Neuroendocrinology Letters Volume 40 No. 5 2019
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

REVIEW ARTICLE

Molecular biomarkers in the diagnostic of patients with colorectal cancer

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Submitted: 2019-06-05 Accepted: 2019-09-10 Published online: 2019-12-12

Key words: **colorectal cancer; molecular biomarkers; *Ras* oncogenes; microsatellite instability; Lynch syndrome; mismatch repair system**

Neuroendocrinol Lett 2019; **40**(5):215–221 **PMID:** 32112545 NEL400519R02 • **www.nel.edu**

Abstract

Colorectal cancer (CRC) is currently a well-known and studied issue in experimental research. Worldwide it is the third most common cancer in men and the second most common cancer in women. 70–80% of cases occur sporadically. Most CRCs develop from adenomas. The transition from normal epithelium to adenoma and finally into carcinoma is associated with acquired molecular events. In 5–10 % of cases, CRC develops from germline mutations in cancer-predisposing genes. 15% of patients have a family history of CRC that suggests a hereditary contribution, common exposures or shared risk factors among family members. Genetic alterations in cancer-related genes represent prognostic and predictive CRC biomarkers. Genetic testing of individuals with newly diagnosed CRC as well as of asymptomatic relatives can lead to improved outcomes for the patient and at-risk family members. Discovery of circulating cell-free tumor DNA (ctDNA) promises an improvement of the CRC diagnostics. ctDNA shares common genetic alterations with the primary tumor so it allows non-invasive monitoring of the disease over time.

This review is focused on the principal molecular biomarkers associated with CRC and on the key characteristics of initiation and progression of CRC including chromosomal instability, microsatellite instability and signaling pathways where this deregulation leads to tumorigenesis.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide. Understanding the molecular mechanisms of cancer development enables a more targeted approach for the prevention and treatment of this cancer (Takayama *et al.* 2006). Genetic alterations in cancer-related genes have been considered as potential CRC molecular markers because they can provide the clinician with diagnostic, prognostic and predictive treatment response information. The ideal molecular marker should have high sensitivity and specificity. A robust biomarker should detect genomic alterations or variations in protein expression that specifically correlate to the disease. Molecular markers can also be used to assess the risk of future disease, the aggressiveness of the malignancy over the time, and the probability that a patient will respond to a particular treatment, thereby helping the clinician make personalized treatment decisions (Gonzalez-Pons & Cruz-Correa, 2015).

The majority of colorectal cancers (70–80%) occurs sporadically by the progressive accumulation of mutations in oncogenes and tumor-suppressor genes (Patel & Ahnen, 2012). Approximately 20–30% of CRC cases are due to genetic factors. 20–25% of cases are estimated to

have an associated hereditary component, which has not yet been well-established and is known as familial CRC (Binefa *et al.* 2014). Only 5–10% of CRC develop as a result of inherited mutations in known cancer-related genes (Patel & Ahnen, 2012).

A better understanding of carcinogenesis pathways has allowed the development of diagnostic and prognostic markers as well as the investigation of new therapeutic targets and predictors of response to cancer treatments.

Recently, circulating cell-free tumor DNA (ctDNA) has received much attention as a cancer biomarker for its ability to track the progression of the advanced disease, predict tumor recurrence and reflect the complex genetic heterogeneity of cancer (Myint *et al.* 2018).

HEREDITARY COLORECTAL CANCER

Because the predisposition is due to an inherited germline mutation, the onset of cancer occurs at a much earlier age than in sporadic cancer. Thus, identification of the hereditary trait in a patient offers a chance for prevention or early detection of cancer in other family members by appropriate genetic workup, endoscopic surveillance of asymptomatic family members and, in some cases, by prophylactic surgery (Järvinen, 2004). Hereditary colorectal cancer includes familial adenomatous polyposis (FAP), MUTYH-associated polyposis (MAP), Lynch syndrome (LS), Lynch-like syndrome (LLS), constitutional mismatch repair deficiency syndrome (CMMRD), polymerase proofreading associated polyposis (PPAP), familial colorectal cancer type X (FCCTX), Peutz-Jeghers syndrome (PJS), serrated polyposis syndrome (SPS) and juvenile polyposis syndrome (JPS).

Familial adenomatous polyposis represents 1% of all CRC cases and refers to an autosomal dominant disease. An inherited mutation in the *APC* (Adenomatous Polyposis Coli) gene is the cause behind it. Clinical manifestation of FAP includes the development of many (hundreds to thousands) polyps generally in colon and rectum. Yet, they may occur also in the extra-colonic area (Half *et al.* 2009). Nowadays, a gene panel-based high-through-put targeted next-generation sequencing for the molecular genetic study of the patient and family members allowed identification of a novel single nucleotide heterozygous germline insertion [c.3992_3993insA; p.Thr1332Asnfs*10] in exon 16 of the *APC* gene. This novel insertion of *APC* gene leads to frameshift by a premature stop codon which finally results in the formation of a truncated APC protein of 1,342 amino acids, almost half a length compared with the wild type APC protein consisting of 2,417 amino acid. Therefore, this mutation is a loss-of-function mutation causing disease following the haploinsufficiency. This frameshift mutation is cosegregated well with the FAP phenotype among all the affected members in an autosomal dominant mode of inheritance (Wang *et al.* 2019).

MUTYH-associated polyposis is an autosomal recessive disorder resulting from germline mutations in both alleles of the *MUTYH* gene. *MUTYH* gene encodes enzyme MYH glycosylase, a member of base excision repair (BER) system which handles the repair of the oxidative DNA damage (Poulsen & Bisgaard, 2008). An increased risk for CRC and multiple adenomatous polyps that can mimic FAP characterizes MAP. Although the predominant polyp type in patients with biallelic mutations of the *MUTYH* gene is an adenoma, many hyperplastic or sessile serrated polyps may occur (Syngal *et al.* 2015).

The classification of hereditary nonpolyposis colorectal cancer (HNPCC) is based on the diagnostic Amsterdam I or II criteria and more recently, revised Bethesda guidelines. HNPCC includes diseases characterized by defects in the mismatch repair system (MMR) and microsatellite instability (MSI) and diseases without MMR deficiency.

Inherited conditions that prove MMR deficiency involve Lynch syndrome, Lynch-like syndrome and constitutional mismatch repair deficiency syndrome. MMR-proficient syndromes include polymerase proofreading associated polyposis and familial colorectal cancer type X (Carethers & Stoffel, 2015).

According to the initial set of Amsterdam I diagnostic criteria, at least three relatives in two or more generations have to be affected, one is a first-degree relative of the other two, at least one of the family members with CRC is diagnosed before 50 years of age and familial adenomatous polyposis has been excluded. On the other hand, the diagnosis of Lynch syndrome requires a germline mutation in at least one of the MMR genes. In this way, a monoallelic germline mutation in MMR genes has a crucial role in the differentiation between Lynch syndrome and other HNPCC diseases (Stoffel & Kastrinos, 2014).

Lynch syndrome is the most prevalent inherited colorectal cancer condition. It is associated with DNA mismatch repair deficiency. The MMR system repairs replication and recombination errors arising from DNA polymerase defects. MMR system includes several genes such as *MLH1* (mutL homolog 1), *MLH3* (mutL homolog 3), *MSH2* (mutS homolog 2), *MSH3* (mutS homolog 3), *MSH6* (mutS homolog 6), *PMS1* (postmeiotic segregation 1) and *PMS2* (postmeiotic segregation 2) (Fukui, 2010). Mutations in MMR genes may result in the hypermutable phenotype known as microsatellite instability. Microsatellites are regions of repetitive DNA and are thriving in the human genome. Replication errors that occur in the microsatellite regions lead to an altered microsatellites length as compared with the parent cells. MSI is detected in about 15% of all colorectal cancers; 3% are of these are associated with Lynch syndrome and the other 12% are caused by sporadic, acquired hypermethylation of the promoter of the *MLH1* gene, which occurs in tumors with the CpG island methylator phenotype (CIMP) (Boland &

Goel, 2010). Microsatellite instability occurs in a majority of Lynch syndrome tumors. That makes it a suitable preselective biomarker for families with suspected LS. MSI status is also a useful prognostic and predictive sporadic CRC marker because it may predict responsiveness to adjuvant chemotherapy. Reports from clinical trials, retrospective case series, and meta-analysis have reported that patients with MSI tumors do not benefit from 5-fluorouracil (5-FU) adjuvant chemotherapy compared to patients with microsatellite-stable tumors (MSS). MSI is associated with increased patient survival and a favorable prognosis (Gonzalez-Pons & Cruz-Correa, 2015).

Lynch syndrome is transmitted in an autosomal dominant inheritance pattern. Hallmarks of LS families include a family history of the disease, young age of onset, accelerated adenoma to carcinoma progression, predominantly right-sided tumors and increased extracolonic manifestations (malignancies of the gastrointestinal tract, endometrium, ovaries, urinary tract, brain, and skin) (Lynch *et al.* 2015).

Point mutations, deletions, and rearrangements of *MLH1* and *MSH2* genes account for 90% of mutations identified in patients with Lynch syndrome (Giardiello *et al.* 2014). It is becoming the standard of care at many centers that all individuals with newly diagnosed CRC are evaluated for Lynch syndrome through molecular diagnostic tumor testing assessing MMR deficiency. A universal screening approach to tumor testing is supported, in which all CRC cases are evaluated regardless of age at diagnosis or fulfillment of existing clinical criteria for Lynch syndrome.

Small intestinal adenocarcinoma (SIAC) is the initial manifestation in about half of LS patients. Identifying LS in patients with SIAC can be beneficial for early detection and treatment of other LS-related cancers in patients and even their relatives (Jun *et al.* 2017).

Many genetic testing laboratories offer multigene (panel) tests that simultaneously test for pathogenic variants in all the Lynch syndrome-associated genes (and often extra genes associated with inherited cancer susceptibility). Individuals with early-onset CRC have been shown to have a high frequency and wide spectrum of germline pathogenic variants, indicating that panel testing in this population may be beneficial. Multigene tests are currently not recommended for universal screening for Lynch syndrome among all newly diagnosed CRC patients, but they may be very useful in selected populations, such as those with early-onset CRC or from familial, high-risk clinic-based populations. It is also important to note that pathogenic variants may be detected in other cancer-associated genes beyond Lynch syndrome, such as *BRCA1*, *BRCA2*, *APC*, *MUTYH*, and *STK11* [i1].

Lynch-like syndrome refers to the condition which shares common clinical manifestations with Lynch syndrome, but there is a significant difference at the molecular level. In Lynch-like syndrome, a germline mutation in MMR genes is absent. It is presumed that Lynch-like patients could have Lynch syndrome with undetectable inherited MMR mutation in a promoter or intronic regions. Another explanation is based on the inactivation of MMR genes in a sporadic manner (for example loss of heterozygosity) resulting in similar phenotypic characteristics with Lynch syndrome (Carethers, 2014).

Constitutional mismatch repair deficiency syndrome represents a very rare genetic disease characterized by a biallelic germline mutation in MMR genes and mutator phenotype is marked by microsatellite instability. Tumor development occurs at a very young age. A typical symptom of all CMMRD patients is cutaneous „café-au-lait" spots (Bakry *et al.* 2014).

Polymerase proofreading associated polyposis syndrome is an autosomal dominant genetic disorder that leads to the development of multiple adenomas and carcinomas that exhibit microsatellite stability. It is caused by inherited mutations in genes that encode exonuclease (proofreading) domains of two DNA polymerases – *POLE* that encodes DNA polymerase ε or *POLD1* that encodes DNA polymerase δ1. Adenomatous polyps appear typically in the second decade of life. PPAP is also associated with a higher risk of endometrial tumors (Palles *et al.* 2013).

Familial colorectal cancer type X syndrome represents a great clinical overlap with Lynch syndrome but it lacks MMR deficiency and MSI. FCCTX is characterized by the age of onset of approximately 60 years, left-sided tumors and slower adenoma to carcinoma progression compared with Lynch syndrome. While genetic etiology of FCCTX remains unknown, several studies describe an association of germline mutations in some genes including *BMPR1A* (Bone Morphogenetic Protein Receptor type 1A) gene, *RPS20* (Ribosomal Protein S20) gene (Lindor *et al.* 2005) and *BRCA2* gene (Garre *et al.* 2015).

Peutz-Jeghers syndrome is an autosomal dominant inherited disease characterized by mucocutaneous pigmentation and multiple gastrointestinal hamartoma polyps. PJS is a very rare disease, with an incidence of about 1/25000 (Duan *et al.* 2018). Patients with PJS have a germline mutation in a gene encoding the serine-threonine kinase 11 (*STK11*), a tumor-suppressor gene. Adults with PJS not only have a high risk of developing gastrointestinal cancer but also non-gastrointestinal cancers, especially breast cancer (Bogaert & Prenen, 2014).

Serrated polyposis syndrome, before known as hyperplastic polyposis, is a rare condition characterized by multiple serrated polyps (SPs) spread throughout the colon and rectum (Kim *et al.* 2017). Diagnostic criteria for SPS were first described in 2000 and redefined in 2010 by the World Health Organization (WHO): (1) ≥5 serrated colon polyps proximal to the sigmoid colon with 2 or more of these being >10 mm, (2) any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with SPS, or

(3) >20 serrated polyps of any size distributed throughout the colon (not all in the rectum). Although SPS was initially considered to be non-inherited, familial clustering and high risk (up to 50%) of CRC in first-degree relatives of SPS patients has been described. In 2017, Horpaopan *et al.* published the first study which performed an exome sequencing in a number of serrated polyps from a single patient to identify potential novel drivers of serrated tumorigenesis. Somatic mutations beyond the well-known driver mutations seem to be rare events in early *BRAF/KRAS*-related serrated lesions of SPS patients. No affected genes and no enrichment of specific pathways have been observed. Thus, other alterations such as non-coding variants or epigenetic changes might be the major driving force of tumor progression in SPS (Horpaopan *et al.* 2017). The association between SPS and personal and familial CRC risk is well-established, although the genetic nature and natural history of this syndrome remain unknown (Kim, 2018).

Juvenile polyposis syndrome is a rare autosomal dominant hereditary disorder characterized by many distinct juvenile polyps in the gastrointestinal tract and an increased risk of colorectal cancer. Juvenile polyposis syndrome is defined by the presence of five or more juvenile polyps in the colorectum, juvenile polyps throughout the gastrointestinal tract or any number of juvenile polyps, and a positive family history of juvenile polyposis. About 50–60% of JPS patients have a germline mutation in the *SMAD4* or *BMPR1A* gene (Brosens *et al.* 2011).

SPORADIC COLORECTAL CANCER

The definition of colorectal cancer is based on the adenoma-carcinoma sequence theory postulated by Fearon and Vogelstein in 1990. According to this theory, CRC is a multistep process of accumulation of mutations in oncogenes and in tumor-suppressor genes. Genomic changes correlate with the progression of colon normal epithelium to invasive carcinoma.

The first molecular event involves a mutation in the *APC* gene (Fearon & Vogelstein, 1990).

APC gene is located at chromosome region 5q21. *APC* is a tumor-suppressor gene that controls cell division and acts as an important member of Wnt signaling pathway that regulates cell growth and proliferation. *APC's* mutations occur in sporadic and also in inherited colorectal cancers (Coppedè *et al.* 2014). The important role of APC in predisposition to colorectal tumors is supported by the association of *APC* germline pathogenic variants with familial adenomatous polyposis [i1].

Besides *APC* mutations, the transformation of adenoma to carcinoma requires a number of additional mutations. This genetic alterations involve loss of *TP53* gene nicknamed guardian of the genome loss of heterozygosity of the long arm of the chromosome 18 where *DCC* (Deleted in Colorectal Cancer) gene is located and mutations in *RAS* oncogenes: *KRAS* (Kirsten rat sarcoma viral oncogene homolog) and *NRAS* (Neuroblastoma RAS viral oncogene homolog) (Fearon & Vogelstein, 1990).

TP53 gene is a very important tumor-suppressor gene that encodes tumor-suppressor p53 protein. The p53 protein is a transcription factor inducing G1 cell cycle arrest, senescence, and apoptosis under cellular stress. Patients with mutant *TP53* gene are often resistant to current therapies, conferring poor prognosis (Li *et al.* 2015).

Genetic alterations in multistep colorectal tumorigenesis include loss of heterozygosity of the putative tumor-suppressing *DCC* gene on chromosome 18q. Although allelic deletions are infrequent in early or intermediate stage adenomas, about 50% of advanced stage adenomas and more than 70% of CRCs show LOH of chromosome 18q. Furthermore, 18q LOH correlates with an increased likelihood of distant metastasis (Schmitt *et al.* 1998). This chromosomal instability (CIN) is common in 60–70% of sporadic colorectal cancers. It occurs during the early stages of tumorigenesis.

Acquisition of genomic instability is associated with the activation of signaling pathways that regulate cell proliferation and survival. The best-characterized signaling pathways involve the mitogen-activated protein kinase pathway (RAF/MEK/ERK MAP kinase pathway) and Wnt signaling pathway (also known as APC/β-catenin pathway) (Pino & Chung, 2010).

The outcome of colorectal cancer may be improved by targeting pathways involved in colorectal cancer formation, such as anti-epidermal growth factor receptor (EGFR) therapy (Yiu & Yiu, 2016). *RAS* oncogenes are the most utilized predictive biomarkers for response to monoclonal antibody-based therapies, namely cetuximab, and panitumumab (Di Nicolantonio *et al.* 2008). They are members of the mitogen-activated protein kinase pathway (MAPK) that transduces signals from the cell surface to the nucleus through the EGFR (Endothelial Growth Factor Receptor). This signaling pathway regulates cell proliferation, differentiation, senescence, and apoptosis. EGFR is a transmembrane tyrosine kinase. Binding of EGF (Endothelial Growth Factor), the ligand to the EGFR, to an extracellular domain of EGFR promotes receptor dimerization. Subsequent autophosphorylation of the intracellular domain of EGFR leads to the activation of downstream members of the MAPK pathway including RAS, RAF, and MEK. *KRAS* gene is located at the chromosome region 12p12.1. It encodes a small GTP-ase that mediates hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP). When bound to GTP, KRAS is in an active conformation and the signal is transduced to the nucleus. The GDP-bound state represents an inactive KRAS conformation and thus the activation of downstream effectors is disrupted. Mutated *RAS* oncogenes lead to constitutively active proteins independently of upstream EGFR signals.

Mutant *KRAS* is found in about 35–45% of CRCs, and codon 12 and 13 are two hotspots, which account for about 95% of all mutation types, with approximately 80% occurring in codon 12 and 15% in codon 13. Other mutations in codons 61, 146 and 154 occur less frequently in CRC, accounting for 5% of all mutation type (Tan &Du, 2012).

Therapeutic drugs for patients with cancers such as metastatic CRC (cetuximab and panitumumab) have limitations in their usage due to the poor efficacy or the insensitivity in patients harboring *KRAS/NRAS* mutations. Therapies targeting both the Wnt/β-catenin and EGFR-RAS-ERK pathways, especially those lowering the levels of β-catenin, RAS, and EGFR, can be ideal approaches for the treatment of CRC. Lee at al. characterized and tested the effects of small molecules KYA1797K that suppress the growth of CRC cells via the destabilization of both β-catenin and RAS. KYA1797K dose-dependently inhibited the growth of various CRC cells regardless of their *KRAS* mutational status. Treatment with KYA1797K overcame the ineffectiveness of cetuximab for inhibiting the colony formation ability and growth of CRC cells harboring *KRAS* mutations (Lee *et al.* 2018).

BRAF gene is another important component of the MAPK pathway. BRAF protein belongs to the RAF family of serine/threonine protein kinases. The most common activating *BRAF* mutation, V600E (Val-600Glu), is found in 10% of sporadic CRCs. In the MAPK pathway, *BRAF* is downstream of *KRAS*. Mutations in the *BRAF* oncogene are linked with less benefit when treated with anti-epidermal growth factor receptor antibodies in metastatic colorectal cancer (mCRC) and also are associated with poor prognosis (Sanz-Garcia *et al.* 2017). The *BRAF* V600E mutation has been associated with microsatellite instability and the CpG island methylator phenotype in sporadic colon cancer (Samowitz *et al.* 2005).

Furthermore, *BRAF* somatic missense mutations are found in 66% of malignant melanomas (Davies *et al.* 2002). Selective inhibition of the MAPK pathway with either BRAF or MEK inhibition has emerged as a key component for the treatment of *BRAF*-mutant metastatic melanoma (Dossett *et al.* 2015).

Wnt signaling pathway plays a critical role in the maintenance of intestinal stem cells in their undifferentiated state. It regulates the transcription of genes responsible for tumor growth and its invasion (Armaghany *et al.* 2012).

Aberrant Wnt signaling occurs in many human diseases, especially in gastrointestinal cancers including colorectal cancer. The major player in the Wnt signaling is β-catenin. The frizzled receptor at the cell surface (Frz receptors) and its co-receptor, low-density lipoprotein receptor-related protein (LRP) (Novellasdemunt *et al.* 2015), transduces the signal.

In the absence of Wnt ligand, destruction complex mediates ubiquitination and proteasomal degradation of cytoplasmic β-catenin and thus prevents transcription of target genes. The destruction complex consists of scaffold Axin protein, APC gene product, casein kinase 1 (CK1) and glycogen synthase kinase 3 (GSK3) (Rubinfeld *et al.* 1996).

When Wnt ligand is present, it inhibits the formation of destruction complex. It promotes the accumulation of β-catenin, its translocation to the nucleus and later transcription of the target genes (Novellasdemunt *et al.* 2015).

Non-mutated *APC* gene acts as a negative regulator of the Wnt pathway, it promotes the degradation of β-catenin. But, *APC* mutated cells induce accumulation of undifferentiated epithelial cells and this can lead to neoplasm formation (Armaghany *et al.* 2012).

Inactivation of the *APC* gene can be due to frameshift or nonsense mutations. But Wnt pathway defects appear also in sporadic colorectal tumors with wild-type *APC* gene. In these cases, *APC* promoter hypermethylation or mutation of the β-catenin structure occurs (Morin *et al.* 1997).

CIRCULATING CELL-FREE TUMOR DNA (CTDNA) AS A NEW POTENTIAL COLORECTAL CANCER BIOMARKER

Routinely used diagnostic methods for the diagnostic of CRC are invasive and are not effective in the monitoring of the disease over time. Molecular biomarkers that serve as prognostic factors are already in use and specific genomic mutations serving as predictive biomarkers are examined in formalin-fixed tumor tissues. However, ongoing research for the identification of noninvasive biomarkers may lead to a new era in diagnosis, risk prediction and choice of treatment (Coppedè *et al.* 2014).

Circulating cell-free DNA (cfDNA) seems to be an interesting way to the improvement of the CRC monitoring. cfDNA was discovered in 1948 by Mandel and represents fragmented DNA that is released from cells and circulates in the bloodstream. Mechanisms by which DNA enters the bloodstream remain still unclear. Several investigations have led to the conclusion that cfDNA originates from apoptosis or necrosis depending on fragments length (Mouliere *et al.* 2014).

Circulating cell-free tumor DNA (ctDNA) is a part of circulating cell-free DNA (cfDNA) that is directly derived from the tumor. Consequently, ctDNA is genetically identical to a corresponding primary tumor. Furthermore, advanced stages of disease show increased ctDNA levels in comparison with the early stages of cancer. Accordingly, ctDNA could be used also as a marker of malignant progression (Antonatos *et al.* 2006). Furthermore, postoperative detection of ctDNA is a marker of residual disease and very strong predictor of future relapse risk. It also indicates that serial ctDNA assessments during patient follow-up may allow early detection of relapse and enable assessment of the response to relapse intervention (Schøler *et al.* 2017).

Generally, the potential value of ctDNA as CRC biomarker has to be verified. A ctDNA analysis could be performed by using plasma or serum sample. According to several studies, serum contains a higher level of ctDNA than blood plasma. It is due to the contamination arising from genomic DNA released by white blood cells. According to this, plasma is a more accurate biological sample for ctDNA analysis (El Messaoudi *et al.* 2013).

There are two types of ctDNA-based biomarkers: ctDNA quantification and detection of gene mutations (Oliveira & Hirata, 2018).

Nowadays, in lung cancer patients, the liquid biopsy could capture the molecular diversity of the disease, whereas the ease of serial testing facilitates the monitoring of its spatial and temporal genomic evolution (Matikas *et al.* 2016). Postoperative detection of ctDNA is a marker of residual disease and very strong predictor of future relapse risk. Furthermore, it indicates that serial ctDNA assessments during patient follow-up may allow early detection of relapse and enable assessment of the response to relapse intervention (Khakoo *et al.* 2018).

In colorectal cancer patients, ctDNA have the potential to become a valid CRC biomarker, but ongoing research and the development of sensitive methods are required.

CONCLUSION

Colorectal cancer represents a very serious disease worldwide. It is resulting from the instability of the human genome. Genomic instability in CRC involves chromosomal instability (CIN), microsatellite instability (MSI) and CpG islands methylator phenotype (CIMP) (Grady & Markowitz, 2000). MSI is the principal hallmark of many colorectal tumors and it is a consequence of the DNA repair deficiency. Approximately 75% of CRC cases are, due to somatic mutations in genes, involved in signaling pathways that regulate cell growth and proliferation. Deregulation of the Wnt signaling pathway or mitogen-activated protein kinase pathway leads to the formation of adenomatous polyps that could become malignant (Pino & Chung, 2010)

RAS oncogenes involved in the MAPK signaling pathway represent a predictive marker of anti-EGFR-based therapy. Biomarkers currently play an important role in the detection and treatment of patients with colorectal cancer. Risk stratification for screening might be augmented by finding new biomarkers which alone or as a complement of existing tests might recognize either the predisposition towards development or early stage of the disease. Biomarkers have also the potential to change diagnostic and treatment algorithms by selecting the proper chemotherapeutic drugs across a broad spectrum of patients. There are attempts to personalize therapy based on the presence or absence of specific biomarkers (Lech *et al.* 2016).

Hereditary CRC syndromes represent about 5–10% of cases and result from mutations in known cancer-related genes. Many other families exhibit aggregation of CRC or adenomas, but with no clear association with an identifiable hereditary syndrome, and are known collectively as familial CRC [i1]. Lynch syndrome is the major hereditary CRC syndrome caused by MMR defect and is associated with extracolonic manifestations (Lynch *et al.* 2015).

Discovery of a circulating cell-free tumor DNA brought completely new insight into the diagnostic of CRC by the non-invasive manner. Still, further investigation about its effectiveness is required (Antonatos *et al.* 2006).

The aim of future research is to fully identify those biomarkers that can provide a non-invasive and cost-effective diagnosis, as well as to recognize the best prognostic panel of biomarkers, and define the predictive biomarkers for treatments available to future patients.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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