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TOPIC HIGHLIGHT

2016 Colorectal Cancer: Global view

Colorectal cancer tumour markers and biomarkers: Recent therapeutic advances

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Abstract

Colorectal cancer (CRC) is the second most commonly diagnosed cancer among females and third among males worldwide. It also contributes significantly to cancer-related deaths, despite the continuous progress in diagnostic and therapeutic methods. 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 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 personalise chemotherapy based on presence or absence of specific biomarkers. In this review, we update review published last year and describe our understanding of tumour markers and biomarkers role in CRC screening, diagnosis, treatment and follow-up. Goal of future research is to identify those biomarkers that could allow a non-invasive and cost-effective diagnosis, as well as to recognise the best prognostic panel and define the predictive biomarkers for available treatments.

Key words: Colorectal cancer; Biomarker; Microsatellite instability; *KRAS* mutation; *BRAF* mutation; *PIK3CA* mutation; Chromosome 18q loss of heterozygosity; Anti-epidermal growth factor receptor therapy; Colorectal cancer biomarkers; Carcinoembryonic antigen

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Core tip: This review summarizes data concerning clinical utility of biomarkers in colorectal cancer patients. Authors focus primarily on currently available diagnostic, prognostic and predictive biomarkers of the disease. Great attention is also paid to the advances achieved in personalized therapy of colorectal cancer.

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INTRODUCTION

Worldwide, colorectal cancer (CRC) annually affects more than one million men and women and causes more than half a million deaths[1]. In Europe in 2010, CRC was the third most common malignant cancer in both men and women[2]. There are 250000 cases of colorectal cancer diagnosed on an annual basis in Europe only. Five-year survival was 54 percent among adult Europeans diagnosed with colorectal cancer between 1995 and 1999[3]. More recent available data report that the overall five-year relative survival can achieve 65 percent, but varies depending on stage of cancer disease[4].

The number of biomarkers used for tests continues to grow. The National Institute of Health defines a biomarker as a biological molecule found in blood, other body fluids, or tissues that is a sign of normal or abnormal process, or of a condition or disease[5]. A definition of biomarker mostly refers to DNA, RNA, microRNA (miRNA), epigenetic changes or antibodies. A term tumour marker, by some researchers considered as a synonym of biomarker, refers to substances (most typically proteins, glycolipids) representing biological structures, which can be attributed to the development of normal cells or carcinogenesis at different cell development stages *e.g.,* tumour-associated antigens (TAAs) which are the largest group of clinically significant markers. As a result, the concentration of TAAs typically correlates with the number (or mass) of specific neoplastic cells.

In daily clinical practice, in the process of diagnosis and therapy, there are several parameters in use of long-established high sensitivity, specificity and positive predictive value. These parameters have been selected from among tens of molecules produced by cells in long-term laboratory tests, observational studies and clinical trials. The concentrations of tumour markers tested at the diagnostic stage are believed to assist in early cancer diagnosis and to be used in screening tests. Some of them are currently found to be more important during treatment and long-term follow-up. On the other hand, for some types of tumours, markers are also considered important in monitoring the progress of treatment, efficacy of neo-adjuvant therapy, surgery, adjuvant chemotherapy and radiation therapy and follow-up for possible recurrence. Long-term observational studies also point to the fact that, apart from determining antigen concentration, it can be also important to trace its progress and dynamics. In this review, we have updated a review published in 2014[6]. We examine molecular (genetic, epigenetic, protein) biomarkers associated with CRC and discuss their role in cancer screening, early detecting of disease recurrence and as prognostic and predictive factors.

BLOOD AND STOOL MARKERS FOR COLORECTAL CANCER SCREENING AND FOLLOW UP

Blood and stool genetic and epigenetic markers

Several authors have investigated molecular non-invasive screening tests for early detection of CRC. DNA, RNA and other molecules derived by tumour in stool, as well as their concentrations in blood have been studied extensively. Colorectal process of carcinogenesis is characterized by genetic and epigenetic alteration transforming normal cells into cancer cells. Most studies concerning molecular markers in stool have focused on the detection of tumour DNA. These investigations have concentrated on the detection of mutated *KRAS*, *TP53*, *APC* and markers for microsatellite instability (MSI)[7-9]. A faecal DNA test targeted at molecular biomarkers has been commercially available for twelve years, with reported sensitivity for cancer ranging from 25% up to 92% for the latest tests based on BEAMing technology, and 94%-98% specificity[10-12]. Apart from genetic alterations, the DNA promoter hypermethylation silencing the tumour suppressor genes has been widely investigated. Epigenetic changes, depending on the markers or their combinations evaluated, have been detected in CRC patients with 70%-96% sensitivity and 72%-96% specificity[9,13,14]. Many combinations of genetic and epigenetic markers have been studied, but until now, the results have not endorsed their use in clinical practice. Using blood instead of stool as a screening material could offer some obvious advantages. Several studies have evaluated potential plasma DNA genetic and epigenetic biomarkers in CRC detection. The overall sensitivity ranges from 30% to 87%, with specificity of up to 96%. The use of RNA biomarkers in stool has not been investigated as extensively as was the case for DNA biomarkers, mainly because stool environment is responsible for mRNA degradation, although improving laboratory retrieval methods seems to solve this problem. Koga *et al*[15] analysed mRNA expression of *MMP7*, *PTGS2*, *TP53* and *MYBL2* in colonocytes isolated from stool by quantitative real-time RT-PCR, to find out that these markers can identify CRC patients with 58% sensitivity and 88% specificity. Sensitivity was found to depend on tumour size and tumour location, but not cancer stage[15]. Most recently, the so called transcriptomic studies have investigated the expression of miRNAs - short, non-coding 18-22 nucleotide RNA molecules in

stools of CRC patients. The most extensively studied miR21, miR106a, miR135, miR17-92 were found to be overexpressed in CRC patients compared with healthy individuals[16,17]. As was the case with RNA markers in stool, many studies have been evaluating mRNA of different tumour genes in whole blood, plasma or circulating tumour cells to identify new CRC screening markers. Most of them investigated mRNA molecules of CK19, CK20, or Carcinoembryonic antigen (CEA). The overall sensitivity of these markers was up to 72%, specifically when combinations of these markers were used[18,19]. The specificity was very high with healthy control samples or much lower when compared to other cancer or inflammatory bowel diseases samples[20]. Recent studies have indicated that circulating miRNAs may be involved in the process of oncogenesis. The use of miRNA as a biomarker is now being evaluated. A large number of miRNA molecules have been assessed, with a focus on miR145, miR143, miR135, miR17-92. More specifically, Huang *et al*[21] has found that plasma miR29a and miR92a demonstrated a significant diagnostic value for advanced neoplasia with 83% and 84% sensitivity and specificity, respectively, in discriminating CRC patients. These studies need to be validated in randomised trials to define their value in CRC screening.

Blood and stool protein markers

Protein markers for screening and early detection of CRC can be divided into tumour TAAs, antibodies against TAAs, and other CRC-relevant proteins. CEA was discovered almost 50 years ago, in 1965, and it still remains the only tumour marker of recognised efficacy in monitoring CRC patients' therapy[22]. CEA was first considered specific for CRC, but elevated CEA levels were later detected in other neoplasms too, *e.g.* gastric and pancreatic cancers, and in inflammatory conditions. Elevated CEA concentrations are only rarely identified in CRC stage I. Moreover, CEA does not differentiate benign versus malignant polyps. According to The European Group on Tumor Markers, European Society of Medical Oncology and American Society of Clinical Oncology guidelines[2,23,24], CEA is not recommended for use in screening tests. Recently, some studies have investigated the advantages of mRNA molecules encoding CEA for the detection of CRC, but the results were not superior to those of CEA[19].

In some studies, high CEA concentrations in patients with CRC stage Ⅱ and Ⅲ were found to be potentially indicative of more aggressive types of cancer[25,26]. Earlier, the Colorectal Working Group of American Joint Committee on Cancer proposed to include CEA baseline concentration to the traditional TNM classification as the so-called C-stage. C-stage was proposed to be divided into Cx, C0 (CEA < 5 ng/mL) and C1 (CEA > 5 ng/mL) substages[27]. The meaning of CEA as an independent prognostic factor was also confirmed in a recent retrospective analysis of 17910 patients with CRC, with a mean 27-mo follow-up, with longer survival periods for patients with ⅡA C0 and ⅢA C0 *vs* Ⅰ C1, ⅢA C0 *vs* ⅡA C1, and ⅢB C0 *vs* ⅡB-C C1, respectively[28]. No study, however, has shown that CEA concentration level can be used to select those patients with stage Ⅱ CRC who would benefit from adjuvant chemotherapy. From a prognostic point of view, it appears reasonable to determine CEA levels before surgery in patients with disseminated CRC. The roles of CEA in determining life expectancy was confirmed in several studies on patients with liver metastases[29,30]. Recent study proved that combined use of CEA and serum amyloid A (SAA) is able to identify patients with favourable and poor prognosis. In addition to tumour baseline parameters, routine analysis of CEA together with SAA provides improved prognosis value on cancer specific survival and disease-free survival in resected rectal cancers[31]. CEA half-life is known to last approximately 7 d. After R0 resection surgery, CEA levels should return to normal within 4 to 6 wk. Sustained elevated CEA levels can be indicative of infiltration or metastases. Slow increase in CEA concentrations after surgery is a typical sign of local recurrence, whereas dynamically increasing levels can be symptomatic of metastases, most probably in the liver[24,32]. Testing CEA levels is considered most cost-effective in detecting post-surgery recurrences[24]. Please note that CEA levels tested every 3 mo for the first 3 years and thereafter every 6 mo for subsequent 2-3 years is a golden follow-up standard after CRC therapy recommended by a number of scientific associations[2,23,33]. It appears particularly important in asymptomatic patients, in whom chemotherapy can be used, with a much longer life expectancy as compared to treatment administered after the onset of symptoms of recurrence. CEA is a marker of choice in monitoring disseminated disease during systemic therapy. Constant increase in CEA levels is typically associated with a progression of the disease, even though radiological tests may prove otherwise[23,24]. However, chemotherapy can also result in temporary increase in CEA concentration, which must be also taken into account. Therefore, it is not recommended to test CEA levels within 2 wk of chemotherapy, whereas in patients on oxaliplatin, tests can be carried out after 4 to 6 wk.

Cancer antigen 19-9 (CA 19-9) is a glycoprotein whose relevance in CRC diagnosis still remains an issue. The majority of researchers arrived at the conclusion that CA 19-9 sensitivity is much inferior to that of CEA, and that elevated CA 19-9 levels is a poor prognostic factor[2,23,34-36]. Other carbohydrate antigens: CA 195, CA 50 have been also investigated, but with comparatively disappointing results. CA 72-4 is a biomarker with poor sensitivity ranging from 9% to 31% and better specificity ranging from 89% to

Table 1 Recommendations for use of tumour markers and biomarkers in colorectal cancer by groups of experts

Biomarker	Applications	ASCO[23,98,99]	ESMO[2,33]	NCCN[100,101]
CEA	Screening	No	None published	None published
	Prognostic factor	Yes	Yes	Yes
	Follow up	Yes	Yes	Yes
CA 19-9	All	No	No	None published
CA 72-4	All	None published	None published	None published
CA 242	All	None published	None published	None published
CA 195	All	None published	None published	None published
CYFRA 21-1	All	None published	None published	None published
MSI	Prognostic factor	No	Yes	Yes
18qLOH	Prognostic factor	Yes	Yes (potentially)	None published
p53 gene	Prognostic factor	No	Yes (potentially)	None published
KRAS	Prognostic factor	None published	Yes (potentially)	None published
	Predictive factor	Yes	Yes	Yes
BRAF	Prognostic factor	None published	Yes	Yes
	Predictive factor	Yes	Yes (potentially)	Yes (potentially)
PIK3CA	Predictive factor	None published	Yes (potentially)	None published
PTEN	Predictive factor	Yes (potentially)	Yes (potentially)	None published
UGT1A1	Predictive factor	Yes	Yes (only in case of severe toxicity of irinotecan)	No
VEGF	All	None published	None published	None published
TPA, TPS	All	None published	None published	None published
Ezrin	All	None published	None published	None published
DNA ploidy	All	No	None published	None published
TS	Prognostic factor	No	Yes (potentially)	None published
	Prognostic factor	Yes (potentially)	Yes (potentially)	None published
TP	All	No	None published	None published
DPD	Prognostic factor	No	Yes (only in case of severe toxicity of 5-FU)	None published

CEA: Carcinoembryonic antigen; MSI: Microsatellite instability; 18qLOH: Chromosome 18q loss of heterozygosity; VEGF: Vascular endothelial growth factor; TPS: Tissue polypeptide-specific antigen; TPA: Tissue polypeptide antigen.

95% in patients screened for CRC. The diagnostic information in recurrent CRC provided by CA 72-4 has borderline significance, by far worse than CEA. All authors conclude that CA 72-4 sensitivity is rather low and specificity incomplete in screening and following up in patients with CRC. On the other hand, an algorithm based on combination of CEA, CA 19-9, CA 72-4, CA 242, CYFRA21-1 improves the diagnostic accuracy compared with these biomarkers alone[34-39]. Among other protein markers examined for screening purposes, two have been extensively investigated: the tumour specific M2 isoform of pyruvate kinase (M2-PK) in stool and tissue inhibitor of matrix metalloproteinase 1 (TIMP1). M2-PK measured in stool showed relatively high sensitivity for CRC up to 91%, and much lower for adenomas[40,41]. Plasma level of TIMP1 is reported to be elevated in CRC patients and prospective studies have been carried to assess its utility as biomarker. The results of the study included more than 4500 patients screened by endoscopy for CRC demonstrated that TIMP1 is not significantly superior to CEA marker in cancer screening and is not suitable for the detection of premalignant lesions[42]. Tissue polypeptide-specific antigen (TPS) and tissue polypeptide antigen (TPA) which detects the fragments of cytokeratines 8, 18 and 19 due to lack of sensitivity and specificity can not to be recommended in CRC screening. The majority of investigators have found that increased levels of TPA and TPS are observed in metastatic stage of CRC. A further studies has suggested that combination of TPA and CEA rises the sensitivity of these biomarkers in identifying the patients with CRC recurrence[34,37,43,44]. Other biomarkers, such as: thymidine phosphorylase (TP), DNA ploidy were determined to be insignificant in detecting, staging and following-up of patients with CRC[23].

MOLECULAR PROGNOSTIC AND PREDICTIVE BIOMARKERS

With the recent progress in understanding the molecular mechanisms of cancer development, dissemination, resistance to chemotherapy, and radiation therapy, it is now easier to select the most proper strategy for managing CRC. Clinical prospective and retrospective studies open the door for biomarkers use in clinical practice to assist in selecting the best drugs, both standard, such as 5-fluorouracil, oxaliplatin or irinotecan, and new generation targeted drugs: cetuximab, panitumumab, or bevacizumab. Biomarker identification is particularly important for patients with CRC stage Ⅱ. In this group of patients, the risk of recurrence is only 20 percent. It is also desirable to use adjuvant therapy in this type of patients. There

are attempts to select this group of patients based on genetic tests, or to personalise chemotherapy based on specific biomarkers. The following markers discovered throughout the recent years continue to be closely examined: MSI, chromosome 18q loss of heterozygosity (18qLOH), *p53*, *KRAS*, *BRAF*, *NRAS*, *PIK3CA* mutations, *PTEN* expression, *UGT1A1* gene polymorphism, and ezrin protein (Table 1).

MSI

MSI denotes changes in coding and non-coding sequences of microsatellite chromosomes, *i.e.* repeated DNA sequences. These sequences are particularly exposed to errors in the mutation repair system that consist in the loss or multiplication of nucleotide sequence repetitions, which results in shortening or extension of microsatellite regions in neoplastic cells. Mutations arising out of these processes are eliminated by mismatch repair genes (*MMR*) such as *MSH2*, *MSH6*, *PMS2* and *MLH1*, which makes some researchers believe that MSI can be caused by mutations in these genes[45]. Microsatellite instability can be classified into microsatellite instability-high (MSI-H), and microsatellite instability-low (MSI-L), depending on the percentage of loci that correlate to MSI characteristics. Tumour 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 (OS), irrespective of the progression (stage) of cancer. A lower incidence of lymph node metastases and distant metastases as compared to MSI-L or MSS cancer cells was also observed[46-49]. MSI status is currently recommended in the WHO classification of mucinous-type CRC - MSI-H indicates good prognosis, MSI-L or MSS - poor outcome. However, MSI should be considered more of a prognostic rather than predictive factor. This conclusion is based on equivocal results of studies evaluating the efficacy of 5-FU-based chemotherapy in groups of patients with MSI-H and MSI-L or MSS. Ribic *et al*[48] examined tumour specimens collected from 570 patients with CRC stage Ⅱ and Ⅲ and correlated the test results with chemotherapy outcomes in these patients to reveal a tendency for shorter overall survival in patients with MSI-H on adjuvant therapy. Significant improvement was observed in patients with MSS tumours. A recent pooled analysis of randomized clinical studies revealed significant decrease in the overall five-year survival rate for patients with CRC stage Ⅱ and MSI-H on 5-FU-based chemotherapy. 5-FU-based chemotherapy was found to improve therapeutic outcomes only in patients with CRC stage Ⅲ and MSI-L or MSS[50]. Some studies indicated potentially negative effects of 5-FU-based chemotherapy in patients with MSI-H. A longer survival rate as compared to patients on 5-FU-based adjuvant chemotherapy was observed in a reference group of patients undergoing surgical treatment. Resistance of MSI-H tumours to 5-FU was also confirmed in *in vitro* studies[51]. A completely different conclusion can be drawn from earlier studies of Elsaleh *et al*[52,53], which confirmed the efficacy of 5-FU in patients with CRC stage Ⅲ and MSI-H. Recent study also proved that prognostic value of MMR mutation was similar in the presence or absence of fluorouracil and folinic acid chemotherapy[54]. Beragnolli *et al*[55] revealed that a higher rate of overall 5-year progression-free survival was observed in patients with CRC stage Ⅲ and MSI-H on 5-FU and irinotecan *vs* 5-FU-based chemotherapy. To recap, the results of MSI studies and clinical experience in patients with CRC stage Ⅱ indicate that the degree of microsatellite instability may be of significance as a prognostic factor. Also, adjuvant 5-FU-based chemotherapy was proved to provide no benefits (or potentially cause adverse reactions) in patients with MSI-H. Further research is needed to investigate whether the MSI status can predict benefit (in high-risk patients) from irinotecan-based treatment or oxaliplatinum-based therapy.

Chromosome 18q loss of heterozygosity

A number of studies were dedicated to another prognostic factor in patients with CRC stage Ⅱ and Ⅲ - chromosome 18q loss of heterozygosity in the coding place of, inter alia, SMAD 4 proteins specific to CRC. In these studies, the overall 5-year survival was poorer for patients with CRC stage Ⅲ and 18qLOH as compared to non-18qLOH patients[56]. A meta-analysis of data from 27 studies and 2189 patients by Popat *et al*[57] confirmed that poorer survival was correlated with 18q chromosome deletion. Two years later, the same research team questioned these findings after re-examining the same data[58]. Likewise, no correlation was identified between the presence of 18qLOH and 5-year survival in patients with non-MSI-H phenotype[59]. The role of 18qLOH in predicting response to standard chemotherapy has not been yet fully confirmed. Watanabe *et al*[60] demonstrated better response to 5-FU-based chemotherapy in patients with CRC stage Ⅲ and MSS and with the absence of 18q chromosome deletion vs. patients in whom 18q chromosome deletion was present. The recently published results of the same research team can be a proof that in patients with CRC stage Ⅱ and Ⅲ and MSS-H (> 33%), the level of LOH of four chromosomes, including 18, is correlated with significantly poorer survival rate as compared to patients with MSS and LOH-L or MSI-H phenotype[61].

Based on the available data, 18q chromosome deletion cannot be the sole basis for any therapeutic decisions, however, it is being more closely examined under ECOG 5202 study, featuring molecular markers identified so far in selecting the most proper adjuvant post-surgery treatment, by prospectively analysing the role of MSI and 18qLOH in prognosis and therapeutic

decisions in patients with CRC stage Ⅱ. Patients with good prognosis (with MSI-H and w/o 18qLOH) were followed-up, and patients with poor prognosis (with MSI-L or MSS and 18qLOH) were randomized to one of two groups on chemotherapy (FOLFOX alone or FOLFOX and bevacizumab). The results of E5202 are expected in the next few years. No conclusion can be drawn from this study about the possible inefficacy of chemotherapy in patients with MSI-H, however, the study will include a multifactor analysis of biomarkers that can assist in taking therapeutic decisions in other groups of patients[62].

P53 mutation

Mutation in the tumour suppressor gene *p53* (chromosome region 17p13) occur in 50%-70% of all CRC and is associated with worse outcomes, including disease free survival and overall survival[63]. Results obtained from a study that included more than 3500 CRC patients confirm the prognostic value of *p53* mutation, which seems to be determined by the primary tumour site. Patients with *p53* mutation and tumour of proximal colon had better OS when treated with adjuvant chemotherapy compared to those treated by surgery alone[64].

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) signalling pathways. *KRAS* gene mutation on short arm of chromosome 12 at codon 12 (80% of patients) or, to a lesser extent, codon 13 is believed to be of use as a biomarker in patients on cetuximab or panitumumab[65]. These mutations are one of the most common in proliferative diseases (37% and 13%, respectively), and their significance in CRC carcinogenesis has been examined in much detail[66]. As these mutations are present in EGFR signalling pathway, they can be a predictive factor for therapy with anti-EGFR antibodies. In studies performed so far, *KRAS* mutation was found to be correlated with non-responsiveness to cetuximab and panitumumab[67,68]. CRYSTAL and OPUS data indicate that the effectiveness of FOLFOX or FOLFIRI alone is no inferior to that of cetuximab in patients with KRAS in combination with chemotherapy according to FOLFIRI and FOLFOX regimen, respectively. However, in non-KRAS patients, cetuximab improves the therapeutic outcome[69,70]. The same conclusions can be drawn from the results of other large clinical studies: COIN, NORDIC Ⅶ or PRIME[71-73]. Yet, the effects of *KRAS* mutation at codon 12 or 13 on tumour biology were found to differ. In two studies, the survival rate was higher in patients with an uncommon G13D mutation at codon 13 on cetuximab *vs* patients with other mutations, and similar to patients with no KRAS mutations identified[65,74]. It is presently believed that anti-EGFR antigens should not be used in patients with tumours indicative of G12V mutation of KRAS at codon 12. For bevacizumab, KRAS mutation was found to be of no use as a predictive factor[75].

The same applies to BRAF mutations found in 8%-13% of patients with CRC, which are mutually exclusive with KRAS mutations. The most frequently observed BRAF mutation is V600E mutation. BRAF mutations make the tumour to a large extent resistant to anti-EGFR monoclonal antibodies, and significantly worsen prognosis, especially in patients with MSI-L and MSS[66,70,76-78]. Based on the available data, National Comprehensive Cancer Network (NCCN) suggests considering BRAF mutation testing when KRAS is mutation negative. Interestingly, good prognosis was reported even in those MSI-H CRC patients who had coincident BRAF mutations[78]. In one of studies, the OS period was shown to be slightly longer in patients on cetuximab even if the BRAF mutation was present[69]. Very limited response to vemurafenib, recently approved for metastatic melanoma patients harboring *BRAF* (V660E) mutation, was demonstrated in CRC patients. Researchers reported that by adding cetuximab strongly synergistic reaction with BRAF inhibitors was observed[79]. NRAS is another member of RAS proto-oncogenes which was found to be rarely mutated, while BRAF is mutually exclusive with KRAS mutations. Since NRAS mutation can predict resistance to EGFR therapy, NCCN suggests considering NRAS mutation testing when KRAS is mutation negative. To date, NRAS mutation does not appear to be associated with the prognosis[80].

Phosphatidylinositide-3-kinases (PI3K) are kinases that promote cellular proliferation. Mutations in *PIK3CA* gene encoding p110α catalytic subunit of PI3K have been identified in different human solid tumours, including CRC. *PIK3CA* gene is mutated in 10%-20% of CRC tumours. *PIK3CA* gene encodes the kinase that regulates, alongside with KRAS, downstream signalling pathways of EGFR. Moreover, PI3K-initiated signalling is inhibited by phosphatase and tensin homologue deleted on chromosome 10 (*PTEN*). Recent studies have revealed an increase in colon cancer-specific mortality in patients with PIK3CA-mutated tumours, as compared with patients with PIK3CA wild-type tumours[81,82]. However only the coexistence of *PIK3CA* exon 9 and 20 mutations but not *PIK3CA* mutation in either exon 9 or 20 alone has been reported to be associated with the worse prognosis[82]. Among patients with KRAS wild-type tumours, the presence of PIK3CA mutation correlated with a significant increase in CRC specific mortality. In contrast, PIK3CA mutation did not significantly affect mortality among patients with KRAS-mutated tumours. Thus, the effect of PIK3CA mutation may be potentially limited to patients with KRAS wild-type tumours[81]. Following

the fact that only patients with KRAS-wild type CRC may respond to anti-EGFR antibodies, several studies have investigated the role of PIK3CA mutations on CRC cells response to cetuximab or panitumumab. The data collected so far indicate that CRC with PIK3CA mutations are significantly resistant to anti-EGFR antibodies. When only KRAS wild-type tumours are analyzed, the correlation is even stronger[83-85]. Changes in PIK3 signalling and loss of *PTEN* expression have been generally linked with the lack of response to EGFR-targeted therapy[86,87]. Recent studies have found that inhibition of cyclooxygenase-2 by regular use of aspirin after CRC diagnosis was associated with longer cancer specific survival time among patients with mutated as opposed to wild-type PIK3CA. The authors conclude that PIK3CA mutations may serve as a predictive biomarker for adjuvant aspirin therapy[88]. Further studies involving KRAS mutated CRC patients are necessary to establish the role of aspirin in PI3K pathway.

Biomarker of the potential toxicity of irinotecan

Irinotecan is a chemotherapeutic agent that inhibits topoisomerase I, thereby inhibiting replication and stimulating cell apoptosis. Advanced neutropenia and intensive diarrhoea caused by damaged intestinal epithelium are the most common adverse effects of irinotecan, which significantly limit its use. *UGT1A1* gene polymorphism is a very useful biomarker of the potential toxicity of irinotecan. It appears that the use of genetic tests is reasonable before treatment initiation with irinotecan to avoid severe adverse effects - mainly neutropenia in women. Genotyping for *UGT1A1* can be carried out to select a group of sensitive patients with UGT1A1*28 allele, of whom lower initial doses would be recommended. Hopefully, it will also allow to administer a higher accumulated dose of the drug, divided into smaller portions, to limit its toxicity[89,90]. However, according to a recent meta-analysis, genotyping for *UGT1A1* has no predictive value in terms of responsiveness to various doses of irinotecan among patients with CRC[91]. On the other is recommended by ESMO for patients with several toxicity reaction in whom irinotecan in high doses should be used[33]. Furthermore, homozygosity for the UGT1A1*28 has been linked with improved efficacy of FOLFIRI[92].

Potential biomarkers of vascular endothelial growth factor - targeted therapy

Since the vascular endothelial growth factor (VEGF) - targeted therapy has been integrated into CRC treatment protocols, some anti-angiogenic drugs have been introduced (bevacizumab, regorafenib, aflibercept). However, a patient selection strategy to identify those patients who benefit most from this therapy has yet to be developed. To date, a predictive biomarker for bevacizumab - the most commonly administered anti-angiogenic drug in CRC therapy - has not yet been identified. Several studies on the identification of predictive biomarkers of bevacizumab have been performed. Jürgensmeier *et al*[93] evaluated retrospectively, using samples from randomised trial HORIZON Ⅲ, the prognostic/predictive value of VEGF and soluble VEGF receptor-2. High baseline values of VEGF were associated with worse progression free survival (PFS) and overall survival. These data have revealed that baseline VEGF levels were not predictive of PFS or OS outcome in bevacizumab-treated patients[93]. Other studies have demonstrated that plasma VEGF-A may serve as a prognostic marker, but is unable to predict response to VEGF-targeted therapy in advanced CRC[94,95]. At the same time, KRAS mutation was found to be of no use as a predictive factor for bevacizumab[75].

Ezrin

Ezrin protein, a part of ezrin/radixin/moesin family may play an important role in tumour invasion process. Recent studies has found that overexpression of ezrin protein correlates with CRC aggressiveness, its metastatic potential and worse prognosis. High ezrin expression was also identified as marker of early local recurrence of rectal cancer[96,97]. Although further investigation is needed, ezrin may represent a relevant biomarker and target for personalized anti-metastatic therapies.

CONCLUSION

The recent studies result in a better understanding of colorectal cancer and assist in the development of new treatment regimens, especially in advanced CRC stages. The new predictive factors, molecular imaging, or even commercial genome tests increasingly facilitate tumour genome testing and assist in selecting targeted therapies. Adjuvant targeted therapy with anti-EGFR antibodies is required in advanced CRC patients and absence of *KRAS*, *BRAF*, *NRAS* and *PIK3CA* genes mutation. Tests for MSI or MSS tumour phenotype and the presence or absence of 18q chromosome deletion is very much desirable in standard therapy based on 5-FU. Genotyping of UGT1A1 alleles is reasonable before treatment initiation with irinotecan to avoid severe adverse effects. Further studies are necessary to identify predictive biomarker of bevacizumab. Targeted therapy against membrane receptors appears to be the future of CRC therapy. Some promising studies are now carried out in this area, dedicated to, inter alia, other EGFR ligands, insulin-like growth factor receptor 1, platelet-derived growth factor receptors and c-MET inhibitors. The aim of future research is to identify those biomarkers that can provide a non-invasive and cost-effective diagnosis, as well as to recognise the best prognostic panel of biomarkers and define the predictive biomarkers for available treatments.

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