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Colitis-associated colorectal cancer: pathways of carcinogenesis and biomarkers

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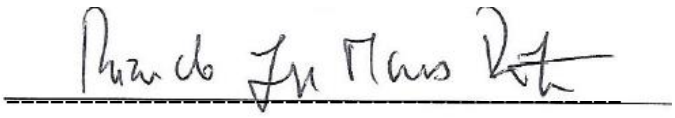

(Ricardo Jorge Marcos-Pinto, MD PhD)

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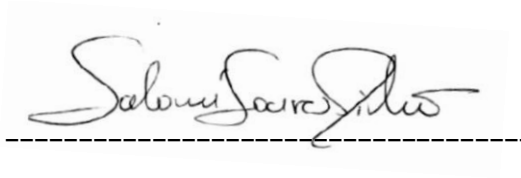

(Salomé Pinho, DVM, PhD)

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Resumo

Introdução

A doença inflamatória intestinal está associada a risco acrescido de cancro colorretal. Embora a carcinogénese associada a esta doença ainda seja mal conhecida, sabe-se que os mecanismos não são idênticos à sequência de adenoma-carcinoma do cancro esporádico e que as alterações genómicas são semelhantes, mas diferem na frequência e na ordem de eventos carcinogénicos. De forma a poder intervir precocemente, através de estratégias preventivas do desenvolvimento tumoral, é essencial a compreensão dos perfis genéticos e moleculares por detrás da carcinogénese, para permitir diagnóstico precoce, prevenção e tratamento mais eficazes, como também prever a resposta terapêutica e identificar biomarcadores prognósticos. Até agora, poucas moléculas estão propostas como possíveis biomarcadores, mas é provável que a Glicomedicina traga respostas nesta área.

Objetivos

Revisão científica relativa à associação entre doença inflamatória intestinal e cancro colorretal, com destaque para as alterações a nível histológico e molecular que diferenciam a carcinogénese nestes casos da dos cancros esporádicos, bem como para os novos biomarcadores, sublinhando a importância que os glicanos têm vindo a ganhar nesta área.

Metodologia

Revisão de publicações científicas acedidas, maioritariamente, através da base de dados informatizada PubMed. De entre os artigos originais e de revisão publicados entre 2008 e 2019, em inglês, selecionei aqueles considerados de maior relevância, atualidade e rigor científico. As bibliografias destes artigos foram também analisadas para possivelmente identificar outras publicações a incluir na revisão.

Conclusões

A abordagem dos doentes com doença inflamatória intestinal tem ainda questões por resolver, incluindo necessidade de melhor tratamento e prevenção de complicações, nomeadamente o cancro colorretal. Em relação à prevenção, existem dados contraditórios sobre o efeito quimioprofilático dos anti-TNF, 5-ASAs e tiopurinas e, considerando que aumentam o risco de infeções e de alguns cancros, é importante avaliar a relação risco/benefício do seu uso crónico. Novas terapias estão a ser aprovadas, mas sua eficácia e segurança a longo prazo ainda não são bem conhecidas. Colonoscopias de vigilância também são altamente recomendadas, mas há dúvidas quanto à melhor técnica e altura em que devem ser realizadas, uma vez que alguns cancros se desenvolveram antes do início da vigilância, o que pode explicar por que não há redução da mortalidade associada.

Foram feitas algumas descobertas sobre a patogénese do cancro colorretal associado à doença inflamatória intestinal, que destacam o papel da resposta inflamatória e imunológica crónica como potenciadora das alterações genéticas carcinogénicas e que apoiam a possibilidade da contribuição do microbioma intestinal, visto que casos de cancro têm mais disbiose do que indivíduos saudáveis.

Em relação aos novos biomarcadores, a Glicomedicina parece ser muito promissora, tendo já revelado que níveis de *N*-glicanos ramificados, anticorpos anti-glicano e glicosilação das imunoglobulinas G podem constituir biomarcadores prognósticos precoces, e que a *N*-acetilglucosamina pode ser uma estratégia imunomoduladora específica e não tóxica para tratamento e quimioprofilaxia do cancro colorretal.

Palavras-chave

Biomarcadores; Cancro associado a Doença Inflamatória Intestinal; Cancro colorretal; Carcinogénese; Doença Inflamatória Intestinal; Glicanos.

Abstract

Introduction

Inflammatory bowel disease is associated with higher risk of colorectal cancer. Although colitis-associated cancer' pathogenesis remains unclear, it is known that the carcinogenic mechanisms differ from the adenoma-carcinoma sequence of sporadic cancer and that genomic alterations are similar but differ in frequency and in order of carcinogenic events. More than trying to prevent cancer, by improving disease treatment and surveillance, it is important to better understand the genetic and molecular profiles behind these cases' carcinogenesis, allowing earlier diagnosis, more effective prevention and treatment strategies, predicting therapy response and identifying prognostic biomarkers. So far, there are very few molecules proposed to be possible biomarkers, but it is likely that Glycomedicine research will bring new answers to this question.

Objectives

Scientific review on the association between colorectal cancer and inflammatory bowel disease, highlighting the histological and molecular changes that differentiate these cases carcinogenesis from that of sporadic cancers, as well as the new biomarkers, underlining the importance that glycans have been gaining in this matter.

Methods

Review of scientific publications accessed, mainly, through the computerized PubMed database. Among the original and review articles published between 2008 and 2019, in English, I selected those considered of greater relevance and scientific rigor. Those articles references were also analyzed to possibly identify other publications to be included in the review.

Conclusions

Inflammatory bowel disease' management still has unresolved issues, including need for better treatment and prevention of complications, namely colorectal cancer. Regarding prevention, there is conflicting data about the chemoprophylactic effect of anti-TNF, 5-ASAs and thiopurines and, considering they raise the risk of infections and of some cancers, it is important to assess the benefit/risk ratio of their long-term use. New therapies are being approved, but their long-term efficacy and safety are not well known yet. Colonic surveillance is highly recommended, but doubts remain regarding the best technique and timing in which colonoscopies should be performed, since some cancers developed before surveillance started, which may explain why there is no mortality reduction associated with it.

There are some new answers about the pathogenesis of colitis-associated colorectal cancer, which highlight the role of the chronic inflammatory and immune response as a potentiator of the carcinogenic genetic alterations and that support the possibility of intestinal microbiome contribution in carcinogenesis, since cancer cases have more dysbiosis than healthy individuals.

Regarding new disease biomarkers, Glycomedicine seems very promising in this matter and already revealed that levels of branched *N*-glycans, anti-glycan antibodies and immunoglobulin G glycosylation can constitute early prognostic biomarkers, and that *N*-acetylglucosamine can be an inexpensive and nontoxic targeted-specific immunomodulatory strategy for treatment and chemoprophylaxis.

Key Words

Biomarkers; Carcinogenesis; Colitis-Associated Cancer; Colorectal Cancer; Glycans; Inflammatory Bowel Disease.

List of abbreviations:

5-ASA – aminosalicylates
ACCA – anti-chitobioside carbohydrate IgA
AGA – American Gastroenterological Association
ALCA – antilaminaribioside carbohydrate IgG antibodies
AMCA – anti-mannobioside carbohydrate IgG antibodies
anti-TNF – anti-tumor necrosis factor
ASCA – anti-Saccharomyces cerevisiae
CAC – colitis-associated colorectal cancer
CADASIL – cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CD – Chron's disease
CIN – chromosomal instability
CRC – colorectal cancer
CSC – cancer "stem cells"
DCE – dye-spray chromoendoscopy
DFS – disease-free survival
dMMR – mismatch repair pathway defects
ECCO – European Crohn's and Colitis Organization
GlcNAc – *N*-acetylglucosamine
HD – high definition
HGD – high-grade dysplastic
IBD – inflammatory bowel disease
IgG – immunoglobulin G
JAK – janus kinase
LGD – low-grade dysplasia
MSI – microsatellite instability
NBI – narrow-band imaging
OS – overall survival
pANCA – perinuclear antineutrophil cytoplasmic antibody
PSC – primarily sclerosing cholangitis
ROS – reactive oxygen species
S1P – sphingosine 1 phosphate
SD – standard definition
SCENIC – Surveillance for Colorectal Endoscopic Neoplasia detection and management in IBD patients
TCR – T cell receptor
UC – ulcerative Colitis
WLE – white light endoscopy

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Introduction

Inflammatory bowel disease (IBD) – including Chron's disease (CD) and Ulcerative Colitis (UC) – constitutes a global public health challenge, associated with high morbidity, mortality and health-care costs[5,6]. Overall, patients with IBD have higher risk of colorectal cancer (CRC) than healthy individuals, which is one of the most feared complications of the disease[7–10].

Although the pathogenesis of colitis-associated colorectal cancer (CAC) remains unclear, some risk factors have been identified, and it seems that the inflammatory burden is the most important[11,12]. Therefore, a successful anti-inflammatory treatment, promoting high mucosal healing rates is essential in cancer prevention[12]. However, as a life-long illness, IBD has periods of relapse and remission and some patients do not respond to treatment or lose the initial response, so the inflammatory burden differs between individuals with the same disease duration[11,13–21]. Current therapies are based on anti-inflammatory drugs, immunomodulators and biologic agents that suppress the immune system but have undesired risks when used chronically[22–24]. Besides treatment, an important concern on this matter is cancer screening and surveillance, since the high mortality is partially due to diagnosis at late stages[7]. Colonic surveillance of IBD patients is highly recommended, in order to detect potentially resectable dysplastic lesions or potentially surgically curable early stage CAC, reducing its morbidity and mortality[9,24–28]. Nevertheless, more than trying to prevent cancer by improving IBD treatment and surveillance, it is important to better understand the genetic and molecular profiles behind these cases' carcinogenesis, not only for development of effective early diagnosis, prevention and treatment strategies, but also for identifying prognostic biomarkers.

So far, it is known that CAC develops from an inflammatory non-dysplastic mucosa, which evolves into dysplasia, to invasive adenocarcinoma and the carcinogenic events differ from the typical adenoma-carcinoma sequence of sporadic CRC[29–32]. Genomic alterations detected in sporadic CRC were also recognized in CAC, like Wnt/β-Catenin pathway activation, p53 mutations and MYC amplification, but they differ significantly in frequency and in order on the sequence of carcinogenic events[33–38]. Moreover, considering that chronic inflammation secondary to chronic infection may lead to carcinogenesis, like what happens between *Helicobacter pylori* infection and gastric cancer[39–41], in IBD, the

intestinal microbiome may also contribute to carcinogenesis through its interaction with the host damaged mucosa, metabolism and immunity[3,4]. Nevertheless, the immune and inflammatory response in IBD has definitely a key role in dysplasia and cancer development, since, prolonged over time, it leads to DNA damage[42].

Regarding prognostic biomarkers, few molecules are possible candidates, but none has cost-effectiveness evidence supporting its active search in all patients. Glycans have been reported to change significantly in many diseases[43–45] including IBD[46,47] and cancer[48], so, it is likely that Glycomedicine research will yield future translational opportunities, including predict therapeutic response and find new immunomodulatory strategies for IBD treatment and CAC chemoprophylaxis.

Objectives

This scientific review aims to gadder the latest knowledge about the association between colorectal cancer and inflammatory bowel disease, highlighting the histological and molecular changes that differentiate these cases carcinogenesis from that of sporadic cancer, as well as the new diagnostic and prognostic biomarkers, underlining the importance that glycans and Glycomedicine have been gaining in this matter.

This work is framed in a review article prepared by the IPATIMUP/i3S research group on Immunology, Cancer and Glycomedicine, of which I am coauthor, and whose purpose is to serve as the scientific basis for an investigation intended to better understand the carcinogenesis related to inflammatory bowel disease and to identify biomarkers in each stage of evolution from inflammation, through various degrees of dysplasia, to carcinoma.

Methods

Review of scientific publications accessed, mainly, through the computerized PubMed database, using the keywords (MeSH terms of Index Medicus) 'Biomarkers', 'Carcinogenesis', 'Colitis-Associated Cancer', 'Colorectal Cancer', 'Glycans' e 'Inflammatory Bowel Disease'. Among the original and review articles published between 2008 and 2019, in English, I selected those considered of greater relevance and scientific rigor. Those articles references were also analyzed to possibly identify other publications to be included in the review.

COLITIS-ASSOCIATED COLORECTAL CANCER

Epidemiology and Risk factors

IBD – including CD and UC – constitutes a worldwide public health challenge, associated with high morbidity, mortality and health-care costs[5,6]. During the 20th century, IBD was mainly a western disease, with incidence rising in USA, Canada, western Europe, Australia and New Zealand. However, in the 21st century, an epidemiological shift occurred: studies from 1990 to 2016 show that IBD incidence plateaued or decreased in the western world, and it is rapidly rising in the newly industrialized countries of Asia, Africa, eastern Europe and South America[5,49–53]. This alteration might be due to a diminished exposure to some risk factors in western countries, that, oppositely, are increasing in the new industrialized society. Therefore, it is urgent to decipher which are the risk factors that could explain those geographical alterations[54].

The onset of IBD can occur in any age, but both CD and UC are most often diagnosed in late adolescence and early adulthood, between 15 and 35 years of age, with no gender predominance[13,53,55,56]. In Europe, the estimated incidence of CD and UC range from 0.5 to 10.6 cases and 0.9 to 24.3 cases per 100,000 person-years, respectively. Despite the lowering trend in IBD incidence, the global disease burden remains high, which could be expectable considering the early age of onset and lower mortality due to improved treatment over the years. The estimated prevalence of IBD in Europe is around 0,3% – about 2.7 million people –, with 1.52–213 cases of CD and 2.42–294 cases of UC per 100,000 persons. This translates into a direct annual healthcare cost of 4.6–5.6 billion euros. As in incidence, there also appears to be a north-west gradient in the prevalence of IBD[6,53]**.** In Portugal, as well as in Europe, there are approximately 150 cases of IBD per 100,000 inhabitants, equally distributed by CD and UC, with a perception of increasing incidence; CD is more prevalent between 15 and 39 years of age, while UC highest prevalence ranges from 40 to 64 years of age; in both IBD entities, females are more affected[57].

CRC is one of the most feared complications of IBD. Although CAC accounts for a small percentage of all CRCs, it has worse prognosis, with 19% to 55% estimated 5-year survival rates, being responsible for 10–15% of annual deaths of IBD

patients[7–10]. This high mortality is partially due to diagnosis at later stages – around 50% of CAC patients have nodal or distant metastases at presentation[7] – and also because mean age at onset of CRC in patients with IBD (estimated to be 43.2 years[58]) is about 14 years earlier than onset of sporadic cases[10,13,38,59].

In general, patients with IBD have higher risk of CRC than healthy individuals, but this ratio varies accordingly to each patient's characteristics. Epidemiologic studies are not fully consensual regarding the risk to develop CAC: most of them refer higher risk in UC than in CD cases, while some consider similar risk, especially when comparing CD involving the colon (as opposed to pure ileitis) to UC; others suggest a declining incidence of CRC in IBD patients over the last decade, while some found it stabilized. This can be attributed to differences in environmental factors, lifestyle and genetic susceptibility, or simply to studies design, insufficient study size, differences in diagnostic criteria or access to diagnostic procedures – countries with the latest endoscopic imaging technology may have earlier detection of dysplastic lesions and CAC – and different treatment guidelines, including criteria for performing colectomy. Comparing the meta-analyses/cohort studies by Eaden et al. (2001)[58], Rutter et al. (2006)[60] and Jess et al. (2012)[61], it seems that cumulative incidence of CRC according to UC duration has been diminishing in recent years. Jess et al. surprisingly did not find an increased incidence of CRC in UC cases compared to healthy controls, which may be attributable to the higher rates of colostomies in UC patients in Denmark[62]. Therefore, this apparent decreased incidence of CAC in developed countries over the years can be related to improved medical and surgical treatments.

Although the pathogenesis of CAC remains unclear, some risk factors have been identified: family history of CRC – increases risk of CRC as in individuals without IBD; inflammatory activity – persistently active IBD is associated with higher risk than predominantly quiescent cases; extent of affected mucosa – after 40 years of disease, risk of CRC is 10-15 fold increased in patients with pancolitis, and only 2 fold increased in patients with left sided colitis (ulcerative proctitis, where inflammation is most severe, seems to be the exception, in which CRC risk appears not to be significantly increased)[13,14]; presence of inflammatory complications – like perianal abscess, anal fissure, colonic strictures or inflammatory pseudopolyps; and concomitant primarily sclerosing cholangitis (PSC)[15–19] – these patients have increased risk of CRC, compared to those with IBD alone, even after liver transplant (the mechanism behind this is unclear)[11,14]. Young age at diagnosis of IBD (<15

years) and longer disease duration are also reported as risk factors for CAC – estimated risk at 10, 20, and >20 years of disease is 1-3%, 2-8%, and 5-18%, with comparable risk associated with UC and CD[58,63]. However, as a life-long illness, IBD course presents periods of relapse and remission, as well as variable therapy response, which can influence the inflammatory damage, that may differ between individuals with the same disease duration. A large single-centre study from 2017 showed that inflammatory burden in UC, estimated from a mean severity score calculated from all colonoscopies performed in preceding 5 years, was significantly associated with CAC risk, while the most recent colonoscopy evaluation alone was not[11]. Importantly, it seems that the cumulative inflammatory activity, more than disease duration, is a determining risk factor for CAC development. In fact, there are evidences suggesting that CAC incidence decreases when there are efficient surveillance strategies and successful anti-inflammatory treatment (e.g. aminosalicylates [5-ASA]), promoting high mucosal healing rates[12].

Larger and well-designed studies are needed to quantify the real burden of the disease. Nevertheless, more than trying to prevent CAC by improving treatment and follow-up of patients with IBD, it is still important to better understand the genetic and molecular profiles behind dysplasia and cancer development, in order to identify individuals at higher risk, providing earlier chemoprevention and therapy.

CAC Vs. sporadic CRC: two distinct entities

HISTOLOGY

CAC's mechanisms of carcinogenesis remain unknown, but it is recognized that it develops from an inflammatory non-dysplastic mucosa, which evolves into dysplasia, to invasive adenocarcinoma, diverging from the typical adenoma-carcinoma sequence of sporadic CRC. IBD dysplastic lesions are usually multiple and flat, and, unlike sporadic CRC exophytic lesions, are difficult to resect endoscopically, often requiring colectomy because the total extent of the precancerous area is difficult to determine[29–32].

High-grade dysplastic (HGD) lesions are strongly related to CAC development and an indication for colectomy[64,65], however, this approach is questionable in case of

low-grade dysplasia (LGD), because the progression rate to HGD or cancer is variable. Recently, it was shown that, among UC patients with LGD, the annual incidence of CRC is 0.8% and the risk of dysplasia progression increases if there is concomitant PSC and/or the lesions are multifocal, distally located or not macroscopically visible (flat)[66]. In addition, other study demonstrated a high prevalence of aneuploidy in HGD lesions (93.3%), and an association between aneuploidy in LGD lesions and their progression to HGD or CRC (in 40.5% cases). Therefore, aneuploidy may constitute a prognostic marker, allowing better management of patients with LGD[67].

Two patterns of dysplasia with different natural histories can be found in IBD patients: adenoma-like dysplasia – in areas not yet affected by the disease, where carcinogenesis may be considered sporadic – and non-adenoma-like dysplasia – where carcinogenesis is associated with the underlying chronic inflammation. While the first may be treated with polypectomy, the second usually requires colectomy, considering its higher risk of concurrent malignancy. Although they are not easily distinguished endoscopically or histologically, the well characterized genetic and molecular profiles of CAC can allow differentiate it from sporadic CRC, improving patients' management[31].

INFLAMMATORY ENVIRONMENT

The inflammatory environment of IBD has a key role in dysplasia and CRC development. In fact, some inflammation-associated genes (e.g. COX-2 and NOS-2) are overexpressed in IBD and in CAC[42]. Inflammation promotes releasing of reactive oxygen species (ROS) and inflammatory mediators (IL-6, IL-10, IL-17, IL-23, TNFα, VEGF), causing oxidative stress, increased cell turnover, anti-apoptotic activity, neoangiogenesis, migration and invasion of tumor cells[13]. Moreover, the immune and inflammatory response prolonged over time leads to DNA damage, which results in chromosomal instability (CIN) and microsatellite instability (MSI). This fact explains the increased risk of CAC with duration, extension and degree of inflammation, and why it can be attenuated by an effective disease control. The reason why CAC risk appears not to be increased in ulcerative proctitis remains unclear[13,14].

GENOMIC ALTERATIONS

Genomic alterations detected in sporadic CRC were also recognized in CAC[33–37]. DNA hypermethylation, commonly detected in sporadic CRC, is also common in CAC, involving frequently MLH1 (up to 46%) and p16 (up to 100%), and can be found early, in chronically inflamed mucosa without visible dysplasia[68,69]. However, other alterations differ in frequency and in order on the sequence of carcinogenic events. Recent studies[37,38] found significant differences in genomic alterations in CAC tissues in comparison to the evidences reported about sporadic CRC in The Cancer Genome Atlas[70] and the Foundation Medicine database[71]:

- In sporadic CRC, Wnt/β-Catenin pathway activation is the initiating event in 90% of cases, mostly due to APC and K-RAS mutations, with early APC tumor suppressor protein loss of function (in dysplastic tissue) and later activation of K-RAS protein (in adenoma), that promotes NF-kB mediated cytokine secretion, associated with inflammatory response, increased ROS release, neovascularization, cancer growth, invasion and metastization. In CAC, these mutations are rarer and occur later: K-RAS amplification in 40% of cases, in HGD, and APC mutations in 21% of cases, later in the dysplasia-carcinoma sequence[10,38].
- p53 mutations occur late in sporadic CRC, during transition from adenoma to carcinoma, and are found in about 50% of cases[70], or up to 75% in advanced CRC[71]. But in CAC, p53 is the most commonly altered gene (in 63-89% of cases[37,38]) and mutations occur early, in 50% of chronically inflamed patients before mucosal dysplasia develops[10,13,33–38]. Mutant p53 loses the tumor suppressing function, allowing cytokine mediated DNA damage and prolonged TNFα-induced NF-kB activation. Actually, in mice harboring a germline p53 mutation, there is severe tissue damage and HGD, rapidly progressing to invasive carcinoma[72].
- The most common copy number alteration in CAC is MYC amplification (in up to 26% of cases) – its constitutively upregulation leads to increased expression of many genes, some of which are involved in cell proliferation, contributing to the formation of cancer –, while in sporadic CRC it is rare (4%)[38,70,71].
- Several CAC cases have alterations in the Notch gene family (34%)[38], which proteins are transmembrane receptors implicated in a signaling pathway which regulates cell-to-cell interactions that influence many cell fate decisions in various cell types, important during fetal development of almost all organs and

tissues[73]. There is no evidence in the literature of an association between notch mutations and CRC yet. However, other diseases and developmental changes are described to be associated with these mutations, such as Adams-Oliver, Alagille and Hajdu-Cheney syndromes, neurodegeneration, aortic valve disease, CADASIL (Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy), head and neck squamous cell carcinoma and T-cell acute lymphoblastic leukemia[73,74].

INTESTINAL MICROBIOME

Recent data reported that intestinal microbiome may support carcinogenesis through its interaction with the host damaged mucosa, metabolism and immunity[3,4]. It is already well known that chronic inflammation secondary to chronic infection may lead to carcinogenesis – one example is the association between gastric cancer and *Helicobacter pylori* infection[39–41] – so, this possibility may also explain some of the CAC development.

The possible influence of commensal flora or specific bacteria in CAC risk was first suggested when it was noticed that mice raised under germ-free conditions or treated with antibiotics did not develop neither IBD or CAC and that, comparing genetically identical mice, cancer only occurred in some animal facilities[75,76].

Some studies show that CAC cases' microbiota differs from cancer-free controls[4,77], but the doubt remains if this difference is cause or consequence of the cancer. CRC patients have more frequently gut dysbiosis, namely increase of *Fusobacterium nucleatum* and decrease of butyrate-producing bacteria[4,78–80]. In fact, several studies support a potential active role of *Fusobacterium*, specifically *F. nucleatum*, in CRC development[81]. Other pathogens have been suggested to be initial triggers in cancer development in several animal models, most of them opportunistic: *Escherichia coli, Enterococcus faecalis, Streptococcus gallolyticus and enterotoxigenic Bacteroides fragilis*[4,82,83]. A study comparing fecal bacterial diversity in CRC patients and healthy volunteers concluded that CRC patients had more *Bacteroides fragilis*, in contrast to healthy individuals microbiota, enriched in *B. vulgatus* and *B. uniformis*[4]. It is interesting to see that different *Bacteroides* strains may influence the host homeostasis in different ways, therefore, further studies are warranted to ascertain the impact of specific species strains from the gut in CRC pathogenesis.

There is no conclusive study showing that elimination of these specific organisms of the gastrointestinal tract prevents CRC[80]. However, the possible influence of commensal flora in CRC risk might partially explain the rising incidence of CAC in the newly industrialized countries[5,49–53], with recently adopted western alimentary habits, since diet is directly related to microbiota composition (for example, high-fat and low-fiber diet is associated with greater cancer risk, because it induces dysbiosis)[82]. It is important to consider that diet potentially alters IBD and CAC risk. Moreover, if new studies achieve significant results about specific microbes that are protective against CRC development, probiotics or even fecal transplantation could be considered as preventive measures.

IMMUNOMODULATION THERAPY

The current IBD therapies are based on anti-inflammatory drugs (5-ASA – sulfasalazine, mesalazine), immunomodulators (corticosteroids, thiopurines – azathioprine, 6-mercaptopurine – and methotrexate) and biologic agents (mainly anti-tumor necrosis factor [anti-TNF] agents – Adalimumab, Infliximab), which suppress the immune system and can also contribute to reduce the risk of CAC, depending on disease location and duration[22–24]. However, approximately 30% of patients do not respond to treatment and about 40% lose the initial response[20,21].

On the other hand, long term therapy with these agents is controversial, considering they may raise the risk of infections, including serious opportunistic infections, and promote carcinogenesis, due to reduced physiologic immunosurveillance of malignant cells[22]. The cancers considered secondary to chronic immunosuppression include mainly lymphomas and skin cancer, but also acute myeloid leukemia, myelodysplastic syndromes and urinary tract cancer[22,84]. The risk to develop any of these types of cancer vary according to the immunosuppressing drug class and to the patient's age and gender. For example, thiopurines are associated with higher risk of postmononucleosis lymphoma and hepatosplenic T-cell lymphoma in younger males, but in older patients, they are more related to urinary tract cancers[85–88]. Moreover, while anti-TNF agents increase the risk of melanoma, thiopurines are associated with nonmelanocytic skin cancers[22,89,90]. In fact, the impact of some drugs in promoting this differential risk susceptibility to develop cancer in specific tissues is not yet explained.

To date, new therapies are being approved, involving new drug mechanisms, with the goal of effective disease control with fewer negative effects and finding solutions for the high percentage of non-responders. These include anti-adhesion agents (Natalizumab, Vedolizumab, Ertolizumab, AJM 300 and PF-005476659), anti-interleukin inhibitors (Ustekinumab and Risankizumab), janus kinase (JAK) inhibitors (Tofacitinib and Filgotinib) and sphingosine 1 phosphate (S1P) receptor modulator (Ozanimod). Their main advantages include no increased risk of malignancy (except with Tofacitinib) and of serious infections (with Ertolizumab, AJM 300, PF-005476659, Risankizumab and Ozanimod), and a less invasive administration, such as the oral administration of JAK inhibitors[1].

Recently, it was described that a gene (SERPINE-1/PAI-1) linked to blood clotting is highly expressed at sites of intestinal inflammation and it is specific for IBD patients with active disease non-responders to biologics[91]. In addition, a new compound (MDI-2268) was developed to suppress that gene, which resulted in decrease of IBD symptoms in mice. Thus, this compound might be another new IBD treatment alternative with less undesirable risks[91].

In conclusion, to properly manage each IBD patient, it is important to assess the benefit/risk ratio of long-term use of immunomodulators and biologic agents, considering patients' age, gender and disease phenotype. On the other hand, besides new drugs' advantages and how promising they seem, it is important to consider that few have been available for some years (like Natalizumab), and others are new or still in development, so, their long-term efficacy and safety are not well known yet[1].

CLINICAL MANAGEMENT: THE UNMET NEEDS

Prevention Strategies

Prevention consists of a series of measures taken to avoid disease or its burden, defined according to successive stages of primary, secondary and tertiary prevention[92].

PRIMARY PREVENTION – CHEMOPREVENTION

The most striking preventive measure is primary prevention, which aims to avoid the onset of diseases. Translating to CAC, considering the increasing evidence that chronic inflammation favor's the development of cancer in IBD, improved mucosal healing with 5-ASA, immunomodulators or biologics may prevent it[93].

Most evidence comes from observational studies of chemoprevention in UC using 5-ASA, which effects may go beyond inflammation control[94]. 5-ASA, including sulfasalazine and mesalazine, are the most commonly prescribed anti-inflammatory drugs in IBD and, despite some conflicting evidence, data suggests that they may confer protection against the development of colonic neoplasia[93], as recently shown in a systematic review with meta-analysis[95].

Thiopurines (azathioprine and 6-mercaptopurine) – immunomodulators which inhibit purine synthesis – have demonstrated efficacy in a broad range of IBD complications. Once again, there is conflicting data regarding the chemoprophylactic effect of these drugs on dysplasia and CAC development, but the most recent studies suggest a trend toward a protective effect[24,96,97].

The evidence on the chemoprevention role of anti-TNF (infliximab and adalimumab) is even more scarce. Although animal models suggested that these biologic agents may prevent the development or progression of dysplasia and cancer[98], only a few population-based studies within IBD have shown a lower frequency of colorectal cancer among those treated with infliximab[99,100].

SECONDARY PREVENTION – CANCER SCREENING AND SURVEILLANCE

Assuming a key role as well, secondary prevention seeks to identify and treat diseases in the earliest stages before the onset of signs and symptoms. CRC screening programs have shown to reduce its incidence and related mortality[101]. In IBD patients, surveillance colonoscopy also achieved positive outcomes regarding CAC prevention[24,26,27].

Considering that IBD patients present higher risk of developing CRC, even though the reported risk estimates vary widely between studies, colonic surveillance is highly recommended[9], in order to detect potentially resectable dysplastic lesions or potentially surgically curable early stage CRC, reducing its morbidity and mortality[9,24–28]. However, mortality reduction due to periodic follow-up has not been

clearly established[102–105], which means that better techniques and surveillance programs are needed. Doubts remain regarding the best endoscopic technique and the right timing in which surveillance colonoscopies should be performed.

The usual IBD surveillance method has been, for more than thirty years, standard definition (SD) colonoscopy with targeted and random biopsies every 10cm throughout the colon[25,31,66]. However, this technique inspects less than 5% of all the mucosal surface[106] and a large retrospective analysis reviewing 1010 colonoscopies during ten years of surveillance quantified a small rate of dysplasia detection (0.19)[107]. With high definition (HD) endoscopes and monitors, dysplasia detection increased three-fold[28,108], which changed these patients' management, favoring more conservative approaches regarding colectomy[108].

On the other hand, using the traditional white light endoscopy (WLE), dysplastic lesions may be macroscopically visible (adenoma-like) or invisible (non-adenoma-like/flat lesions)[31,109], but some of those invisible lesions, which have higher risk of concurrent malignancy[31,110,111], are identified in dye-spray chromoendoscopy (DCE)[25,31,66]. In fact, the diagnostic reliability of WLE is defied in a recent review, which found a sensitivity of only 76% for this technique[112], while another study revealed that DCE increases dysplasia detection sensitivity[113–117], showing a detection rate of 9.3% with WLE, and of 21.3% with both WLE and DCE[118]. Taking into consideration the DCE success and its operational barriers, this technique was sought to be mimicked without the use of dye. Thus, virtual chromoendoscopy appears, facilitating the visualization of tissue abnormalities by filtration of some wavelengths of light – narrow-band imaging (NBI). However, compared to conventional WLE and DCE, the advantages of NBI are controversial[119–121].

To date, the most recent SCENIC (Surveillance for Colorectal Endoscopic Neoplasia detection and management in IBD patients: International Consensus[122] – endorsed by American Gastroenterological Association [AGA] and other societies in North America, Europe and Asia) recommendations advocate the use of HD – rather than SD – colonoscopy, and DCE – rather than WLE or NBI –, with targeted biopsies of all identified lesions only[28,64,110,111,122,123], as chromoendoscopy allows more targeted biopsies and diminishes the necessity for random ones[122]. Therefore, if there are no limitations of the diagnostic efficiency (poor preparation, presence of post-inflammatory polyps or active mucosal inflammation), random biopsies on macroscopically normal lumen are not recommended[111,124]. Alternatively, when

resources and expertise for performing chromoendoscopy are not available, HD WLE with random biopsies is accepted[110,122,123].

Even though most of the evidence favor's DCE as the gold standard technique for IBD patients' surveillance, this may change in the future, since there is recent conflicting data. Two studies from the past 2 years presented similar dysplasia detection rates and shorter withdrawal times for HD colonoscopy and NBI, when compared to DCE[125,126]. Future studies are needed to answer the remaining questions concerning practical aspects, like the cost-effectiveness of the different procedures and the real and long-term impact of modern techniques utilization to increase dysplasia detection on overall CAC mortality.

Regarding the timing of screening, the different programs are similar. When comparing the most recent guidelines from AGA[110] and form European Crohn's and Colitis Organization (ECCO)[123], both recommend commence surveillance 8 years after onset of symptoms (not the official IBD diagnose moment), with the exception that, according to AGA, patients with concomitant PSC should undergo annual surveillance starting from the time of diagnosis. AGA recommends 1-2 years interval between colonoscopies and, after 2 negative exams, it can be extended to 1-3 years, while ECCO recommendations regarding surveillance intervals range from 1-5 years, depending on the individual risk stratification: once a year for high risk patients, every 2-3 years for intermediate risk and every 5 years for low risk patients[110,123].

Alarmingly, a 16 years' study, including seven university medical centers in The Netherlands, demonstrated that a substantial part of all CACs occur before colonic surveillance should start according to these guidelines[2]. They found out that CAC could appear between 0 to 45 years since IBD diagnosis and that 17% of patients developed cancer before the first surveillance[2]. In conclusion, strict adherence to these guidelines will lead to late detection of some early cancers, which may reduce the efficacy of colonic surveillance in IBD and explain why there is no mortality reduction associated with it. It is necessary to rethink if initial screening colonoscopy should be recommended before 8 years of disease duration for all IBD patients, to reassess disease extent, as colitis may progress over time.

Prognostic Biomarkers

Greater knowledge about the pathogenesis of CAC at the molecular level is needed not only for development of effective early diagnosis, prevention and treatment strategies, but also for predicting therapy response and identifying prognostic biomarkers. So far, there are very few molecules proposed to be possible prognostic biomarkers and none has cost-effectiveness evidence supporting its active search in all IBD patients.

As previously mentioned, the percentage of IBD patients that do not respond to treatment or lose the initial response is high[20,21]. Regarding CRC, there is also a significative resistance to chemotherapeutic drugs[127,128] which is one of the major reasons for the increased mortality. A very recent study[128] showed that cancer "stem cells" (CSCs) – a subset of cancer cells linked to chemoresistance[129,130] – that express CD44v6 – a CD44 (cell-surface glycoprotein involved in cell-to-cell interaction, adhesion, and migration[131]) variant – are associated with more aggressive CRC phenotype, with higher cellular self-renewal capacity and metastatic potential[132,133], increased resistance to chemotherapeutic agents and, therefore, lower disease-free survival (DFS) and overall survival (OS). In fact, inhibition of CD44v6 resulted in resensitization to chemotherapy and diminished self-renewal capacity of CSCs. In those CD44v6+ cells, a distinct panel of miRNAs with dysregulated profiles was noted, with miR-1246 upregulation being particularly related to the enhanced tumorigenicity. This suggests that specific miRNAs, like miR-1246, may represent potential prognostic biomarkers and therapeutic candidates to target CSCs[134,135].

Furthermore, a recent meta-analysis reported that, without any adjustment for molecular subtypes, the overall 5-year survival of CAC does not differ from sporadic CRC[136]. Another recent study[137] demonstrated that different cancer mutational patterns have an impact on survival outcomes. It showed that proximally located CRC is related to higher mutational rates, associated with mismatch repair pathway defects (dMMR), mostly due to MLH1 protein loss of expression. Patients with these types of cancer had a significantly better survival rate, compared with non-hypermutated ones. Moreover, this study found that the increased survival of sporadic CRC cases with MSI[138] has also been observed in CAC. The neo-epitopes generated by the high mutational rate cause an anti-tumor infiltrating lymphocytes response that is thought to have an important role in the improved survival of these dMMR/MSI cancers[137].

Additionally, it would be interesting to consider an analogous molecular profiling for CAC as used in gastric cancer, considering that both are inflammation-associated[139]. Thus, future studies are required to determine whether combinatory analysis of p53 alterations – the most commonly altered gene in CAC[37,38] – and MLH1 loss of expression in the biopsies' tissue taken during IBD patients colonoscopic surveillance could identify patients with higher risk of developing CAC[139].

Overall, there is an urgent need for recognizing the molecular landscape behind CAC. Information concerning prognostic biomarkers is scarce, and new studies are needed in this area, to clarify the different treatment responses and help clinicians give a more reliable prognostic estimate to patients.

Can Glycomedicine have a role?

Most extracellular and membrane proteins are glycosylated, and glycosylation alterations significantly affect the protein structure and function[141,142]. The cellular profile of glycans has been reported to change significantly in various diseases[43–45] including IBD[46,47] and cancer[48], so it is likely that Glycomedicine research will yield future translational opportunities.

Particularly, complex branched *N*-glycans are known to play key roles in many glycoproteins in cancer[48,143–145] and in autoimmune disorders[146,147], through T cell activation (via T cell receptor [TCR] signaling) and function. Mucosal T lymphocytes from patients with active UC were previously shown to display a deficiency in branched *N*-glycosylation, and mice models with absence of this type of glycans develop early-onset and severe disease[148]. The i3s research group recently demonstrated in human *ex vivo* models that supplementation of mucosal T cells (isolated from patients with active UC) with *N*-acetylglucosamine (GlcNAc) lead to an enhancement of branched *N*-glycosylation on the TCR, which was associated with more controlled T cell-mediated immune response. Nonetheless, mice with severe colitis treated with GlcNAc revealed a clinical improvement, namely better clinical scores and suppression of disease progression[148]. These results repurposed GlcNAc as an inexpensive and nontoxic targeted-specific immunomodulatory strategy for IBD treatment, and eventually a potential chemoprophylaxis agent to primary prevention of CAC.

Remarkably, glycans can also be useful for IBD prognosis. Recently, the i3s research group showed that they can discriminate, early in the disease course, which patients are most likely to develop severe colitis and fail to respond to standard therapy, benefiting from other therapeutic strategies initially (e.g. biologics). This study demonstrated that low levels of branched *N*-glycans around time of diagnosis is an independent predictor of non-response to conventional therapy with 75% of specificity, and, correspondingly, high levels of branched *N*-glycans predict 78% of UC patients that will have a favorable disease course exclusively under 5-ASA treatment with more than 5 years disease[149].

Other interesting serologic markers that can differentiate CD from UC and help determine the course of the disease are anti-glycan antibodies – like anti-mannobioside carbohydrate IgG antibodies (AMCA), anti-chitobioside carbohydrate IgA (ACCA) and antilaminaribioside carbohydrate IgG antibodies (ALCA) –, alone or combined with anti-*Saccharomyces cerevisiae* (ASCA) and perinuclear antineutrophil cytoplasmic antibody (pANCA), which can be seen in a subgroup of patients with IBD[150–152]. High levels of these antibodies can be related with a high probability to develop complicated IBD, leading to increased risk of IBD-related surgery, as shown in a recent study, that found anti-glycan antibodies, especially AMCA, to be valuable biomarkers to promptly classify patients into high risk for severe disease[151–153]. Another recent study investigated the same antibodies – pANCA, ASCA, AMCA, ACCA and ALCA – in newly diagnosed, untreated IBD patients, concerning disease phenotype and course, and analyzed their presence over time. This study concluded that anti-glycans may fluctuate under the influence of immunosuppressive treatment, which is congruent with the hypothesis that they may be risk biomarkers. Also, it showed that antibody profiles at diagnosis, namely pANCA and ASCA, enable good discrimination between CD (ASCA+, pANCA-) and UC (ASCA-, pANCA+), and that pANCA status over time is associated with UC activity, having the potential to be used in disease monitoring[154]. A meta-analysis/systemic review designed to evaluate the diagnostic value of anti-glycan biomarkers and their association with IBD complications and need for surgery, confirmed these results, concluding that ASCA had the highest diagnostic value for differentiating IBD from no disease and CD from UC, and that ACCA had the highest association with complications. Moreover, it showed that combination of 2 markers had a better diagnostic value as well as higher association with complications and need for surgery than any individual marker alone[155].

It is also known that immunoglobulin G (IgG) Fc-glycosylation levels affects IgG effector functions. Interestingly, IgG in mice can have pro-and anti-inflammatory activity, depending on its glycosylation status[156,157]. A recent retrospective analysis of plasma samples from patients with IBD and healthy individuals (controls), found an association between IgG Fc-glycosylation levels with disease and its clinical features: patients with IBD had lower levels of IgG glycosylation than controls, namely low galactoslation, which was associated with more severe CD or UC and related to the need for surgery[158]. This brings to light the possibility of a minimally invasive biomarker for IBD, by measuring serum protein glycosylation levels.

In conclusion, this new era of glycomics revealed a new window of opportunities for the management of IBD patients and consequently, prevention of CAC. The promising role of Glycomedicine, namely, new serological biomarkers in stratification of patients according to disease phenotype and risk of complications, can restructure the actual prevention programs by helping predict the disease course and therapeutic response.

Conclusions

Despite the lowering trend in IBD incidence, the global disease burden remains high. Epidemiologic studies are not fully consensual regarding the risk to develop CAC, nevertheless, it is one of the most feared complications of IBD, being responsible for 10–15% of annual patients' deaths[7–10] and this high mortality is due to cancer early onset and late diagnosis[7,10,13,38,58,59].

The pathogenesis of CAC remains unclear, but the inflammatory burden seems to have a key role[11,12]. Although there is conflicting data about the chemoprophylactic effect of 5-ASAs and thiopurines, recent studies suggest they are protective[24,96,97]. The evidence about anti-TNF on cancer prevention is more scarce[98–100]. Alarmingly, though these therapies may reduce the risk of CAC[22–24], chronically, they raise the risk of infections and promote carcinogenesis[22], so, to properly manage patients, it is important to assess the benefit/risk ratio of long-term use of these drugs. To date, new therapies are being approved to find solutions for the high percentage of non-responders[20,21], which is a great clinicians' concern. Their main advantages include less undesired risks, in addition to less invasive administration[1,91]. Besides how promising these drugs seem, they are recent or still in development, so, their long-term efficacy and safety are not well known yet[1].

Also regarding CAC prevention, colonic surveillance is highly recommended[9]. The most recent guidelines advocate the use of HD DCE, with targeted biopsies of all identified lesions only (SCENIC)[28,64,110,111,122,123], and commence surveillance 8 years after onset of symptoms, with 1-2 years (AGA) or 1-5 years interval (ECCO)[110,123]. However, doubts remain regarding the best technique and right timing in which colonoscopies should be performed, since some CACs occurred before colonic surveillance started[2], which may explain why there is no mortality reduction with surveillance[102–105]. Future studies are needed to clarify the remaining doubts, like the real long-term impact of improved dysplasia detection on overall CAC mortality.

Greater knowledge about CAC's pathogenesis at the molecular level is needed, for effective early diagnosis, prevention, treatment and prognosis. Though new discoveries about CAC carcinogenesis have recently been made, it remains incompletely understood. So far, it is known that CAC develops from an inflammatory non-dysplastic mucosa, that evolves into dysplasia, to invasive adenocarcinoma, which differs from the typical adenoma-carcinoma sequence of

sporadic CRC. In fact, recent studies showed that HGD lesions are strongly related to CAC development[64,65], and that LGD lesions have higher risk of progression if the patient has concomitant PSC, if there is high prevalence of aneuploidy and/or the lesions are multifocal, distal or flat[66,67]. Other studies found that some inflammation-associated genes are overexpressed in IBD and in CAC[42] and that the chronic immune and inflammatory response leads to DNA damage, explaining the increased cancer risk with duration, extension and degree of inflammation. The reason why CAC risk appears not to be increased in ulcerative proctitis remains unclear[13,14]. The genomic alterations detected in sporadic CRC were also recognized in CAC[33–37], but with significant differences, for example, in CAC Wnt/β-Catenin pathway activation is rarer and occurs later[10,38] and p53 is the most commonly altered gene and mutations occur earlier[10,13,33–38]. Another interesting finding was the percentage of CAC cases with alterations in the Notch gene family (34%)[38], since, though other diseases and developmental changes are associated with these mutations, there is no evidence in the literature of an association with CRC yet[73,74].

The possibility of an intestinal microbiome' role in carcinogenesis was supported recently[3,4], since CRC patients have more gut dysbiosis than healthy individuals, namely increase of *F. nucleatum*, decrease of butyrate-producing bacteria[4,78–80], and more *B. fragilis*, in contrast to healthy individuals microbiota, enriched in *B. vulgatus* and *B. uniformis*[4]. Therefore, further studies are needed to establish the impact of specific species strains in CRC pathogenesis and discover microbes that may be protective against CRC development, so that probiotics or even fecal transplantation could be considered as potential preventive measures.

Regarding disease biomarkers, recent studies found some candidates. Specific miRNAs may help in prognosis and treatment, since a distinct panel of miRNAs with dysregulated profiles was noted in CSCs CD44v6+, cells associated with more aggressive cancer phenotype and chemotherapy resistance[128,134,135]. Also, high dMMR/MSI mutational rates are associated with improved survival[137]. Glycomedicine seems very promising in this matter. Recent studies showed that levels of branched *N*-glycans[149] and anti-glycan antibodies (AMCA, ACCA and ALCA)[150–153,155] can constitute early prognostic biomarkers and that GlcNAc can be an inexpensive and nontoxic targeted-specific immunomodulatory strategy for IBD treatment and CAC chemoprophylaxis[159]. IgG Fc-glycosylation levels were also associated with IBD and its clinical features, since low galactoslation was associated

with more severe disease phenotype[158], raising the possibility of a minimally invasive biomarker, by measuring serum protein glycosylation levels.

Though information about biomarkers is scarce, this new era of glycomics opens a new window of opportunities, since Glycomedicine may find new serological biomarkers to help in patients' stratification according to IBD phenotype and risk of complications, by helping predict the disease course and therapeutic response.

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