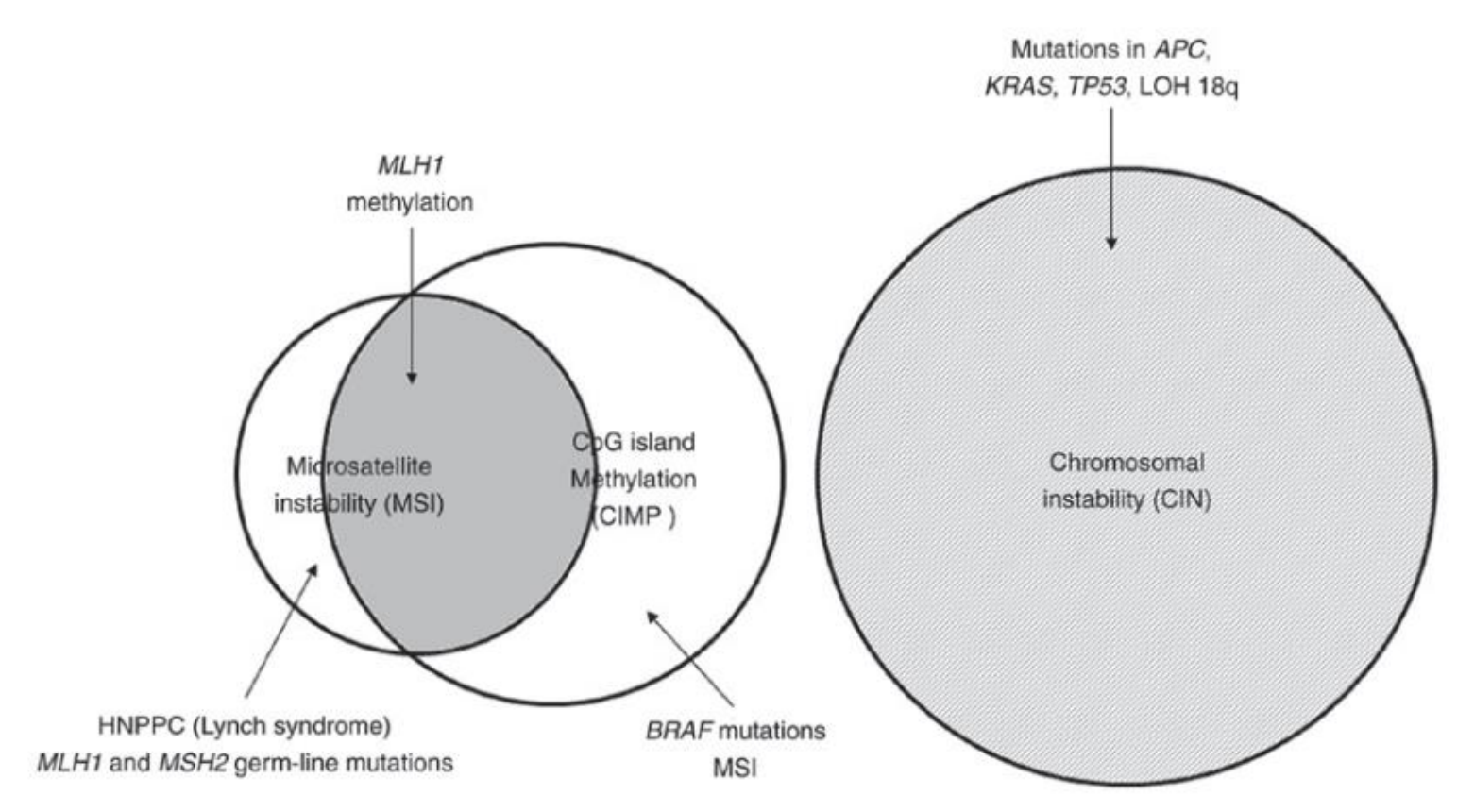
It is now accepted that MSI is associated with post-replicative DNA MMR deficiency, primarily involving MLH1 and MSH2. Impairment of MMR genes can occur by either mutational inactivation or by epigenetic inactivation through CpG island methylation of the promoter of the genes.

Loss or insufficiency of MMR activity leads to replication errors with an increased mutation rate and a higher potential for malignancy.

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. The CpG island methylator phenotype has been identified in 30-35% colorectal adenoma cases, and is considered as an early event of colorectal tumorigenesis.

Notably, sporadic MSI colorectal tumors are almost exclusively associated with CIMP-associated methylation of MLH1 leading to inactivation of this gene. 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).


Mutations in *APC*, *KRAS*, *TP53*, LOH 18q
MLH1 methylation
Microsatellite instability (MSI)
CpG island Methylation (CIMP)
Chromosomal instability (CIN)
HNPPC (Lynch syndrome) *MLH1* and *MSH2* germ-line mutations
BRAF mutations MSI

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. 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.

Particularly, the frequency of BRAF mutations in CIMP+ tumors is significantly higher compared with their CIMP- counterparts.

Shen et al 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%).

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. 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. Several known mutations are considered to be associated with a poorer patient outcome and/or failure of response to a certain therapy.

Patients with inactive TP53 mutations are at an increased risk of mortality compared with their counterparts, but this mutation does not appear to affect the outcome of chemotherapy.

However, the presence of somatic KRAS mutations has been considered as a predictor of resistance to anti-EGFR therapy. 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.

APC is another commonly affected gene whose mutations generally appear in the early stage of CRC development. 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. Therefore, APC mutations are being considered as good diagnostic markers for identifying individuals at risk of CRC.

CRC with MSI are more likely to occur in the proximal colon. Evidence has suggested that MSI is a favorable prognostic biomarker for CRC.
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, while others reported the beneficial effects. Des Guetz et al 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.

The more improved survival rate of MSI-H patients was due to a better prognosis rather than the benefit of chemotherapy. MSI may be considered as a predictive marker of chemoresistance. The MSI status among patients with CRC, thus, is highly valuable in prognosis and therapy of CRC, and should be thoroughly evaluated 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 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.

Sadanandamet al 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, exhibited an improved response to the combination chemotherapy regimen FOLFIRI (5-FU with irinotecan), whereas transit-amplifying- and goblet-like-subtype tumors, with markedly better prognosis, did not appear to benefit from these treatments.

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.

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.

Thanks for watching