

Tumor Markers of Colorectal Carcinoma: New Perspectives

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Abstract Colorectal carcinoma (CRC) represents one of the most commonly diagnosed malignant tumors worldwide. In spite of the continuous progress in diagnostic and therapeutic tools, CRC significantly contributes to cancer-related deaths all over the world. Early detection and treatment of patients with CRC may have a positive impact on prognosis, as the delay of diagnosis carries the risk of poor prognosis. Recently, new tumor biomarkers were discovered which can alone or together with the existing tests recognize either the predisposing factors or the early stages of CRC. Also, these biomarkers have the potential to determine the appropriate treatment algorithms by selecting the proper chemotherapeutic agents that can induce remission in a broad spectrum of patients. This review throws light on the tumor markers of CRC and their role in early diagnosis and determination of prognosis and the suitable lines of management of patients with CRC.

Keywords: colorectal cancer, tumor markers, diagnosis, management

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1. Introduction

Colorectal cancer (CRC) is one of the common malignancies that may affect one million male and female patients annually and causes more than half a million deaths worldwide [1]. In Europe in 2010, CRC was considered to be the third most widespread malignant tumor in both males and females [2]. The recently available data report that the overall five-year relative survival rate may reach about 65%, but it varies depending on the stage of the tumor [3].

The National Institute of Health defines a biomarker as a biological molecule found in blood, body fluids, or tissues that represents a sign of abnormal or normal process, or of a condition or a disease [4]. Some researchers considered a tumor code marker as an alternative to biomarkers [5]. It refers to substances representing biological structures, which can be attributed to the development of normal cells or carcinogenesis at different cell development stages e.g., tumor-associated antigens (TAAs) which are the large group of clinically significant markers [6]. Consequently, TAAs concentrations typically correlate with the number or mass of neoplastic cells [7].

Recently, the researchers are constantly searching for new diagnostic methods that would be able to early detect CRC. Neoplastic markers can be able to determine a quantity of glycoproteins and glycolipids in blood of patients with CRC [8]. The neoplastic markers have participated in diagnosis of gastrointestinal neoplasms,

mainly in early post-operative detection of repetition of neoplastic disease and in evaluation of the efficacy of surgery [9]. As demonstrated in Figure 1, various biomarkers are commonly used in the clinical practice for proper diagnosis, monitoring of therapy, and prediction of prognosis of CRC.

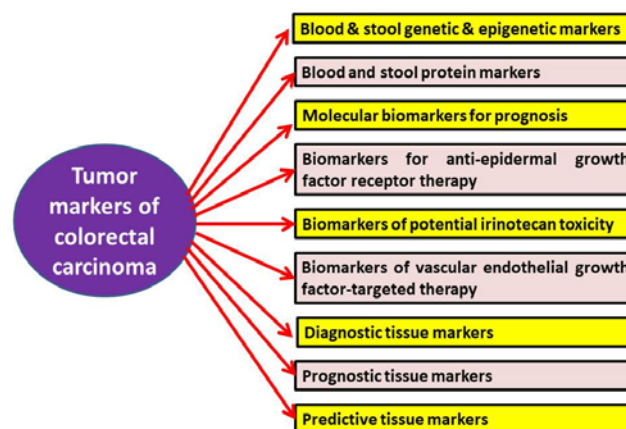


Figure 1. Tumor markers of colorectal carcinoma

According to the European Group on Tumor Markers (EGTM), carcinoembryonic antigen (CEA) is the main marker used in detection of CRC [6]. However, approximately 10-15 % of patients are unable to produce CEA or could secrete a little amount. Therefore, the use of cancer antigen (CA) 19-9 as a tumor marker in diagnosis of CRC has been proposed [10]. This review sheds light on the tumor markers of CRC and their role in early

diagnosis and determination of prognosis and the suitable lines of management of patients with CRC with special emphasis on the most commonly used biomarkers.

2. Blood and Stool Genetic and Epigenetic Markers

Many authors had investigated molecular noninvasive examination tests for early detection of CRC. DNA, RNA and other molecules derived by tumor in feces, as well as their concentrations in blood have been elaborated extensively [11]. Colorectal carcinogenesis is described by genetic and epigenetic factors that transform normal cells into cancer cells [12]. Epigenetic changes have been detected in CRC patients with 70%-96% sensitivity and 72%-96% specificity [13]. Numerous combinations of genetic and epigenetic markers had been studied. Using blood in place of stool as a screening material could offer some advantages [14].

The use of RNA biomarkers in stool has not been investigated as widely as was the case for DNA biomarkers, fundamentally because the responsible cause for degradation of mRNA is stool environment, although improving laboratory recovery methods appear to solve this problem [15]. Sensitivity was thought to be more related to the tumor size and its location, rather than to the tumor stage [16]. Several studies had evaluated mRNA of different tumor genes in complete blood, plasma or circulating tumor cells to recognize new CRC screening markers. Most of them investigated mRNA molecules of CK19, CK20, or carcinoembryonic antigen (CEA) [17]. Recent studies had indicated that circulating miRNAs may be involved in the process of oncogenesis [16]. The use of miRNA as a biomarker is now under-evaluated. A major number of miRNA molecules have been assessed, with a focus on miR145, miR143, miR135 and miR17-92 [18].

3. Blood and Stool Protein Markers

Protein markers for screening and early detection of CRC include tumor-associated antigens (TAAs), antibodies against TAAs, and other CRC-relevant proteins [19]. CEA was discovered about 55 years ago and is still the only recognized tumor marker for monitoring efficacy of CRC patients' therapy [20]. High CEA concentrations are only rarely identified in CRC stage I. Furthermore, CEA does not differentiate benign versus malignant polyps [21]. According to The European Group on Tumor Markers, CEA is not recommended for use in screening tests [22]. In some studies, elevated CEA concentrations in patients with CRC stage II and III were found to be potentially indicative of more aggressive kinds of cancer. Previously, the Colorectal Working Group of American Joint Committee on Cancer suggested to include CEA baseline concentration to the traditional TNM classification as the so-called C-stage [6].

The role of CEA in prediction of life expectancy was approved in several studies on patients with liver metastases [23]. It was demonstrated that the use of CEA and serum amyloid A (SAA) as combination is able to determine patients with favorable and poor prognosis [24]. CEA half-life is known to last about 7 days. Slow

elevation in CEA concentrations after surgery is an irregular sign of local recurrence, while dynamically rising levels can be symptomatic of liver metastasis [25]. CEA is the first marker of choice for observation of disseminated disease during systemic treatment [20]. CEA concentration can also be elevated temporarily as a result of using chemotherapy [26]. So, it is not recommended to test CEA levels within 2 weeks of chemotherapy, while in patients on oxaliplatin, tests can be carried out after 4 to 6 weeks [6].

The plurality of researchers concluded that CA 19-9 sensitivity is much inferior to that of CEA, and that increased CA19-9 levels is a poor prognostic value [27]. CA 72-4 is a biomarker with poor sensitivity ranging from 9% to 31% and better specificity ranging from 89% to 95% in patients screened for CRC [28]. Many authors concluded that CA 72-4 sensitivity is partly low with incomplete specificity in screening and following up in patients with CRC [20].

4. Molecular Biomarkers Used for Prediction of Prognosis

With the recent progress in understanding the molecular mechanisms of cancer development, dissemination, resistance to chemotherapy, and radiation therapy, the selection of the most appropriate strategy for managing CRC is now easier than before [6]. Clinical prospective and retrospective studies open the gate for biomarkers use in clinical practice to support in choosing the best drugs. Biomarker identification is predominately important for patients with CRC stage II [29]. The markers discovered during the recent years include microsatellite instability (MSI), chromosome 18q lack of heterozygosity (18qLOH), p53, PTEN expression, UGT1A1 gene polymorphism, and ezrin protein [30].

4.1. Microsatellite Instability (MSI)

MSI indicates changes in coding and non-coding sequences of microsatellite chromosomes. Microsatellite instability can be categorized into microsatellite instability-high (MSI-H), and microsatellite instability-low (MSI-L), based on the percentage of loci that correlate to MSI characteristics [31]. Tumor cells that lack MSI features are designated as MSS. In retrospective studies and meta-analyses in patients with CRC stage II and III, MSI-H was shown to be a predictive factor that improved overall survival, irrespective of the stage of cancer [32].

MSI status is presently recommended in the WHO classification of mucinous-type CRC. MSI-H indicates good prognosis while MSI-L or MSS indicates poor outcome [31]. However, MSI should be considered more of a prognostic rather than predictive value [6]. This conclusion depends on results of studies evaluating the efficacy of 5-fluorouracil based chemotherapy in patients with MSI-H and MSI-L or MSS tested tumor specimens collected from 570 patients with CRC stage II and III and correlated the test results with chemotherapy outcomes in these patients to show a tendency for shorter overall survival in patients with MSI-H on adjuvant therapy. Significant improvement was observed in patients with MSS tumors [33].

4.2. Chromosome 18q Loss of Heterozygosity

Many studies were dedicated to another prognostic factor in patients with CRC stage II and III - chromosome 18q lack of heterozygosity in the coding place of, inter alia, SMAD 4 proteins specific to CRC [34]. In these studies, the total 5-year survival was poorer for patients with CRC stage III and 18qLOH as compared to non-18qLOH patients. Furthermore, no correlation was identified between the presence of 18qLOH and 5-year survival in patients with non-MSI-H phenotype [35]. Depending on the available information, 18q chromosome deletion cannot be on a single basis for any therapeutic decisions [36].

4.3. P53 Mutation

About 50%-70% of all CRC patients are vulnerable to mutations in the tumor suppressor gene p53 [37]. This mutation is also associated with worse outcomes, including disease free survival and overall survival rates [38]. Patients with p53 mutation and tumour of proximal colon had preferable overall survival rate when treated with adjuvant chemotherapy in comparison to those treated with surgery alone [6].

5. Biomarkers Suitable in Anti-Epidermal Growth Factor Receptor Therapy

A number of currently tested markers have been discovered in the course of studies on epidermal growth factor receptor (EGFR) signaling pathways [39]. KRAS gene mutation on short arm of chromosome 12 at codon 12 or, to a lesser extent, at codon 13 is believed to be used as a biomarker in patients on cetuximab or panitumumab [40]. These mutations are one of the most common in proliferative diseases and their significance in CRC carcinogenesis has been examined [6]. As these mutations are present in EGFR signalling pathway, they can be a predictive factor for therapy with anti-EGFR antibodies. Cetuximab improves the therapeutic outcomes with non-KRAS patients [41].

V600E mutation was the most frequently observed BRAF mutation. BRAF mutations make the tumor resistant to anti-EGFR monoclonal antibodies, and significantly worsen prognosis, especially in patients with MSI-L and MSS [42]. Strongly synergistic reaction when adding cetuximab with BRAF inhibitors was observed. Since NRAS mutation can predict resistance to EGFR therapy, NRAS mutation testing should be considered when KRAS is mutation negative [43].

Phosphatidylinositol-3-kinases (PI3K) are kinases that enhance cellular proliferation. Mutations in PIK3CA gene have been identified in several human solid tumors, including CRC [44]. PIK3CA gene is mutated in 10%-20% of CRC tumors. Recent studies have detected an increase in colon cancer-specific mortality in patients with PIK3CA-mutated tumors, as compared with patients with PIK3CA wild-type tumors [45].

The presence of PIK3CA mutation was reported to be correlated with a significant increase in CRC specific mortality, through patients with KRAS wild-type tumors

[46]. Recent studies have found that suppression of cyclooxygenase-2 by regular use of aspirin after CRC diagnosis was associated with longer cancer specific survival time through patients with mutated compared to wild-type PIK3CA [47]. PIK3CA mutations may serve as a predictive biomarker for adjuvant aspirin therapy [46].

6. Biomarkers of the Potential Toxicity of Irinotecan

Irinotecan is an antineoplastic enzyme inhibitor primarily used for treatment of CRC. It is a derivative of camptothecin that inhibits the action of topoisomerase I, and inhibiting replication and stimulating cell apoptosis [48]. The most common adverse effects of irinotecan are intensive diarrhoea caused by damaged intestinal epithelium and advanced neutropenia. Polymorphism of the UGT1A1 gene is a very useful biomarker of the potential toxicity of irinotecan [6]. According to a recent meta-analysis study, genotyping for UGT1A1 has no predictive value in terms of responsiveness to different doses of irinotecan in patients with CRC [49].

7. Potential Biomarkers of Vascular Endothelial Growth Factor-Targeted Therapy

Since the vascular endothelial growth factor (VEGF)-targeted therapy has been incorporated into CRC treatment protocols, several anti-angiogenic drugs have been introduced (e.g. bevacizumab) [50]. Various studies on identification of predictive biomarkers of bevacizumab have been performed. High baseline values of VEGF were associated with worse progression free survival and the overall survival rate [51]. Other studies had proven that plasma VEGF-A may serve as a prognostic marker, but is incapable to predict response to VEGF-targeted therapy in advanced CRC [6].

Ezrin protein, a part of ezrin/radixin/moesin family may play a significant role in tumor invasion process [52]. Recent studies have found that overexpression of ezrin protein correlates with CRC aggressiveness [53]. Ezrin may represent a relevant biomarker and target for personalized anti-metastatic therapies [54].

8. Diagnostic Tissue Markers

Immunohistochemistry (IHC) is usually used for diagnosis of gastrointestinal neoplasms to facilitate accurate tumor classification [6]. IHC is used to affirm tumor analysis by barring morphologic mimickers and to recognize the most sensible tissue or organ of source in cases of metastatic carcinoma of unknown primary [55]. Also, the results of IHC give important prognostic data and even anticipate responsiveness to standard chemotherapy or novel molecular targeted therapy. Analytic tissue biomarkers accordingly give extra and essential data that complement clinical colonoscopy findings [56].

8.1. Cytokeratins

The cytokeratins (CKs) that belong to the group of intermediate filaments along with glial filaments, neurofilaments, desmin, and vimentin, are proteins communicated by epithelial cells [57]. Non-neoplastic colonic mucosa proximal to the rectum shows a CK7-/CK20+ phenotype, as do 90% of CRC [58]. The main other neoplasms to exhibit this CK7-/CK20+ phenotype in a significant extent of cases are Merkel cell carcinoma and a fraction of gastric adenocarcinomas [6].

8.2. CDX2

CDX2 is an interpretation factor encoded by CDX2, a part of the caudal subgroup of homeobox genes [59]. It is fundamental for embryonic and lifelong maintenance of a cellular intestinal phenotype [60]. CDX2 is strongly expressed in epithelial cells of the normal small intestine, appendix, colon, and rectum as well as the centroacinar and interacinar ductal cells of the pancreas [61].

8.3. Villin

Villin is a differentiation marker of the brush border which has a diagnostic role in human renal cell carcinoma. The specificity of villin as a marker of intestinal origin is limited [62]. This may be attributed to the fact that similar CDX2 appearance might be observed in adenocarcinomas with intestinal separation emerging from an assortment of sites including the stomach, lung, and ovary and additionally, seldom, in malignancies of other places such as the endocervix and liver [63].

8.4. Carcinoembryonic Antigen

Monoclonal CEA may be communicated in a wide assortment of adenocarcinomas, including those emerging from the colon [64]. Although this lack of specificity bounds its general utility as an indicative marker of CRC, it remains a helpful marker for diagnosis of CRC [6].

8.5. Alpha-methyl Acyl-CoA Racemase

Alpha-methyl acyl-CoA racemase (AMACR) is a peroxisomal and mitochondrial enzyme that is implicated in beta-oxidation of branched-chain fatty acids [65]. Among the different types of malignancies, AMACR protein expression is frequently seen in adenocarcinomas of the colon and prostate [66]. Increased AMACR expression in the colonic adenomas and carcinomas implies that it has an important role in colorectal carcinogenesis [67]. Interestingly, decreased AMACR expression in high grade colorectal carcinomas may be taken as a proof of its role in malignant differentiation of the colonic tissues [68].

8.6. Cadherin 17

Cadherin-17 (CDH17) is a calcium-dependent transmembrane glycoprotein that mediates cell-cell adhesion in intestinal epithelium [69]. Expression of

CDH17 was reported in gastric, pancreatic, and colorectal adenocarcinomas [6]. It is seldom found in adenocarcinomas from outside the gastrointestinal tract. Strangely, although CDH17 is transcriptionally controlled by CDX2, few researchers observed it to be slightly more sensitive and specific than CDX2 in distinguishing colorectal adenocarcinomas [70].

9. Prognostic Tissue Markers

High Special AT-rich sequence-binding protein 2 (SATB2) expression was related with good prognosis in colon cancer and might balance affect ability to chemotherapy and radiation, while decreased expression of SATB2 in colorectal adenocarcinomas was observed to be related to poor prognosis, containing tumor invasion, lymph node metastasis, and distant metastasis [71]. In addition, loss of CDX2 expression is related with proximal location, infiltrative growth, advanced stage and is an independent poor prognostic factor of general survival and progression-free survival [72].

10. Predictive Tissue Markers

10.1. BRAF V600E IHC

In CRC, BRAF V600E is the most common mutation [26]. BRAF V600E mutation in MSI CRCs can virtually exclude Lynch syndrome, and mutation positive tumors are resistant to anti-epithelial growth factor receptor (EGFR) therapy [73]. BRAF V600E IHC is a sensitive and reliable assay, generating outcomes that correlated well with those from molecular detection methods [13].

10.2. Epithelial-to-Mesenchymal Transition (EMT) and Drug Resistance

An increasing number of findings suggest that tumors undergoing EMT resist conventional drug therapy [18]. In clinical practice, tumor specimens taken from CRC patients who have received preoperative chemotherapy followed by radical surgery display phenotypic changes characteristic of and molecular changes consistent with the EMT [27]. Consistently, loss of E-cadherin stimulates drug resistance in CRC patients and the recurrent tumors have a significantly EMT-like signature [74].

10.3. EGFR Expression and EGFR Targeted Therapy Predictive Markers

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein with an intracellular tyrosine kinase domain [41]. Binding of ligands to the EGFR promotes tumor growth and progression by controlling transcription, cell cycle progression, apoptosis and differentiation through the EGFR downstream signaling pathway [75]. EGFR monoclonal antibodies (e.g. cetuximab and panitumumab) are active drugs in combination chemotherapy or as monotherapy for metastatic CRC [76].

10.4. CD133 As a Potential Marker for Traditional and Targeted Treatment

CD133 is a surface protein commonly used for isolation of colon cancer stem cells. It is associated with tumor angiogenesis and recurrence [77]. The patients with high gene expression levels of CD133 showed a significantly greater tumor response than patients with low expression levels, independent of the expression of VEGF or its receptors [78].

10.5. P53

P53 is encoded by TP53 gene, located on the short arm of chromosome 17 [79]. P53 is a transcription factor and one of most commonly investigated tumor suppressor genes [80]. Many functions have been recognized for p53 including repair of damaged DNA, block of cellular cycle, and induction of apoptosis. Its alterations are frequent at early stage in natural history of many cancers [81]. However, its main disadvantage is of lack of specificity [35].

10.6. Familial Adenomatous Polyposis (FAP)

FAP is an autosomal dominant condition characterized by hundreds of adenomas in the colon and rectum [82]. It has been proven to be responsible for about 0.5% of all cases of CRC [83]. Approximately 70–80% of patients with classical FAP harbor germline mutations in the APC gene [6]. Screening for FAP should commence with a detailed family history. For individuals with suspected FAP, genetic testing can be used both to confirm diagnosis and to assess the risk in presymptomatic family members [84]. Provided that the mutation responsible for FAP within a family is known, testing for APC mutations can be considered for at risk family members [41].

11. Conclusion

The tumor markers of CRC play a major role in better understanding of this disease and help in the development of new therapeutic strategies, especially in advanced metastatic cases. These markers include blood and stool genetic and epigenetic markers, blood and stool protein markers and biomarkers used for prediction of prognosis. Implementation of the primary care services may effectively decrease the burden and improve the prognosis of CRC.

Conflict of interest

The authors had no conflict of interest to declare.

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