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Review Article

Prognosis in colorectal cancer beyond TNM☆

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ABSTRACT

Introduction: Colorectal cancer is one of the neoplasms with the greatest social impact. Given the great molecular heterogeneity and diversity of pathophysiological mechanisms, it is difficult to define prognostic factors that could guide therapy.
Objectives: To identify the molecular prognostic factors that may be of interest in clinical practice and to synthesize the existing evidence.
Material and methods: The search for the articles was carried out using the PubMed platform and the keywords "sporadic colorectal cancer and prognosis", for articles published between 2014 and 2019. We selected all articles published on studies in humans and written in English or Portuguese. Of the 215 articles found, 35 articles were selected to perform this review.
Results: Current evidence supports the use of four molecular markers in clinical practice – KRAS, NRAS and BRAF (EGFR signalling pathway) and the mismatch repair status.
Conclusion: The use of molecular biomarkers in clinical practice to define prognosis is still little supported by the existent evidence. The studies are slightly contradictory, so new projects and international collaborations must be carried out in this area to obtain more robust evidence.

Prognóstico no carcinoma colorretal para além do TNM

Palavras-chave:
Carcinoma colorretal
Prognóstico
Instabilidade de microssatélites
Instabilidade cromossómica
Biomarcadores

RESUMO

Introdução: O carcinoma colorretal é uma das neoplasias com maior impacto social. Dada a grande heterogeneidade molecular e diversidade de mecanismos fisiopatológicos, torna-se difícil definir fatores de prognóstico que orientem a terapêutica.
Objetivos: Identificar os fatores de prognóstico moleculares que poderão vir a ter interesse na prática clínica e fazer uma síntese da evidência existente.

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Material e métodos: A pesquisa dos artigos foi realizada recorrendo à plataforma *PubMed* e utilizou-se as palavras-chave "*sporadic colorectal cancer and prognosis*", para artigos publicados entre 2014 e 2019. Foram selecionados todos os artigos publicados sobre estudos em humanos e escritos em inglês ou em português. Dos 215 artigos encontrados, foram selecionados 35 artigos para realizar esta revisão.

Resultados: A evidência atual apoia a utilização de quatro marcadores moleculares na prática clínica – KRAS, NRAS e BRAF (via de sinalização do EGFR) e o estado *mismatch repair*.

Conclusão: A utilização na prática clínica de biomarcadores moleculares para definir o prognóstico é ainda pouco apoiada pela evidência disponível. Os estudos são algo contraditórios, pelo que novos projetos e colaborações internacionais devem ser realizados neste âmbito para se obter evidência mais robusta.

Introduction

Sporadic colorectal carcinoma (CRC) results from the interaction of somatic genetic mutations with environmental factors, with 85% of cases following the adenoma-carcinoma sequence.[1] It is a very prevalent condition in Western countries, contributing with approximately 10% to the cancer-related mortality.[2] The CRC is the third cancer type with the highest incidence worldwide, being, however, the second leading cause of cancer-related death.[3,4] In Portugal, similarly to other European countries, this neoplasm is a frequent cause of death, being the second most frequent one.[5] The increase in incidence in developed countries seems to be related to population aging, poor dietary habits and an increase in risk factors, such as smoking, physical inactivity and obesity.[2,6]

According to current evidence, tumor extension staging according to TNM remains the most important prognostic factor.[4,7,8] However, several prognostic factors have an impact on the natural history of the disease, namely – demographic, histopathological, immunological, molecular and multidisciplinary intervention factors.[7] This justifies the interindividual difference in response to therapy and prognosis inside the same TNM stage.[9]

The CRC is a very heterogeneous disease with several underlying pathophysiological mechanisms, so it is difficult to precisely define the prognostic factors to guide the therapy.[7] It is increasingly accepted that the CRC should be subdivided into different prognostic groups defined by combinations of molecular biomarkers that reflect the different carcinogenesis pathways.[10] Four main biological pathways are currently known in the sporadic CRC carcinogenesis – Chromosomal Instability (CIN), Microsatellite Instability (MSI), CpG Island Methylator Phenotype (CIMP) and, more recently, the one based on Ribonucleic Acid (RNA) profiles.[1,7,11–13]

The classic model (chromosomal instability) presupposes the adenoma-carcinoma evolution and explains most CRCs.[7,14] Molecularly, this model is based on tumor suppressor gene Adenomatous Polyposis Coli (APC) inactivating mutations and the early loss of regulation by the Wnt signaling pathway, followed by the oncogenes Kirsten Rat Sarcoma viral oncogene homolog (KRAS), phosphatidylinositol-4,5-bisphosphate 3-kinase, Catalytic Subunit alfa (PIK3CA) e B-Raf proto-oncogene (BRAF) activating mutations.[7,13,14] Subsequently, malignant transformation is dependent on mutations in genes such as TP53 and Sterile Alpha Motif Domain containing protein 4 (SMAD4), as well as chromosomal instability, which results in the loss of heterozygosity (LOH).[7,11]

However, about 15% of CRCs do not develop via chromosomal instability.[7] In these cases, there is a deficiency in the mechanisms of DNA Mismatch Repair (dMMR), which results in high levels of MSI.[7,11,15,16] About half of the cases of sporadic CRC with dMMR also have the BRAF gene mutation.[7]

Finally, CIMP is related to the hypermethylation of deoxyribonucleic acid (DNA) in CpG islands (tumor suppressor gene promoting regions) and hypermethylation of MutL protein Homolog 1 (MLH1) often occurs, making it possible to differentiate between two distinct pathways - CIMP-high ($\geq$ 3 of 5 altered markers) is associated with BRAF mutations, while CIMP-low ($\leq$ 2 of 5 altered markers) is associated with KRAS mutations.[1,11–13]

The identification of molecular biomarkers may change the management of patients with CRC, as the provision of individualized and good-quality care is highly dependent on individual prognostic modeling.[7] It is known that the prognosis definition based only on TNM staging is insufficient, namely in stage II and III tumors, resulting in insufficient treatment of stage II tumors and overtreatment of stage III tumors.[17]

Thus, tumor molecular markers have gained prominence as potential prognostic factors and targeted therapeutic approach; however the existing evidence is still very scarce.[7,17] The objective of this review is, therefore, to identify the molecular prognostic factors that may be of interest in clinical practice and synthesize the existing evidence.

Materials and methods

The study was carried out using the PubMed platform and the keywords "sporadic colorectal cancer and prognosis" were used for articles published between 2014 and 2019. All articles published on studies performed in human beings and written in English or Portuguese were selected. The survey was carried out on November 3, 2019 and the best match was selected. In total, 215 articles were found, and the fol-

lowing were excluded from this review –50 did not address prognostic factors; 45 addressed prognostic factors other than the selected ones; 18 addressed early-onset CRC; 53 addressed non-sporadic CRC (hereditary, neuroendocrine or in the context of inflammatory bowel disease); nine did not allow access to the full text; two were written in Japanese; one was written in Russian and two were performed using animal models. Thus, in total, 35 articles were selected to carry out this review. A parallel search was also carried out and five more relevant articles were selected to complete the missing information, namely referring to epidemiological data and pathophysiological mechanisms.

Results

Chromosomal instability pathway

Chromosomal instability is present in approximately 85% of CRC cases.[13] These tumors are characterized by extensive somatic chromosomal changes (numerical and/or structural) throughout the entire genome.[1,18] These anomalies can lead to KRAS activation, loss of APC and TP53 (through chromosome 17p deletion) and the loss of SMAD4 (through alteration in the Transforming Growth Factor Beta – TGFβ tumor suppressor gene), which promotes an increase in clonal diversity.[1,13,14,19,20] The TP53 mutation and subsequent loss of heterozygosity is a fundamental event in the pathogenesis of this pathway, coinciding with the conversion from benign to malignant alteration, constituting a factor of poor prognosis.[1] Although not used in clinical practice, the chromosomal instability phenotype is associated with a worse prognosis (survival) when compared to tumors with MSI.[7,18]

APC mutation

APC is a tumor suppressor gene of which main function is the regulation of the Wnt / β-catenin signaling pathway, which interferes with processes such as DNA repair, cell cycle, chromosomal instability, cell adhesion and apoptosis.[1,11,21] The APC mutation can be found in approximately 70% of sporadic CRCs and is responsible for the constitutive activation of the Wnt/β-catenin pathway, resulting in adenomas.[1,21,22]

The steps of this process are not yet fully known, but inflammation is thought to play a major role.[21] Some inflammation inducers are increased in malignant tissue and their relationship with the regulation of β-catenin expression, which is increased in proliferating cells, has been demonstrated.[21] Interleukin-17A (IL-17A) has a pro- tumorigenic role when it is expressed at high concentrations through stimulation of the Cyclooxygenase-2 (COX-2) / Prostaglandin E_2 (PGE_2) pathway.[21] A recent study demonstrated that increased expression of IL-17A in tumor tissue is associated with increased recurrence and decreased survival, resulting in a worse prognosis.[21] The increase in the expression of Peroxisome Proliferator-Activated Receptor gamma (PPARγ) has a beneficial impact on CRC, as it provides a good prognosis by inhibiting the production of IL-17A, the effects of β-catenin and cell growth.[21] However, these molecules are thought to have an intricate and interdependent relationship during the carcinogenesis processes.[21] COX-2 has the capacity to modulate both the Wnt / β-catenin and the PPARγ pathways.[21] PPARγ activation inhibits β-catenin expression and IL-17A activation.[21]

Dickkopf (DKK)-1 is a cytoplasmic tumor suppressor that modulates cell proliferation and survival, being mutated in cells with the constitutively active Wnt/β-catenin pathway.[22] The presence of DKK-1 in the nucleus seems to control gene expression, being able to inhibit the growth of tumor cells through alternative routes to Wnt/β-catenin.[22] Thus, DKK-1 seems to perform antagonistic actions depending on whether it is found in the cytoplasm (tumor suppressor) or in the nucleus (chemoresistance inducer).[22] A recent study showed that serum DKK-1 levels constitute a predictor of tumor invasion and recurrence in stage II-III tumors, so it can be used as a biomarker to predict the benefit of systemic therapy in metastatic CRC.[22]

Unfortunately, several APC mutations have been described, with different effects on tumorigenesis, which makes it difficult to apply effective strategies in clinical practice.[11] Thus, β-catenin and APC are inadequate prognostic markers, as they are frequently altered in most CRCs, making them markers of poor differentiation.[11]

KRAS and NRAS proto-oncogene (NRAS) mutation

KRAS is a proto-oncogene of the RAS family, involved in the signaling pathway activated by the Epidermal Growth Factor Receptor (EGFR).[11,23] This pathway, responsible for tumor proliferation, invasion, migration and neovascularization, is altered in about 40% of CRC cases and contributes to its pathogenesis at a very early stage, being associated with hyperplastic polyps.[1,11,23,24] The activation of this oncogene appears consistently after the APC inactivation during tumor progression.[1]

The usefulness of KRAS as a prognostic factor is quite contradictory.[11,13] However, some studies say that the activation of this pathway translates into a worse prognosis.[1] Despite the lack of evidence to support its use as a prognostic factor, screening of the KRAS mutation in exon 2 in metastatic CRCs has an impact on the treatment that is offered.[24]

In fact, wild-type tumors for this mutation can be treated with monoclonal anti-EGFR antibodies (such as cetuximab and panitumumab) and are already routinely used in clinical practice.[11,24] However, several studies have also shown that tumors with KRAS mutations have worse disease-free progression and overall survival rates when treatment includes anti-EGFR therapy (such as panitumumab), when compared to patients with wild-type KRAS tumors.[7,11,23] KRAS and NRAS mutations constitutively activate the signaling pathway, which makes it resistant to the action of anti-EGFR antibodies.[13] Several meta-analyses have further demonstrated that KRAS mutations in exons 3 and 4 also predict resistance to therapy with anti-EGFR monoclonal antibodies.[24] Similarly, the NRAS and BRAF V600E mutations also results in a poor response to therapy with anti-EGFR monoclonal antibodies.[23,25] In a retrospective, multicenter analysis of patients treated with cetuximab prior to the selection of the KRAS mutational state, tumors with BRAF V600E mutations,

when compared to wild-type BRAF tumors, had a significantly lower response rate and an equally lower disease control rate, which resulted in lower overall survival.[25] However, several studies have failed to reach statistical significance to determine whether the BRAF V600E mutation is a primary resistance biomarker or not to anti-EGFR monoclonal antibodies used in the treatment of CRC.[25] A CRC with NRAS mutation shows less lymphatic invasion, affects older patients and has a more distal location, which entails a better prognosis, when compared with CRC with KRAS mutation.[23] It is also known that tumors with KRAS mutations have a worse prognosis, even when submitted to chemotherapy with 5-fluorouracil (5-FU).[23] However, it is known that the prognosis varies according to the mutation found in the gene, even if it occurs at the same location in the genome.[11,13]

In any case, according to the recommendations of the Journal of Clinical Oncology, patients with CRC who are candidates for anti-EGFR therapy should be tested for KRAS and NRAS mutations in codons 12 and 13 of exon 2, 59 and 61 of exon 3, and 117 and 146 of exon 4.[26]

Regarding targeted therapies, the results are quite controversial. The BRAF V600E mutation constitutively activates BRAF; hence, drugs such as vemurafenib and dabrafenib can be used to inhibit this signaling pathway.[25] Despite the excellent results achieved in the antitumor activity of these drugs in metastatic melanoma, the same was not observed in patients with CRC and BRAF V600E mutation. These unexpected results have motivated research in this area, but there is still no evidence that could change the clinical practice.[25]

PIK3CA mutation

PIK3CA corresponds to a proto-oncogene that may be mutated in the CRCs and seems to be related to lower survival rates in patients who undergo colon recession, suggesting that these patients may benefit from adjuvant therapy.[1,11]

Some studies suggest that COX-2 has an increased expression in CRC due to a PIK3CA mutation.[1] This enzyme is responsible for converting arachidonic acid into prostaglandins, with a pro-inflammatory and pro-proliferative effect, with this action being blocked by acetylsalicylic acid.[1] The regular use of this drug was effective in reducing the number of deaths among patients with CRC and PIK3CA mutation, when compared with patients who did not take this compound or with wild-type PIK3CA tumors.[1] Therefore, acetylsalicylic acid can be used as adjuvant therapy and as a secondary prevention in patients with PIK3CA mutation, as it inhibits the production of proliferative mediators.[1] However, the prognostic role of the PIK3CA mutation remains to be clarified.[11]

Microsatellite instability pathway and DNA Mismatch Repair (MMR)

MSI is a key biomarker in CRC, with a leading role in the diagnosis and prognosis. MSI corresponds to mutations that occur in specific areas of the genome that contain microsatellites and that result in a defect in the genes involved in the DNA mismatch repair mechanisms – MLH1, MutS protein Homolog 2 (MSH2), MutS protein Homolog 6 (MSH6) and PostMeiotic Segregation increased 2 (PMS2).[9,11,14,27,28] MSI is present in around 12% of sporadic CRCs and is more frequent in the early stages, causing activation of oncogenes and inactivation of tumor suppressor genes in affected cells.[10,11,16,28,29]

The dysfunction in DNA MMR mechanisms in sporadic CRCs is a consequence of the epigenetic methylation of the MLH1 gene promoter, unlike Lynch syndrome, which requires a germline mutation in one of the MMR genes.[9,14] The CIMP phenotype is closely associated with MSI, mutation in BRAF V600E, wild-type KRAS and wild-type TP53.[1,8,14] It is therefore characterized by a tendency towards DNA hypermethylation and genetic silencing, which it is histologically reflected in sessile serrated adenomas.[1,8,14] Due to this overlap between the CIMP phenotype and MSI, patients who develop a sporadic CRC with MSI have a methylation phenotype, in contrast to patients with Lynch syndrome.[8]

According to the National Cancer Institute, it is possible to subdivide CRCs with MSI into three groups according to the Bethesda panel, which analyzes five specific microsatellite markers – if $\geq$ 2 or $\geq$ 30% of the repetitions are unstable, the tumor is classified as H-MSI (High Microsatellite Instability); if only one or <30% of the repetitions is unstable, the tumor is classified as L-MSI (Low Microsatellite Instability); finally, if no repetition is unstable, the tumor is classified as MSS (Microsatellite Stable).[16,19]

The establishment of the tumor pathophysiological mechanism has very important clinical implications, namely in the prognostic prediction, in the increased incidence of metachronous tumors and in the differential response to treatment.[7,9,10,15,28,30] For example, a study published in 2003 demonstrated that, although CIMP+ patients have a lower overall survival than CIMP- patients, patients with CIMP+ tumors treated with adjuvant 5-FU have longer overall survival.[8]

However, the poor prognosis associated with the CIMP phenotype in patients with MSI is thought to be associated with the BRAF V600E mutation.[8] Histologically, tumors with MSI show differentiation in signet ring, marked immune response with evident intraepithelial and peritumoral lymphocytic infiltrate, as well as a poorly differentiated and mucinous phenotype.[9,10,12,30–32] It usually affects older and female patients, with a preferential location in the right colon and with less hepatic involvement.[8,9,28,33]

Several studies have shown that patients with early-stage tumors with dMMR have better stage-adjusted survival and less aggressive clinical behavior when compared to patients with tumors with proficient DNA MMR mechanisms (pMMR).[7,10,12,16,27,33,34] The results of a meta-analysis that included 7,642 patients indicate that tumors with MSI have a significantly better prognosis than tumors without MSI (overall survival 0.65, with a confidence interval of 0.59–0.71).[35] Additionally, they have a lower risk of metastasis and are associated with earlier stages – there is a low incidence of tumors in stages III and IV.[7,9,10,32] A 2016 study evaluated 1250 patients with CRC stages I-II and found that H-MSI was associated with a decreased risk of ganglion and distant metastases, with an improved disease-free survival.[16] However, H-MSI stage III tumors exhibited more aggressive pathological features, including higher rates of lymphovascular and perineural invasion, when compared with H-MSI stage I-II tumors.[16,35]

The change in prognosis with the advancing tumor stage is thought to be dependent on the BRAF V600E mutation.[9,16]

In fact, the better prognosis for H-MSI tumors is more evident in stages I-II of the disease, but this association tends to decrease or even to reverse as the tumor stage progresses.[9,16] Patients with pMMR and BRAF V600E or KRAS mutations have statistically shorter survival lengths when compared to patients with pMMR without the aforementioned mutations.[9,15,27] CRCs with pMMR without BRAF V600E and KRAS mutations have a prognosis similar to that of CRCs with dMMR (either sporadic or family subtype).[15,27] However, the dMMR phenotype in metastatic CRCs is rare (prevalence of 3%–5%) and a predictor of chemoresistance to regimens that use oxaliplatin or 5-FU, although the mechanism is not yet fully understood.[1,28,34] It is thought, once again, that the BRAF V600E mutation may contribute to this poor prognosis, with four phase III studies demonstrating a decrease in disease-free and overall survival.[27,28,34,35]

Metastatic CRCs (stage IV) with a high MSI frequency have an increased expression of Vascular Endothelial Growth Factor (VEGF), when compared to CRC without MSI, which may be the basis for increased angiogenesis and the appearance of liver metastases.[32] Therefore, it is possible to use targeted therapies, such as bevacizumab (anti-VEGF), which decrease the number of circulating endothelial progenitor cells and VEGF levels.[11,32]

However, metastatic CRCs with dMMR respond better to treatment with pembrolizumab, with higher response and survival rates being achieved,[34] when compared with pMMR patients. Thus, H-MSI tumors in the earlier stages have a better prognosis, due to their highly immunogenic properties and the high expression of Programmed cell Death 1 (PD-1), Programmed cell Death Ligand 1 (PD-L1), Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4), Lymphocyte-Activation Gene 3 (LAG-3) e Indoleamine 2,3-Dioxygenase (IDO) and do not seem to benefit from adjuvant therapy with 5-FU or oxaliplatin.[9,11,16,30,33–36]

In fact, CRCs with dMMR have alterations in the membrane expression of HLA class I molecules, suggesting the occurrence of a phenomenon called “adaptive immune escape”.[36] Tumors with dMMR are subject to an immune activity attack, which motivates the selection of tumor cells resistant to the host’s immune system.[36] One study demonstrated that CRCs with dMMR have an increased expression of CD80 (a co-stimulatory molecule), as well as abundant CD8+ and Th1 cell infiltrate, which reflects the importance of immunological mechanisms and their impact on the prognosis of this CRC subtype.[9,31,37,38]

That said, pembrolizumab (a PD-1 inhibitor) seems to play an important role in the treatment of metastatic CRC with MSI, resulting in better response rates and disease-free survival.[33,37] However, tremelimumab (anti-CTLA-4) did not demonstrate efficacy in the performed study.[33] As for nivolumab (anti-PD-1) and nivolumab + ipilimumab (anti-CTLA-4), they are currently being tested in phase II clinical trials in CRC with recurrent metastatic MSI and the preliminary results indicate a benefit of the combined therapy, due to its synergistic effect.[25,33,39] According to the recommendations of the National Comprehensive Cancer Network, all patients with stage II CRC should be tested for MSI, as tumors with H-MSI have a good prognosis and do not benefit from adjuvant chemotherapy with 5-FU.[35] As for the significance of the MSI status as a prognostic factor in patients with metastatic disease, it remains to be clarified, and its screening is controversial.[35]

BRAF V600E mutation

BRAF is an oncogene that encodes a kinase of the RAF family, which is regulated by the activity of the KRAS protein, frequently mutated in CRCs with MSI.[11,14,27] The mutation in this gene results in the activation of the RAS-RAF-MEK pathway and it is present in 5%–10% of CRCs.[11,23,25] Histologically, tumors with BRAF mutations are poorly differentiated and mucinous, with a preferential location in the right colon, a high rate of peritoneal involvement and distant ganglionic metastasis, but with a low frequency of lung metastases.[13,25] The presence of this mutation in dMMR cancers and loss of MLH1 expression virtually excludes the possibility of Lynch syndrome, as it is almost exclusive to sporadic CRC.[9,10,16,27,28,33]

The BRAF V600E mutation can result in CRC with pMMR or dMMR, with MLH1 hypermethylation in codon 600 being the key phenomenon of the pathway that results in Dmmr.[5,9,25] The mutation of codons 594 and 596 are not associated with MSI.[25] A post-hoc analysis of the PETACC-3 study demonstrated that the BRAF V600E mutation was significantly associated with poorer survival in patients with pMMR tumors.[25] A study carried out on a cohort followed at the Mayo Clinic showed that patients with CRC and a BRAF mutation that did not affect codon 600 were less likely to have a high-grade histology (0 vs. 63.5%; $p < 0.0001$) or tumors in the right colon (50 vs. 79%; $p = 0.04$), which gave them a better prognosis.[25]

In the earlier stages, the identification of the BRAF mutation does not seem to have any impact on the therapeutic decision, as the reported results are quite contradictory.[25] However, this mutation confers a dramatic prognosis in more advanced diseases and is used as a stratification factor for adjuvant therapies, due to its impact on the prognosis after recurrence.[25] A retrospective cohort study published in 2018 that included resectable stage III CRC concluded that the establishment of MSI is a predictor of the response to chemo-adjuvant therapy only when the results are interpreted in combination with BRAF status.[40] The presence of BRAF mutations is related to worse survival rates in tumors without MSI, while the presence of a high frequency of MSI cancels the negative effect of the BRAF mutation on survival. [10,11,27,33] Therefore, patients without MSI and with BRAF mutations require more aggressive adjuvant therapy. [10] Particularly in the rectum, the BRAF V600E mutation, although rare, is associated with a very poor prognosis.[10]

In addition, wild-type KRAS tumors with a BRAF mutation do not respond to EGFR inhibitors, which confers a poor prognosis. [11,24] Despite the scarcity of phase III randomized clinical trials, the European Society of Medical Oncology (ESMO) ensures that the current therapy for metastatic CRC with BRAF V600E mutation should be based on the use of FOLFOXIRI (leucovorin + 5-FU + irinotecan + oxaliplatin) in combination with bevacizumab or BRAF inhibitors, in patients

who have never been submitted to chemotherapy. [11,25,27] However, in the future, it may be necessary to stratify patients with BRAF mutations according to their MSI status, aiming to predict the prognosis with more accuracy and establish the most appropriate therapy. [27] According to recommendations published in the Journal of Clinical Oncology and the National Comprehensive Cancer Network, all patients with metastatic CRCs should be tested for the BRAF V600E mutation to achieve better prognostic stratification.[25,26,40]

Discussion

The use of molecular markers in clinical practice to define prognosis is still little supported by the available evidence. However, the identified mutations have a great impact on the treatment provided to patients, always based on the purpose of personalized and targeted medicine.

Within the three main carcinogenesis pathways, the presence of MSI is the most useful biomarker in clinical practice.[1,2] Among the tumor suppressors, APC cannot yet be considered a prognostic marker, whereas SMAD4 and TP53 have the potential for such. With regard to proto-oncogenes, BRAF, KRAS and PIK3CA are strong candidates for prognostic markers.[2,39]

The status of the DNA MMR mechanisms is not only useful in defining the prognosis. This marker is one of the most extensively studied and can play a key role in predicting response to treatment.[7,9,15] For instance, chemotherapy regimens based on 5-FU are frequently used as adjuvant therapy in stage III tumors and are responsible for reducing the risk of recurrence and death.[8,9,35,40] Given the harmful potential of chemotherapy, it is essential to identify biomarkers that allow predicting tumor response and selecting the patients who can really benefit from this treatment.[35,40] In stage II tumors with dMMR, adjuvant chemotherapy with 5-FU has no clinical benefit.[7,9,28] However, the addition of oxaliplatin to fluoropyrimidines is associated with a survival benefit in patients with high-risk stage II tumors and stage-III ones.[28]

Moreover, it is quite often observed that dMMR tumors have an increased expression of immune checkpoints, including PD-1, the therapeutic target of pembrolizumab.[7] Due to the aforementioned reasons, MSI screening is currently recommended in patients who meet the Bethesda guidelines.[12] However, it is accepted that routine dMMR immunohistochemical or molecular MSI analysis is more cost-effective, sensitive and specific for all patients with CRC.[12,33]

Studies have not been able to consistently demonstrate the prognostic impact of the KRAS mutation in CRC stages II and III.[15] BRAF V600E mutations are normally associated with poor outcomes, namely in metastatic CRCs.[15] In summary, according to recommendations published in the Journal of Clinical Oncology, the currently available evidence supports the screening for mutations in the genes involved in the EGFR signaling pathway.[26] Mutations in BRAF and MMR have a clear prognostic value, whereas mutations in KRAS and NRAS can be used as negative predictors of the benefits of anti-EGFR antibody therapy.[1,13,26,27] BRAF mutations are consistently associated with poor outcomes in patients with metastatic CRC, including those who showed recurrence after adjuvant therapy.[13,26] Patients with dMMR and more localized tumors have a better prognosis.[26] The classification based on the CIMP is very useful to understand the pathophysiological mechanisms underlying some CRCs.[1] However, its usefulness as a clinical tool or as a biomarker is still uncertain and cannot be used as an independent prognostic factor.[1,8]

There are some reasons that justify the absence of prognostic biomarkers transversal to clinical practice, namely poorly selected samples, without clinical relevance or without sampling power, as well as the absence of validation and reproducibility for biomarker application in the clinical practice.[4]

Conclusion

The CRC is a biologically very heterogeneous disease. A typical sporadic CRC has about two to eight precursor mutations, which fall into a series of random events, making it a genetic and epigenetically unique entity. Knowledge of the main carcinogenesis pathways of the CRC is essential to provide the best treatment for patients. The conventional treatment for CRC remains the surgical procedure in combination with adjuvant chemotherapy.[14] At the same time, targeted molecular therapy has gained more and more ground due to advances in the knowledge of the pathophysiological mechanisms. Take the example of the treatment of metastatic CRC – the conventional treatment has evolved from 5-FU alone, to a combination of 5-FU, leucovorin and irinotecan (FOLFIRI) or oxaliplatin (FOLFOX) and the use of FOLFOXIRI in combination with biological agents (such as bevacizumab) is currently recommended by ESMO.[24,25,33,39]

However, it is a difficult task to validate the different molecular targets as good prognostic indicators. The CRC has a huge impact on today's society. That being said, there has been a growing effort in the search for new molecular markers that will allow us to stage the disease more accurately and offer a more targeted treatment, avoiding unnecessary costs for society. This action has been reflected in a reduction in CRC mortality rates, partly due to an earlier diagnosis and treatment, together with an increased understanding of the genetic and molecular bases of the disease. However, one should never neglect the importance of the interdependence of the several prognostic factors. As the studies are somewhat contradictory, new studies and international collaborations must be carried out in this context to obtain more robust evidence.

Conflicts of interest

The authors declare no conflicts of interest.

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