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Review Article

Diagnostic and therapeutic biomarkers in colorectal cancer: a review

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Abstract: Colorectal cancer (CRC) is a public health concern and the second most common type of cancer among men and women causing a significant mortality. Biomarkers closely linked to the disease morbidity could holds potential as diagnostic and/or prognostic biomarker for the disease. This review provides an overview of recent advances in the search for colorectal cancer biomarkers through genomics and proteomics according to clinical function and application. Specifically, a number of biomarkers were identified and discussed. Emphasis was placed on their clinical applications relative to the diagnosis and prognosis of CRC. The discovery of more sensitive and specific markers for CRC is an urgent need, and the study of molecular targets is extremely important in this process, as they will allow for a better understanding of colorectal carcinogenesis, identification and validation of potential genetic signatures.

Keywords: Biomarkers, colorectal cancer, prognosis, management

Introduction

Colorectal cancer (CRC) is an important public health problem and the second most common cause of cancer-related deaths worldwide [1]. CRC incidence and mortality rates are increasing rapidly. The disease occurs more commonly in men and women aged 55-85 years where about 80% of CRC cases occur. Common risk factors include obesity, family history, and physical inactivity, among others [2]. Sporadic CRC accounts for approximately 80% of CRC cases and its emergence and progression is related to the aggregation of a variety of genetic and epigenetic changes in epithelial cells that may be responsible for the development of malignant adenocarcinomas [3]. The carcinogenesis process is multifactorial and complex, but it is known to be related to mechanisms that include chromosomal instability (CIN), CpG island methylator phenotype (CIMP) and microsatellite instability (MSI) [4].

There has been a growing interest in the identification of biomarkers that can be effectively used to diagnose and monitor treatment outcome and prognosis [5]. Recent advancements in molecular technologies and proteomics, investigators have made it possible to detect variations in DNA, RNA, protein, and small molecules from limited amounts of tissue [6]. Blood-based biomarkers can also be easily and quickly analyzed when well characterized, and therefore have the potential to improve cancer management efficiency [7].

Evaluation of gene expression of specific markers in CRC is currently an important part of prognosis and treatment strategies [8]. The classic genetic markers reported for CRC are CIN, characterized by the accumulation of mutations in specific genes such as APC, KRAS, BRAF, TP53 being responsible for 65-70% of CRC cases. MSI, another marker is responsible for 15% of cases of the CRC4.

The identification of new biomarkers has become fundamental in molecular diagnosis as well as for the definition of CRC treatment, and to enable personalized medicine [9]. Advances are being made in relation to molecular analysis, but there are still challenges in the inclusion of new biomarkers in clinical practice [10]. The main objective of this paper is to review classical, epigenetic and new biomarkers for CRC that have already been published in scientific articles, focusing on their potentialities in clinical application and the challenges to overcome.

Methods and results

Given the importance of knowing the progress of molecular analysis and knowledge about biomarkers, we carried out a literature review on potential CRC-specific biomarkers. Articles published from 2000 to 2021 were searched with the keywords "colorectal cancer biomarkers", "colorectal cancer screening" and "molecular target in colorectal cancer". The search for studies was carried out in databases that store original scientific articles, to expand the number of journals found. **Table 1** summarizes the markers included in the study indicating the dysregulated pathways in CRC and targeted therapies in use or the most current clinical and preclinical trials.

Progress in the diagnosis of colorectal cancer

In 2012, 14.1 million new cases of cancer were detected, while in 2018 there were 18.1 million worldwide. It is estimated that this number could reach 24 million in 2025. Additionally, younger individuals <50 years are recently presenting cases of CRC, which has led to a decrease in the age of onset (45 years) [11]. These growing incidences show the importance of seeking strategies to fight the disease [2]. The increase in CRC cases observed in the last 30 years reinforces the need for early CRC screening [12] as early detection significantly reduces mortality [13].

There are both invasive and non-invasive screening methods. Within the invasive methods there is the colonoscopy that must be performed every 10 years. This is the gold standard test for the detection of CRC and is useful for both diagnosis and possibility of removing the polyps. Non-invasive methods include fecal immunochemical test (FIT) and occult blood in the stool, fecal DNA test, colonography and sigmoidoscopy. These tests are however limited by lack of specificity and low Sensitivity [11]. Hence, the need for the search for new specific and sensitive biomarkers for CRC diagnosis and prognosis.

More recently, tumor markers are identified as certain proteins or genes expressed in the tumor itself. Some of these markers expressed are prognostic in nature and are therefore important in predicting the malignancy of CRC-related tumor. While the predictive markers are often targeted for treatment, others serve diagnostic purposes [14].

Biomarkers in CRC

CEA: Carcinoembryonic antigen (CEA) is a glycoprotein widely used as a marker in clinical practice. In the 1960s, a radioimmunoassay was developed where it was possible to determine the serum levels of CEA in the human digestive system in patients with CRC [15]. CEA is not expressed in the normal adult mucosa, so it is generally not detected in blood tests, except for smokers or when it is affected by CRC and other types of cancer [16]. CEA is a tumor marker that determines the existence, therapeutic evaluation, development, monitoring and prognosis of different types of tumors. Currently, CRC patients are monitored before and after treatment to investigate possible metastasis [17].

BRAF: The BRAF oncogene is a gene that encodes the BRAF protein, also known as serine-threonine kinase, which is a regulator of the MAPK pathway and is related to cell growth [18], representing a prognostic biomarker and a possible marker for therapies in patients with CRC [19]. The most frequent mutations of the BRAF gene for CRC occur at codon 600 [20]. When conversion of valine to glutamic acid occurs, it generates mutations in the BRAF gene, and 5 to 9 out of every 100 people with CRC have this mutation [21]. There is evidence that cancer progression and development are events that occur when there are mutations in KRAS and BRAF [22]. Studies have shown a high methylation rate in mutated BRAF compared to wild-type BRAF. In addition, it showed an impressive association between BRAF mutation and MSI [23]. Mutated BRAF is more

Table 1. List of markers and dysregulated pathways in CRC and targeted therapies in use or clinical trials

Molecular target	Unregulated signaling pathway in colorectal cancer	Targeted therapy in colorectal cancer	Institution/Trial number	Development phase
APC	Adhesion, carcinogenesis, cell cycle	Cetuximab	Huntsman Cancer Institute-*NCT04853043*	Phase II
Beta Catenin	Cell proliferation, migration, invasion and metastases	Celecoxib	University of Alabama at Birmingham Birmingham, Alabama, United States-*NCT00582660*	Phase II
BMP5	Cell growth and migration	-	-	-
CD26	Metastasis, enhanced invasiveness and chemoresistance	-	-	-
CEA	Cell immortalization	Anti-CEA CAR T	Ruijin Hospital, Shangai, China-*NCT04513431*	Early phase I
CEP55	Genetic instability, aberrant mitotic division and tumorigenesis	-	-	-
CpG Island Methylator Phenotype	Genetic instability	Azacitidine, Capecitabine, Oxaliplatin, Azacitidine MTD	MD Anderson Cancer Center Houston, Texas, United States-NCT01193517	Phase I/II
ctDNA	Recurrence and chemoresistance	ctDNA dynamic monitoring	University of Florida Gainesville, Florida, United States-*NCT04786600*	Phase II
CXCR4	Tumor growth, invasion, angiogenesis and metastasis	Plerixafor	Addenbrookes Hospital Cambridge, United Kingdom-*NCT02179970*	Phase I
FOXD3 and FOXF2	Aberrant DNA methylation in GC	-	-	-
GADD45B	DNA damage repair, cell growth, and apoptosis	-	-	-
Galectin-3	Cell proliferation, apoptosis and adhesion	-	-	-
GAPDH	Metastasis	Diagnostic Test: Stool DNA methylation detection	Tri-Service General Hospital, National Defense Medical Center Taipei, Taiwan-*NCT04823793*	Pre-clinical
GNAO1, GRIA4 and KCNA5	Genetic instability	-	-	-
IGF1R	Cell proliferation and metastases	-	-	-
KI67	Cell proliferation	Metformin ER	University of Texas MD Anderson Cancer Center Houston, Texas, United States-*NCT01816659*	Phase I
KRAS Mutated	Invasion, adhesion, cell growth, differentiation, cell proliferation, angiogenesis, cell mortality, metastases, senescence	KRAS mutated: sorafenib and irinotecan	Institut du Cancer de Montpellier Val d'Aurelle, Montpellier, France-NCT01715441	Phase I
KRAS Wide-type	Invasion, adhesion, cell growth, differentiation, cell proliferation, angiogenesis, cell mortality, metastases, senescence	KRAS wide-type: panitumumab	Beth Israel Deaconess Medical Center Boston, Massachusetts, United States-*NCT00842257*	Pahse II
MACC1	Recurrence	-	-	-
miRNA	Apoptosis, cell differentiation and carcinogenesis	Regorafenib	Georgetown University Washington, District of Columbia, United States-*NCT02402036*	Phase II
MSI	Replication errors with an increase mutation rate	PD-1 Antibody, oxaliplatin, capecitabine	Sun Yat-sen University Cancer Center Guangzhou, Guangdong, China-*NCT04301557*	Phase II
NDST4	Progression, tumor metastasis and shorter survival	-	-	-
PI3K	Differentiation, cell proliferation, angiogenesis, cell mortality, metastases, senescence	Panitumumab, FOLFIRI	Spanish Cooperative Group for Digestive Tumour Therapy, Madrid, Spain-*NCT01704703*	Phase II
PPARG	Tumor initiation	-	-	-

PTEN	Cell division and apoptosis	Akt Inhibitor MK2206	M D Anderson Cancer Center Houston, Texas, United States-*NCT01802320*	Phase II
SARDH		-	-	-
SDF1	Metastases	Bevacizumab and FOLFIRI	A.O. Treviglio-Caravaggio, P.le Ospedale n1 Treviglio, Bergamo, Italy, 24047-*NCT01853813*	Phase II
TBL1XR1	Cell proliferation and metastases	-	-	-
Tetraspanin Co029	Metastases	-	-	-
TGF-β	Immune evasion, progressin, invasion and metastases	Dendritic Cell Vaccine	Hospital Clínic Barcelona Barcelona, Spain-*NCT01413295*	Phase II
TP53	Cell cycle, senescence, apoptosis and metabolism to a variety of stress signals	Cyclophosphamide	Haukeland University Hospital Bergen, Norway-*NCT03149679*	Phase II

prevalent in women and people over 70, located mainly in the right colon, and can affect any part of the colon and rectum. It is recommended to test this mutation in stage IV patients to better target treatment [21].

KRAS: The KRAS oncogene encodes small proteins that bind to guanine triphosphate and is a GTPase transducer. KRAS proteins, also called p21, are located on the cell membrane [22]. KRAS is temporarily activated at the time of signal transduction [23]. Mutations in this gene occur in codons 12 (82-87%) linked to the mucinous CRC and 13 (13%-18%) linked to the non-mucinous CRC, which is more aggressive with a greater occurrence of metastases [24]. When there are mutations in the KRAS gene, they lead to continuous activation of the signal transduction pathway and, as a result, transformation and ineffectiveness of anti-EGFR antibody therapy occurs [25]. Studies have shown that the KRAS mutation targets anti-EGFR therapy, acting as a negative predictive marker, since, for patients with KRAS-WT CRC, anti-EGFR therapy significantly improved overall survival and progression-free survival [26]. Another study showed that patients with KRAS-WT have a better response to treatment when cetuximab was added, compared to patients who did not receive this drug [27]. Patients with mutated KRAS obtained similar results with treatment using FOLFOX alone or combined with cetuximab. In this way, the mutated KRAS can be considered as a predictor in the direction of the best treatment strategies [22].

TP53: The tumor suppressor gene P53 or TP53 encodes a cytoplasmic protein of temporary expression that regulates the cell cycle, apoptosis, senescence and DNA repair, acting as a tumor suppressor. TP53 has a fundamental role in the conservation of stability and prevention of genome mutation. When the gene is mutated, it produces a permanent protein that interferes with the DNA repair system [1]. With the constant expression of the protein, it can lead to the recognition of the immune system and the production of antibodies against TP53, but studies have shown that the dosage of these antibodies in peripheral serum is inconstant and sensitivity is less than 30% [28]. TP53 mutations are seen in approximately 60% of colorectal tumors and may lead to a transition from adenoma to CRC carcinoma. Thus, the identification of this mutation represents a predictive marker in patients with CRC and shows a worse prognosis with short survival [29].

MSI: Microsatellites are short series repeats of DNA sequences present throughout the human genome. Microsatellite instability (MSI) is caused by a deficiency of the DNA mismatch repair system (MMR), especially by inactivation of the four MMR genes (MSH2, MLH1, MSH6, and PMS2) that leads to a failure to correct insertion or exclusion of repetition during DNA replication [30]. It is a hypermutable phenotype. MSI is observed in approximately 15% of all colorectal tumors [31]. CRC with microsatellite instability are mucinous, have poor cell differentiation and strong lymphocyte infiltration, most often in the right colon [32]. Surprisingly, patients with MSI have a better prognosis compared to patients without MSI. This way, it can be considered a potential prognostic marker for CRC patients and MSI status can be assessed on a panel of five specific markers (BAT25, BAT26, D2S123, D5S346 and D17S2720) by polymerase chain reaction (PCR) assay [33].

Approximately, 15% of all CRC patients show MSI, where 75-80% is characterized by methylation acquired in the MLH1 gene [30]. Patients show germinal mutations in 2% to 3% of cases being in one of the MMR genes. MSI is a potential marker for testing, as well as in pointing adjuvant therapeutic choices [34].

CpG island methylator phenotype: Epigenetics means modifications of phenotype or gene expression that do not imply changes in DNA sequence [35]. In this sense, one of the most studied events is DNA methylation, which is one of the CRC biomarkers playing a fundamental role in altering the gene expression observed in carcinogenesis [36]. The CpG island methylator phenotype is located in tumor suppressor genes and the mechanism is gene inactivation. Genetic transcription is inactivated due to changes in chromatin structure [37]. CpG island methylator is capable of inactivating a number of cell pathways including DNA repair system (hMLH1, MGMT), apoptosis (DAPK), angiogenesis inhibition (THBS1), metastasis suppression (TIMP3), cell cycle regulation (p14 ARF, p15 INK 4b, p16 INK4a), and cell adhesion (CDH1, CDH13). Epigenetic alterations of methylated genes can be used as biomarkers [38, 39]. Measurement of aberrant methylation of specific genes in blood samples has been reported to be a potential CRC prognostic biomarker. For example, methylated Vimentin (mVim) is a methylation biomarker currently commercially available [40] for PCR and is able to evaluate the integrity of vimentin and methylated DNA with high sensitivity (83%) and specificity (82%) [41].

APC: Adenomatous Polyposis Coli (APC) is a suppressor gene identified in familial adenomatous polyposis (FAP). This epigenetic change of a mutated APC is responsible for most cases of sporadic CRC, where 70% to 80% of patients have this mutation [42]. APC acts as antagonist of the gene WNT signaling pathway. APC regulates several cellular activities, such as migration, adhesion, transcriptional activation and apoptosis [43]. The evaluation of the association of the three APC polymorphisms (D1822V, E1317Q and I1307K) in the development of CRC, and it was observed that carriers of the E1317Q variant had a low association in the risk of CRC, while for I1307K showed an increase at risk of CRC compared to wild type I1307Q [44]. However, it was observed that there is no association between APC promoter methylation and overall survival of patients with CRC [45]. For patients with APC mutation and high miR-21 expression in advanced CRC, there is worse overall survival. APC mutation and high miR-21 expression can be used in clinical practice as CRC predictors [43]. Different authors understand that hypermethylated APC is an important biomarker in the early diagnosis of CRC, as well as a possible treatment target, being personalized and directed to the mutation involved [42].

miRNA: microRNAs (miRNAs) are small noncoding RNA sequences that can control gene expression at post-transcriptional level [46]. These miRNAs have been found to play key roles in cancer biology and are involved in different cellular processes such as proliferation, apoptosis, differentiation, invasion and metastasis [47]. There is evidence that miRNA-gene abnormalities are involved in carcinogenesis and tumor progression. Thus, miRNAs are important biomarkers for early cancer detection, stratification prognosis, and therapy targeting [48]. One advantage for working with miRNAs is that they can be isolated on different types of samples, including blood, saliva and feces. One human study identified a set of 19 different miRNA expressions. Among these, up-regulations (hsa-miR183-5p and hsa-miR-21-5p) and down-regulations (hsa-miR-195-5p and hsa-miR-497-5p) are associated with CRC by interaction with MMR and transformation of growth factor β, WNT, RAS, MAPK, and PI3K signaling pathways [49, 50].

PI3K: Phosphatidylinositide-3-kinases (PI3K) are a family of enzymes involved in regulating cell functions such as growth, proliferation, differentiation, mobility, survival and intracellular traffic [51]. When expressed, it may present changes which are involved in the development of cancer. PI3K expression is one of the factors for RAS mediation that is involved with tumor proliferation, transformation and progression [52]. PI3K changes are seen in human cancer and mutations in the PIK3CA gene (gene encoding the PI3K p110alpha catalytic subunit) have been described in different cancers, including CRC [53]. In addition, the PIK3CA mutation is also linked to a significant reduction in patient survival. Mutations in PIK3CA (exon 9 and exon

20) are responsible for triggering different biological effects and promoting carcinogenesis [54, 55]. Studies have shown that PIK3CA represents a prognostic marker for survival and treatment targeting [56].

PTEN: The phosphatase and tensin homologue (PTEN) is a tumor suppressor gene that regulates the cell proliferation signaling pathway initiated by PI3K found in almost all tissues of the human body [57]. This gene encodes an enzyme that regulates cell division, preventing cells from growing and dividing uncontrollably and induces apoptosis. It is also involved in migration, cell adhesion and angiogenesis. In addition, it contributes to the DNA repair process [58]. PTEN mutations are associated with advanced and metastatic tumors. Mutations in this gene reduce or eliminate the tumor suppressor function of the PTEN enzyme and this can lead to uncontrolled cell division, leading to the appearance of tumors. It has been observed that in high-grade CRC, hypermethylation of the PTEN promoter occurs very frequently. Mutated PTEN may represent a favorable predictive marker in patients with KRAS-WT treated with anti-EGFR [57].

NDST4: The NDST4 is part of the N-deacetylase/N-sulfotransferase (heparan glucosaminyl) [4] (NDSTs) family that has four isoforms (NDST1 and NDST2, NDST3 and NDST4). NDST4 is a tumor suppressor gene which informs the production of an essential bifunctional enzyme that has the function of carrying out the biosynthesis of heparan sulfate (HS) in the main protein to form heparan sulfate proteoglycans (HSPGs) [59]. The expression of NDSTs is not identified in the human colon, using the RT-PCR technique it was possible to detect the expression of the four NDSTs in the normal colonic mucosa, but only the expression of NDST4 was negatively regulated in most CRC tumors [60]. Studies have shown that most cases of CRC showed a significant decrease in the expression of NDST4 compared to normal colonic mucosa. Decreased expression can lead to loss of NDST4 function, leading to an increase in the invasive capacity of cancer cells, changes in the interaction between cell adhesion receptors and their ligands. The non-expression of NDST4 may represent an adverse prognostic biomarker for patients with CRC [59, 60].

IGF1R: The insulin-like growth factor receptor (IGF1R) is a transmembrane glycoprotein and its activation is involved in cell proliferation, differentiation, angiogenesis and apoptosis [61]. The IGF system is composed of the ligands IGF-1, IGF-2, and by the IGF-1 (IGF-1R), IGF-2 (IGF-2R) receptors [62]. The biological functions of IGF-1 and IGF-2 are mediated mainly by IGF-1R, a transmembrane tyrosine kinase receptor that has more affinity for IGF-1 than IGF-2 [63]. IGF-1 expression is an important marker for the development of CRC and has value in the prognosis [63]. Overexpression of IGF-1 is related to an increased risk of developing CRC [64]. In addition, the expression of IGF-1 stimulates different cell cascades, responsible for the progression of the cell cycle and inhibition of apoptosis, favoring tumor progression. It was observed that metastatic CRC expresses elevated levels of IGF-1R compared to primary cancer [65]. In view of this, IGF1R became the target of treatments, especially with the use of monoclonal antibodies or tyrosine kinase inhibitors [66].

BMP5: Bone morphogenetic protein 5 (BMP5) is a protein encoded by the BMP5 gene in humans. This protein is a member of the TGFβ superfamily. Bone morphogenetic proteins are known for their ability to induce bone and cartilage development [67]. BMP5 has been identified as a new CRC tumor suppressor gene, however, the protein plays an inportant role in the initiation and development of tumors in the digestive tract. RNA sequencing revealed that BMP5 was involved in the Jak-Stat signaling pathway, suggesting that BMP5 loss plays a vital role in the onset and progression of CRC [68]. Studies have shown that BMP5 gene is mutated in 30.4% of CRC samples with 8 mutants identified. BMP5 gene suppresses migration and invasion, and modulates epithelial-mesenchymal transition in CRC cells. Its function however, varies among different types of tumors [69].

Tetraspanin Co029: Approximately 33 proteins are found in the family of tetraspanins in mammalian cells. These proteins have different functions and four transmembrane hydrophobic domains which are expressed in the cytoplasmic and intracellular membranes [70]. Within this family of proteins, Tetraspanin CO029 (TspanCO029) has been considered a poten-

tial tumor biomarker, as it exerts a pro-invasive function, controlling cell-to-cell and cell-to-matrix interactions through its association with other cell adhesion proteins [71]. TspanC0029 is expressed in the colon and stomach epithelia, and has already been identified in different tumors such as colon, liver, prostate, ovary and in cervical cancer [72]. It may present overexpression in human carcinomas, including CRC, where it plays a role in progression and promigratory function in epithelial cancer during interaction with E-cadherin/p120-catenin membrane complex [73]. TspanC0029 mediates the loss of intercellular connections between malignant epithelial cells, important in the development of metastasis, functions as a regulator of cell-matrix and cell-cell adhesion and has a worse prognosis when overexpressed, thus, it can be a potential marker for CRC [74].

SARDH: Sarcosine dimethylglycine (SARDH) has also been associated with tumorigenesis [75] and has been recently identified as a key metabolite produced in cancer and metastatic disease [76]. Studies show that in prostate cancer cell lines, SARDH metabolism regulates invasiveness and could be a potential therapeutic target for cancer treatment as it has been shown to be related to tumor progression and metastatic process [77]. SARDH gene has been identified as a CRC tumor suppressor gene through exome sequencing and has been reported as a potential oncometabolite more than non-proteinogenic amino acid. SARDH expression influences different cancer signaling pathways, especially the 2 chemokine-related genes, CXCL1 and CCL20, and may be associated with the methylation of both genes [75-78].

CEP55: Centrosomal protein of 55 kDa (CEP55) is an essential component of the CEP family and has been identified as a prognostic marker for multiple types of cancer [79] and serves a vital function in mitotic exit and cytokinesis [80]. The wild-type TP53, a well-known tumor suppressor gene, is able to inhibit CEP55 through negative regulation of Polo-like kinase I (PLK1), an important regulator of mitotic process. Neoplasms with TP53 mutations therefore generally elevate the levels of CEP55 and directly impact cell transformation, proliferation, invasion and migration [81]. Overexpression of CEP55 gene has been observed in different types of solid tumors [82], including colon cancer, bladder cancer, hepatocellular carcinoma, gastric cancer, esophageal adenocarcinoma, and ovarian carcinoma [83]. The overexpression of CEP55 gene can also increase the cell cycle transition, the number of multinucleated cells, defects in cytokinesis and ultimately lead to tumorigenesis [83].

FOXD3 and FOXF2: The genes FOXD3 and FOXF2 have been described in few studies in colon or gastric cancer, despite having been identified as tumor suppressors [1-84]. The low expression of these two genes is associated with a significant increase in cell proliferation in colon cancer, thus, affecting the EGFR signaling pathway [85]. Methylation in the promoter region of FOXF2 was previously associated with shorter survival in gastric cancer Patients [86]. In addition, the methylation in both genes could be responsible for their down-regulation, thus disrupting their interaction with other proteins [1].

GNAO1, GRIA4 and KCNA5: GNAO1, GRIA4 and KCNA5 genes have been poorly studied, especially in cancer-related studies [1]. The GNAO1 gene was found to be overexpressed in patients with gastric cancer, whereas in CRC, GNAO1 was negatively regulated [1]. There is an association between high GNAO1 gene expression and tumor size and differentiation, TNM stage and poor prognosis. The low expression of GNAO1 leads to reduced cell proliferation and promotes apoptosis [1]. The GRIA4 gene encodes the subunit of the same name of the AMPA tetrameric receptor complex. Its main function is to act as a cation channel in the central nervous system as seen synaptic communication. Although the knockdown of GRIA4 is related to the dysregulation of genes related to invasion and metastasis, its function in cancer has not yet been fully elucidated. In addition, it has been found that GRIA4-related CGI is highly methylated in CRC and adenomas [87]. The KCNA5 gene encodes a protein involved in tumor cell proliferation that was found in Ewing's sarcoma, but its role in CRC is still unknown [89]. However, one study observed that KCNA5 methylation could be responsible for stable mutation of this gene in CRC, thus, contributing to the proliferation of tumor cells [88]. DYRK2 is a dual-specific tyrosine-regulat-

ed kinase with the ability to induce apoptosis through p53 and inhibit cell cycle through c-Jun/c-Myc. Lower DYRK2 expression in liver metastases correlated with poorer survival rate and possible consideration of DYRK2 as a predictive marker of liver metastases from colorectal cancer [88, 89].

GADD45B: The Growth Arrest and DNA Damage-Inducible (GADD45) family participate in many cellular processes associated with cell growth regulation and stress signaling pathway [90] and are essential mediators of cell survival in cancer cells with implications for cancer chemotherapy and novel drug Discovery [92]. GADD45B shares the common functions of the GADD45 family, which is associated with DNA damage repair, cell growth, apoptosis, and antitumor immune responses [91]. Wang et al. (2012), showed the implicated carcinogenesis function and potential prognostic value of GADD45B for CRC. However, the role of GADD45B expression in the prognostic value and chemotherapy-related predictive significance in CRC remains uncertain [92]. Studies have shown that GADD45B overexpression is associated with worse prognosis for patients with CRC, and it was gradually over-regulated in normal mucosa, primary tumors and liver. GADD45B expression may benefit from adjuvant chemotherapy treatment [93].

TGF-beta: The transforming growth factor-β (TGF-β) signaling pathway plays a key role in controlling tissue development, proliferation, differentiation, apoptosis and homeostasis. Although TGF-β signaling inhibits epithelial growth in normal tissues, it can promote the progression of tumor cells in tissues with advanced cancer [94]. TGF-β has a central role in inhibiting cell proliferation and also modulates processes such as cell invasion, immune regulation, and microenvironment modification. Mutations in the TGF-β type II receptor (TGFBR2) are estimated to occur in approximately 30% of CRC [95]. In this regard, several studies demonstrated TGF-β plays a role in the development of CRC and in its metastatic process [96]. Additionally, the TGF-β germline alterations can confer an increased risk of sporadic colon carcinomas. These mutations have been documented in both carcinomas with microsatellite instability (MSI) and carcinomas with chromosomal instability [97]. As many as 80% of CRC cell lines, depending on their genomic subtype, have a defect in the TGF-β signaling pathway and escape TGF-β-induced growth arrest. Additional mutations have been described in the endoglin gene (ENG), a co-receptor for TGF-β family receptors, but a causative role has not been conclusively proven [98]. Studies of mutational frequencies have shown that TGF-β pathway mutations occur in approximately one-third of tumors [99] which is less than that observed in cell line studies [100].

TBL1XR1: Transducin (b)-like 1 X-linked receptor 1 (TBL1XR1) can regulate the genetic activation of various transcription factors [101] and has been reported to be involved in malignancy development [101]. For patients in stage IV of CRC, high levels of TBL1XR1 expression in liver metastases were identified, indicating overall poor survival [102]. In addition, high TBL1XR1 expressions were predicted for liver metastasis in patients with early stage CRC [103].

SDF1: Stromal cell-derived factor-1 (SDF-1) is a small protein (8-14 kDa) that is expressed as six isoforms [104]. The expression of SDF-1β was associated with the presence of metastases while the expression of SDF-1γ was significantly associated with tumor size [105]. All the six isoforms of SDF-1 were expressed in CRC tissues, and their expressions were found to be associated with metastases, and therefore, were suggested to be possible tumor markers for local tumor progression [106]. High SDF-1 expression in CRC and liver metastasis correlated with advanced clinical stage and lymphatic invasion. Therefore, SDF-1 seems to have indirect prognostic significance, also suggesting that this protein may play a role in promoting the metastatic process [107].

Galectin-3: Galectin-3 is a protein involved in cell proliferation, adhesion, differentiation, angiogenesis, and apoptosis in normal tissues [108]. Recent studies indicate that galectin-3 plays a role in tumor cell transformation and metastasis [109]. Elevated expression of galectin-3 was observed in tissues of multiple solid malignant tumors, whereas low or no expression was observed in normal tissues [110]. Liu and colaborators showed that the risk of CRC progression was significantly higher in patients with positive galectin-3 expression than that of patients with negative galectin-3 expression [111].

Ki67: Ki67 is a nuclear protein expressed in all phases of the cell cycle (G1, S, G2 and M), which, however, is absent in the G0 phase (non-cyclers). The precise function of the Ki67 antigen is still poorly understood, but it has been suggested that this protein is possibly associated with the nucleolus and fibrillar components, and still appears to play an essential role in the synthesis of ribosomes during cell division [112]. Studies have shown that the immunohistochemical expression of the Ki67 protein correlates with the proliferative potential of solid malignant tumors [113]. Studies also indicate that the Ki67 expression index predicts the progression of cancer including CRC, however, it is not used as a predictor of prognosis or therapies when its evaluation does not include the correlation with other markers, since it does not indicate a precise stage and it is not characterized as a treatment marker [114]. Ki67 that correlates with high or low histological grade of numerous neoplasms, is associated with high rates of cell proliferation and favors a shorter disease-free survival time. It has been used as an important index in the assessment of breast cancer prognosis [115]. It has a high level of expression in malignant cells, however, its detection in normal cells is minimal. Its analysis combined with other molecular markers may make it an important biomarker for diagnosis and therapeutic use. Expression of this protein has already been identified in primary tumor tissue and can be used as a marker of liver metastasis [116].

CXCR4: The C-X-C chemokine receptor type 4 (CXCR4) is the chemokine receptor most commonly expressed in tumor growth and metastasis in various gastrointestinal cancers, in particular colorectal, pancreatic, hepatocellular, gastric, and esophageal cancers [117, 118]. The expression of CXCR4 in primary tumor cells correlates with survival, metastasis, and recurrence [119]. Chao and collaborators demonstrated that CXCR4 allows invasion into lymph nodes leading to metastasis. In addition, the chemotactic function of CXCR4 is an effective target for the treatment of tumor metastasis [120]. In CRC, lymph node involvement is a prognostic parameter of clinical importance [121]. A high expression of CXCR4, both nuclear and cytoplasmic, correlated with lymphatic invasion, which can promote liver metastasis from primary colon and rectal tumors [124], being the predominant cause of mortality due to the disease [123]. Assis 2020 demonstrated that e-cadherin, CD133 and Ki67 are regulated positively by CXCR4 in the CRC and also, the disease condition increases their expressions [123].

CD26: The different functions of CD26 are related to processes involved in tumor progression such as migration, adhesion, invasion, apoptosis and immunomodulation [124]. It has also recently been described as a cancer stem cell marker [125]. CD26 interacts with type I and III collagens and fibronectin and results in facilitating metastasis. Based on its ability to regulate biological molecules through its enzymatic activity, CD26 can act as a tumor suppressor or activator [126]. A recent study demonstrated the presence of CD26 cells in portal veins after tumor induction in the murine cecal wall showing liver metastasis [127]. 5-Fluorouracil, oxaliplatin and SN-38 (the active metabolite of irinotecan), as well as cisplatin, methotrexate and vinblastine, elevate CD26 levels in HT-29, T84, HRT-18, SW480 and SW620 CRC cell lines [128]. Studies have shown that patients with metastatic colorectal carcinoma have high levels of this enzyme in the serum when compared to healthy individuals [129]. Assis 2020 demonstrated an intense relationship between the CD26 and GAPDH transcripts in CRC and increasing the risk of tumor progression [123].

GAPDH: Several molecules regulate mRNA levels and affect cancer-related functions of GAPDH (proliferation, tumor formation and chemoresistance) [133]. Although GAPDH is expressed in most types of cells with enzymatic function, it is often used as an endogenous control molecule in the analysis of relative quantification in gene expression. In CRC, it has an intense association with the CD26 gene which suggests a high risk of malignancy [123].

β-catenin: β-catenin is a protein involved in the regulation and coordination of cell-cell adhesion and gene transcription [131]. It helps to maintain the stemness of normal intestinal cells. Furthermore, high-level of β-catenin expression in the cytoplasm and nuclear localization always induces tumorigenic traits and promotes cancer cell proliferation and survival [132]. In more than half of all cancer cases, including colorectal carcinoma, β-catenin accu-

mulates within the nucleus or cytoplasm [133, 134]. Indeed, nuclear accumulation of β-catenin can be observed in 80% of colorectal carcinoma138. Reduction or loss of β-catenin expression in the CRC has been associated with liver metástases [135]. β-Catenin has been shown to upregulate urokinase plasminogen activator (uPA) expression in colorectal tumors to promote invasion, metastasis and dormancy [136].

PPARG: Peroxisome-proliferator activated receptors (PPARs) are ligand-dependent transcription factors belonging to the nuclear receptor superfamily. PPARγ plays critical roles in lipid storage, glucose metabolism, energy homeostasis, adipocyte differentiation, inflammation, and cancer [137]. PPARG is related to β-catenin, an important molecule in CRC carcinogenesis [138, 139]. Studies have shown that PPARG plays an important role in regulating cell growth in CRC [140, 141]. PPARD promotes tumorigenesis and elevated PPARD and COX-2 expression in tumor tissues has been correlated with worse prognosis in patients with CRC [142]. Reduction or loss of PPARG expression in the primary tumor was associated with liver metastases [143].

MACC1: MACC1 is a key regulator of hepatocyte growth factor receptor (HGFR) involved in cell growth, epithelial-mesenchymal transition, angiogenesis, motility, invasiveness and metastasis. The gradual increase in the expression of MACC1 in the early stages of CRC contributes to early invasive growth. In addition, the study suggested that the high expression of MACC1 associated with epithelial-mesenchymal transition (EMT) may be related to distant metastasis [144]. High MACC1 expression at the primary tumor site is associated with liver metastasis [145].

EMT and CSC markers: The EMT is considered a critical mechanism of metastasis [146] due to acquisition of mesenchymal properties, such as increased motility [147]. This involves upregulation of molecules that induce EMT and mesenchymal markers, as well as downregulation of epithelial markers [148]. In CRC, EMT is associated with an invasive or metastatic phenotype [149]. Another group of markers constantly associated with EMTs are Cancer Stem Cells (CSCs) due to the characteristics of self-renewal, infinite proliferation and the potential for multidirectional [150]. A recent study revealed a positive relationship between CXCR4 and CD26 expressions with vimentin, e-cadherin and CD133, EMT and CSC markers, respectively. In addition, colorectal tumors show greater expression of vimentin, especially in the left site [123-151].

ctDNA: Circulating tumour DNA (ctDNA) is a promising biomarker that has received significant attention in recent years. ctDNA are DNA fragments that are released by dying cancer cells into the bloodstream and in theory should contain genetic and epigenetic changes identical to the cancer cells they originated from [152]. Several studies have described the potential uses of circulating tumour DNA (ctDNA) in the care of patients with colorectal cancer [153]. A recent study showed a panel focused on four key areas in which ctDNA has the potential to change clinical practice, including the detection of minimal residual disease, the management of patients with rectal cancer, monitoring responses to therapy, and tracking clonal dynamics in response to targeted therapies and other systemic treatments [154]. Flamini and collaborators showed that the average ctDNA concentration was 25-50 times higher in the plasma of patients with CRC than in the plasma of healthy controls [155]. These results were contrasted with rectal tumors and although the concentration of ctDNA in this case was lower, it still had a value above healthy controls [156, 157].

Discussion

CRC-associated biomarkers: prospects and operational challenges

The biomarkers widely used in the diagnosis of CRC are CEA and APC, however, both are inaccurate and insufficient. CEA has been studied since 1965, when it was identified in adenocarcinoma of the colon and rectum, but absent in normal adult colonic tissue. Its reference values were 3.5 ng/mL and 7 ng/mL in non-smokers and smokers, respectively. Theoretically, in the presence of malignant neoplasia, high levels of CEA are detected in approximately 85% of cases of metastatic colorectal carcinoma. This is however controversial as patients who present invasive tumors also demonstrate low concentration of CEA. Patients undergoing surgery are followed-up for at least five years to

monitor the signs of the disease recurrence [158] using CEA profile as the indicator. Once the increase in CEA level is identified, complementary examinations, such as chest, abdomen and pelvis radiographs, are used to confirm the recurrence. Increased levels in CEA however can also be identified for other types of diseases. Despite being widely used in clinical practice, tests with CEA require greater specificity and sensitivity to identify the disease in early stages [159].

Patients with FAP (familial adenomatous polyposis) may present the mutation in the APC gene. Two groups are identified, those who have inherited the changes in the APC, which require specific follow-up and intervention, and those who have not. Pre-symptomatic genetic diagnosis in at-risk individuals is possible by directly detecting the APC mutation in a family member. The identification of individuals without mutations in the APC removes the need for annual screening [160]. In individuals positive to mutations in APC, annual flexible sigmoidoscopy should be started at age 10. Only 15% of carriers of mutations in APC will develop intestinal polyps at age 10; at 20 years of age, approximately 75% of carriers already have polyposis; at 30 years of age 100% of patients with FAP mutation and without intervention will develop colorectal cancer in the fourth decade of their lives. There is no consensus in literature on screening for other pathological conditions associated with FAP. Similar to CEA, there is a need for improved specificity and sensitivity of APC in order to identify patients with CRC [161].

Molecular markers as CRC therapeutic targets

In CRC patients, KRAS mutations are present in 45% of metastatic tumors [162] and approximately 15-37% of early-stage tumors. Compared with a single chemotherapy regimen, adding anti-EGFR biological drugs, as cetuximab and panitumumab, to the standard chemotherapy regimen can improve survival and reduce the risk of the disease progression [163]. Currently, there is no enough evidence to ascertain that KRAS wild-type/BRAF wild-type tumors and KRAS wild-type/BRAF mutant metastatic tumors respond differently to anti-EGFR treatment [164]. Therefore, data on the response of BRAF mutant CRC to EGFR-targeted drugs are still contradictory [165].

Another potential molecular marker for therapy targeting is the cancer suppressor gene P53 (p53) which plays a key role in coping with stress [166]. Failure of the TP53 pathway is one of the hallmarks of cancer cells, leading to uncontrolled cell growth and proliferation [167]. These changes play an important role in the occurrence and development of CRC, and may represent indicators of response to chemotherapy, radiation therapy, or a combination of both [168]. The mutation status of TP53 is also related to the positive response of patients with stage III CRC to adjuvant 5-fluorouracil therapy. In metastatic CRC, patients with TP53 mutations who received adjuvant therapy have shown poor survival outcomes [169]. In order to determine the role of TP53 as a potential prognosis biomarker in CRC, more research is needed [170].

Another biomarker of great clinical importance is the MSI status. Microsatellites are repetitive sequences of short DNA sequences throughout the genome. MSI status is caused by defects in the DNA mismatch repair (MMR) system, usually due to the inactivation of the four MMR genes (MSH2, MLH1, MSH6 and PMS2) [31].

MSI tumors can be observed in approximately 15% of all CRC patients [171]. Among 15% of people, 3% are related to Lynch syndrome. The other 12% of MSI tumors are caused by occasional hypermethylation of the MLH1 gene promoter. It is worth noting that the prevalence of MSI is related to the stage. In stage II/III CRC, MSI is as high as 15%, while in stage IV CRC only 4%-5% are MSI [172]. The instability of microsatellites is considered to be a possible marker for overall survival, disease-free, and sensitivity to 5-fluorouracil (5-FU) treatment. Recent studies have shown that microsatellite instability is a sign of good response to 5-FU combined with other drugs, especially in the presence of large deletions in HSP110 [173]. MSI can also be related to the potential resistance of tumor cells to methylation agents, such as temozolomide, dacarbazine, procarbazine, and potential sensitivity to methylating agents like nitrosoureas [174]. Although the implications of MSI in the metastatic event are still not well understood, there is an interest in the study and implementation of MSI tests for providing prognostic data and assisting in therapeutic choices [34].

Another factor that has been widely studied is the evident alterations in the composition of the intestinal microbiota in precancerous lesions of the large intestine and in CRC [175]. Several current studies demonstrate the susceptibility and progression of the neoplasm associated with dysbiosis, especially by modulating inflammation and DNA damage, producing metabolites involved in tumor progression or suppression [176]. The proposed mechanisms by which intestinal microbiota dysbiosis could participate in colorectal carcinogenesis are the impairment of intestinal epithelial barrier function, the triggering of pro-inflammatory responses, the biosynthesis of genotoxins that may interfere with cell cycle regulation and the production of toxic metabolites by pathogens [177]. Sánchez-Alcoholado et al. (2020) demonstrated that dietary supplementation with polyphenols and probiotics can be used as a therapeutic approach to reduce the risk of CRC in a primary prevention setting, and can also be used as an adjunct to conventional treatment for CRC, since the gut microbiota can modulate and enhance the response to cancer therapy and reduce toxicity [178].

Studies realized in recent years have shown substantial progress that revealed that aberrant expressions of miRNAs play an important role in the initiation and progression of CRC with a direct impact on the diagnosis, prognosis and therapeutic response of the disease [179]. On the other hand, several studies have indicated that abnormally expressed miRNAs in CRC are often associated with molecular signatures that generally correlate with CRC progression or metastasis [180]. These data support the hypothesis of new opportunities and tools to understand the disease mechanism based on miRNAs and their classification as a potential early detection biomarker and therapy target in colorectal tumors.

The limitations of the existing CRC biomarkers stress the need for discovery of new drug targets for CRC. Prognosis biomarkers remain great tools for identifying response to a specific treatment and guiding decision-making process in the choice of therapeutics [181]. The discovery of new predictive biomarkers becomes increasingly critical for the development of treatment for patients with colorectal cancer and represents a powerful strategy for the discovery and implementation of personalized methods [182].

A large number of molecular targets are commonly identified as prognostic markers for CRC. In this way, combining a panel of several approaches presents a promising alternative tool for medical practices. Large-scale clinical trials are needed to validate the prognostic value of these biomarkers in clinical use [183].

Evidence suggests that liquid biopsy has prognostic and predictive values in the treatment of CRC [13]. A range of prognostic signatures of potential CRC genes have been identified after several studies of global gene expression carried out with microarray technologies and ultra-sensitive transcript sequencing [184]. Advances in technology have led to a greater understanding of the mechanisms underlying colorectal carcinogenesis, resulting in the identification of several molecular targets that can be used as prognostic biomarkers. Those biomarkers that can be used to predict the prognosis are significantly associated with tumorigenesis and progression of CRC and despite the limitation in routine use, it would make the approach more assertive and, consequently, decrease death rates due to the disease [74, 123].

It is therefore imperative that clinicians and researchers look for new biomarkers that are sufficiently sensitive and highly efficient for the diagnosis and prognosis of CRC, and which could serve as therapeutic targets of the disease.

Conclusion

Colorectal cancer is an important public health problem and, hopefully, there is an increasing number of researches aimed at identifying new biomarkers that can be useful for CRC diagnosis, prognosis and treatment. Unfortunately, the real clinical application of these biomarkers is still far from ideal. Although several studies focused on the identification of new targets their application as new strategies to achieve personalized medicine that is applicable to a larger number of people is not yet happening.

Disclosure of conflict of interest

None.

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