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Biomarkers in colorectal cancer: current and future perspectives

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Introduction

Colorectal cancer (CRC) is the third commonest malignancy worldwide with a rapidly rising incidence in many areas of the world[1]. Unfortunately, only 14% of CRC cases are diagnosed at an early stage. Patients with a localized colonic or rectal cancer have a 5-year survival of 91% and 88% respectively[2]. However the overall survival (OS) of patients with distant metastases is only 12.5% at 5 years[2]. Amongst those who have a surgical resection with curative intent have a median survival of 40-55 months and their 10-year survival is only 15-25% when systemic therapy is used alongside surgery[3].

There are inherited, familial and sporadic cases of CRC. The first 2 account for approximately 30% of all CRC cases, and the rest are sporadic[4].

The molecular mechanisms involved in CRC vary and hence make it a heterogeneous disease[4]. Genomic instability is key in tumor development and there are 3 postulated pathways in colorectal carcinogenesis: the chromosomal instability (CIN), microsatellite instability, and CpG island methylator phenotype pathways[5]. Most of the cases arise from the CIN pathway, which is characterized by aneuploidy and loss of heterozygosity (LOH)[5]. Since 1990, when Fearon and Vogelstein first described the multistep genetic model of colorectal carcinogenesis with 7 distinct gene mutations involved in carcinogenesis, further research suggest that there are as many as 80 mutated genes per colorectal tumor[5].

In this review we will discuss current and future perspectives of biomarkers in colorectal cancer.

Blood biomarkers

CEA

Carcinoembryonic antigen is currently used in practice to monitor CRC recurrence and as a prognostic factor[6]. Its overall sensitivity for detecting primary CRC is 37% but varies from 21.4% in stage I to 41.7% in stage III disease[6]. Its overall sensitivity for detecting recurrent CRC is 54.4%, although this improved if there is a history of increased CEA prior to resection of the primary tumor, according to a retrospective study performed by Su BB et al in 2012[6]. Due to its low sensitivity and specificity, it is not used as a diagnostic marker but instead it is currently the most useful marker in early detection of liver metastasis in patients with diagnosed colorectal cancer[7].

Immunological factors

Markers of inflammation are increasingly used in assessing the immunological status of a patient with CRC. A common marker is neutrophil/lymphocyte ratio (NLR); a high NLR due to lymphocytopenia and neutrophilia indicates an impaired cell-mediated immunity in the setting of an acute inflammatory response[8]. A recent meta-analysis showed that a high NLR is associated with shorter OS and PFS rates[8].

Circulating tumor cells

Circulating tumor cells (CTCs) were first reported in 1869. They are derived from either the primary tumor or the metastases[4,9]. They have been extensively researched for their prognostic significance and have found that baseline CTCs is an unfavorable prognostic factor associated with shorter overall survival (OS) and progression-free survival (PFS) in patients with metastatic CRC[10]. However, due to the high variability in blood CTC levels and also the differences in recurrence and mortality rates despite the CTC presence, undermines its use as a prognostic factor[4].

Cell-free DNA

A more practical marker is the presence of cell-free DNA (cfDNA), which are DNA fragments from tumor cells detected in the plasma or serum of patients. They can be examined for mutations and genomic abnormalities, thus giving real-time insight into tumor progression[9]. It is a more accurate measure of tumor burden compared to CTCs and it can be used to detect TP53 and KRAS mutations, MSI or LOH and DNA hypermethylation[9]. Several studies on the detection of aberrantly methylated DNA sequences have been conducted. Recently, detection of methylated SEPT9 in plasma has been evaluated as a diagnostic marker in CRC. Its reported sensitivity in detecting CRC is 90% and specificity 88%[4]. Currently, there are 3 marketed screening tests using SEPT9: Epi proColon 1.0 (Epigenomics), ColoVantage(Quest Diagnostic) and RealTime ms9 (Abbott)[4]. Church TR et al, evaluated its screening potential, as part of a large prospective trial based in the US and Germany, of SEPT9 in 7941 asymptomatic, average risk individuals[11]. They found that its sensitivity was 48.2% and specificity of 91.5% in CRC, whilst its sensitivity for advanced adenomas was low (11.2%)[11]. Clearly, this marker needs further study with each kit to determine the sensitivity and specificity accurately.

Measuring methylated DNA in cell-free circulating nucleosomes by enzyme-linked immunosorbent assay (ELISA) technique gives a sensitivity of 33% with 95% specificity in distinguishing CRC patients from healthy individuals[4,12]. Methylation-based markers with prognostic value include helicase-like transcription factor (HLTF), which is associated with tumor size, metastasis, tumor stage and risk of disease recurrence[13]. Hyperplastic polyposis 1 (HPP1) and the deafness and autosomal dominant 5 gene (DFNA5) have also shown promise as

prognostic markers but need further study to determine their use in clinical practice[14,15].

Circulating RNA

Circulating RNA-based markers have also been investigated but their susceptibility to RNase in blood (especially messenger RNAs), which affects their stability, has been a challenge[4].

MicroRNAs on the other hand are relatively stable and are immune to the RNase activity. They are small, single-stranded, non-coding RNAs which when bound to a target gene, lead to their suppression and depending on the function of the gene, they can act as tumor suppressors or as oncogenes[4]. MiR-21 and miR-92a have shown promise as diagnostic markers for CRC and adenoma, although further study is required due to the variable reported sensitivities and specificities in the literature[16]. The use of panels of microRNAs have been tested, however there is great variability in the results to be clinically useful as diagnostic tests yet[17]. MiR-200c, miR-141, miR-21 and miR-221 have shown potential as prognostic markers[4]. MiR-19a can be useful in predicting resistance to first-line FOLFOX chemotherapy[18].

Tissue biomarkers

Microsatellite instability (MSI)

These are short sequences of 1-6 base pairs in the genome have a high risk of mutations and which are corrected by the MMR systems[19]. MSI is responsible for sporadic and inherited CRC cases. It is reported that localized CRCs with MSI have a better prognosis than the ones with microsatellite stability (MSS)[19,9]. MSI can therefore be used as a diagnostic and a prognostic marker. The use of pembrolizumab which is a monoclonal antibody to programmed death (PD)-1, which is in phase II trials, has shown good rates of response in patients with MSI tumors[8].

KRAS

Mutations to this gene causes an activation of the EGFR pathway. Mutations to this gene confer resistance to anti-EGFR antibodies, cetuximab and panitumumab. It is thus an important predictive factor to response to treatment with EGFR inhibitors[9,19,20]. Some studies showed that even mutations to NRAS have a negative effect to the response to anti-EGFR treatment. Its value as a prognostic factor has also been evaluated with conflicting results.

BRAF

This gene is frequently mutated in CRC, and its most common mutation is V600E, which causes the activation of the MAPK pathway[19]. BRAF V600E is important in that if MSI is detected, then Lynch syndrome can be excluded and in the MSS form, it is associated with a poor prognosis[19,21]. They are found more commonly in right-sided CRC[9]. It can also predict resistance to anti-EGFR therapy. BRAF and RAS mutations are usually mutually exclusive.

APC

Adenomatous polyposis coli is an oncosuppressor gene, whose mutation usually causes Familial Adenomatous Polyposis syndrome (FAP) but is also frequently mutated in most of sporadic CRCs. It is a poor prognostic factor[22].

VEGF

Vascular endothelial factor is a pro-angiogenic factor and the presence of mutations is associated with tumor aggressiveness and metastases, hence poor prognosis[23,19].

EGFR
Epidermal Growth Factor is a transmembrane tyrosine kinase receptor and is a target of 2 currently available monoclonal antibodies: cetuximab[24] and panitumumab. Unfortunately, a few months after treatment resistance to the therapy occurs [9]. HER2 has been implicated in conferring resistance to EGFR treatment. Phase II studies showed that the addition of dual HER2 blockade with transtuzumab and lapatinib, in these patients with HER2-amplified CRC resulted in 35% overall response rate and a median time to progression of 5.5 months[9].
Some studies showed that high EREG which is an EGFR ligand, is a favorable prognostic factor and is associated with longer PFS in patients receiving ant-EGFR treatment[25]. The use of AREG levels as a prognostic factor shows conflicting results.[9]

18q Loss of Heterozygosity (LOH)
This means loss of one parental allele and it is observed in up to 70% of CRCs. It is associated with poor prognosis in patients with stage II or III disease and could benefit from adjuvant chemotherapy[9].

SMAD4
This is an oncosuppressor protein that intervenes in the intracellular pathway of TGF-β. This is related to tumor invasion and poor response to chemotherapy hence can be used as a prognostic and predictive marker[26].

Insulin-like Growth Factor II mRNA-Binding Protein 3 (IMP3)
This is normally produced during embryogenesis and is undetectable in adult patients. It has been reported that expression in CRC, is an important prognostic factor and predictor for metastasis.

TRAF2- and NICK-Interactive Kinase (TNIK)
This is a kinase which is activated when β-catenin binds to it. If there are high levels they are related to distant metastasis in stage II and III tumors.

Telomerase
Telomeres consist of repeats of a DNA sequence and comprise the terminal structures in eukaryotic chromosomes. The length of telomeres is maintained by the enzyme telomerase. Numerous studies have shown that an increased activity of this enzyme and its length can be used as prognostic marker[27]. In blood telomerase can be used as a diagnostic a marker and therapeutic target[28].

Mutated in Colorectal Cancer (MCC)
This is a multi-functional protein in the Wnt and NGkB pathways. Mutations in this gene has been associated with CRC but needs further investigation to establish its value as a prognostic marker.

Phosphatase and Tensin Homolog Protein (PTEN)
PTEN is a tumor suppressor gene in the PI3K pathway. Its loss is associated with aggressive CRCs and is a predictor for nonresponse to cetuximab and favorable predictor factor for wild-type KRAS CRCs treated with cetuximab[19].

Ezrin
This is a cytoskeletal protein that plays a role in cell motility, invasion and metastasis[19]. It is associated with a worse prognosis and is currently under investigation as an antimetastatic treatment target[19].

Stool-based markers
Faecal immunochemical tests and faecal occult blood tests are in use for screening for CRC. The FIT sensitivity, if combined with blood SEPT9 tests has a reported sensitivity of 94% for CRC. However their sensitivity for adenomas in very low[29]. Detection of stool DNA may have promise, however the vast majority of DNA in stool is derived from intestinal bacteria rather than the patient[9]. Patient compliance with the test is also an issue due to aversion in handling stool.

Conclusion

CRC is a heterogeneous disease, which despite the advances in treatment and screening methods, still has an appalling survival rate due to its late detection. It is important to continue with enriching our knowledge of the molecular pathways of this disease to improve our diagnostic, prognostic, predictive and therapeutic therapies.

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