MINIREVIEWS

Novel biomarkers of fibrosis in Crohn's disease

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Abstract

Fibrosis represents a major challenge in Crohn's disease (CD), and many CD patients will develop fibrotic strictures requiring treatment throughout their lifetime. There is no drug that can reverse intestinal fibrosis, and so endoscopic balloon dilatation and surgery are the only effective treatments. Since patients may need repeated treatments, it is important to obtain the diagnosis at an early stage before strictures become symptomatic with extensive fibrosis. Several markers of fibrosis have been proposed, but most need further validation. Biomarkers can be measured either in biological samples obtained from the serum or bowel of CD patients, or using imaging tools and tests. The ideal tool should be easily obtained, cost-effective, and reliable. Even more challenging is fibrosis occurring in ulcerative colitis. Despite the important burden of intestinal fibrosis, including its detrimental effect on outcomes and quality of life in CD patients, it has received less attention than fibrosis occurring in other organs. A common mechanism that acts *via* a specific signaling pathway could underlie both intestinal fibrosis and cancer. A comprehensive overview of recently introduced biomarkers of fibrosis in CD is presented, along with a discussion of the controversial areas remaining in this field.

Key words: Crohn's disease; Fibrosis; Inflammatory bowel diseases; Diagnosis; Biomarkers

Core tip: Fibrosis occurs in a disturbingly large proportion of patients suffering from Crohn's disease (CD), and invasive procedures may be required for both its diagnosis and treatment. Several biomarkers of intestinal fibrosis have recently been proposed. Most of them still need to be validated, but they could be useful for obtaining an early diagnosis of fibrosis, thereby allowing timely treatment and delaying or even avoiding surgery. A comprehensive overview of recently introduced bio-

markers of fibrosis in CD is presented, along with a discussion of the controversial areas remaining in this field.

INTRODUCTION

Fibrosis represents a major challenge in Crohn's disease (CD). Approximately 50% of patients suffering from CD will develop penetrating or fibrotic strictures, and up to 75% of them will eventually need surgery[1-4]. However, fibrosis occurring in the bowel in inflammatory bowel diseases (IBD) is a problem that has been largely neglected by the scientific community, particularly compared with fibrosis occurring in other organs, such as the liver, lung, kidney, and heart[5]. Insufficient resources are allocated for research in intestinal fibrosis, and there is currently no available medical treatment for preventing or reversing fibrosis. All current efforts are focused on improving the ability to obtain an early diagnosis and apply timely treatment, ideally with the aid of noninvasive biomarkers of fibrosis (Table 1, Figure 1).

CHALLENGES IN INTESTINAL FIBROSIS

Several problems should be considered when investigating biomarkers of intestinal fibrosis in CD[1]. No validated quantitative or qualitative scores are currently available for diagnosing the presence of fibrosis and its extent. There is also no agreement on how to perform biopsies in strictured bowel segments, and the number and depth of samples have varied among the published studies. Furthermore, no standard anatomopathological scoring system has been developed, which increases the difficulties of data interpretation. Lastly, no medical treatment is currently able to reverse intestinal fibrosis once it has occurred.

NONINVASIVE BIOMARKERS OF FIBROSIS

Genetic markers of fibrosis

The pathogenesis of CD is complex, involving interactions between host-predisposing factors and environmental agents. Genetic factors controlling the immune system and the intestinal microbiome are likely to be involved, given that several genetic polymorphisms and variations have been associated with an increased susceptibility to IBD. However, genetic variations are present in fewer than one-quarter of CD patients[6]. Rather than considering chromosomes and genes themselves, it

might be better to investigate the mechanisms that control their expression in order to understand and potentially modulate the pathways leading to fibrosis in CD.

There is accumulating evidence that circulating single-stranded, noncoding RNA molecules (microRNA) modulate adaptive immune responses[7]. This is potentially highly significant since it may make it possible to diagnose those patients who are more likely to develop fibrotic strictures at an earlier stage, or to monitor the response to treatment.

NOD2/CARD15 gene polymorphisms are the most widely investigated in intestinal fibrogenesis. They have been associated with a higher risk of developing stricturing CD[8,9], and their expression could be influenced by race[10]. The underlying mechanism could be impairment of barrier function by such genetic mutations[11]. It has been suggested that more than half of the patients carrying an *NOD2/CARD15* mutation will develop stricturing CD, with the findings being similar for patients from Europe[9] and North America[12]. One large study investigated the presence of the SNP13 polymorphism of *NOD2/CARD15* in patients with ulcerative colitis (UC) and CD, and found that homozygosis was only observed in the latter[12]. Most studies have suggested that the risk of developing strictures increases with the number of mutations[13]. Although clinical decision-making in this field is almost completely unexplored, *NOD2* mutations have been associated with a greater need for the resection of strictures and with surgical recurrence[14]. The detection of genetic biomarkers in asymptomatic patients may therefore lead to changes in the management of such patients.

Other genetic and epigenetic factors may also play a role in intestinal fibrogenesis, and they have recently been investigated thoroughly. Genes controlling the expression of several cytokines - particularly interleukin (IL)-10[15] and IL-23[8] - have been associated with an increased risk of bowel stricture, but the evidence is conflicting, and so relying on these genes cannot be recommended for routine clinical practice[13]. Other molecules that are involved in maintaining the homeostasis between profibrotic and antifibrotic mechanisms have been proposed as candidates for diagnosing fibrotic CD at an early stage [*e.g.*, transforming growth factor (TGF) and metalloproteinase][1,5,16], but their possible role as biomarkers needs to be further elucidated.

Obtaining more reliable findings requires prospective, collaborative studies involving several centers across multiple countries aimed at identifying genetic factors underlying fibrosis. Such an approach would lower the costs for each participating unit, make the results more consistent, facilitate the inclusion of a large patient sample, and allow the application of genome-wide analysis to a wide spectrum of genes simultaneously. Moreover, population-based cohorts would be easier to establish and would allow comparison with non-CD individuals.

Table 1 Overview of fibrosis biomarkers and relative references

Biomarkers	Alteration/finding	Ref.
Genetic markers		
NOD2/CARD15	Polymorphisms	Barrett *et al*[8], 2008, Lesage *et al*[9] 2002, Yamazaki *et al*[10] 2002, Buhner *et al*[11] 2006, Abreu *et al*[12] 2002, Jürgens *et al*[13] 2010, Alvarez-Lobos *et al*[14] 2005
Epigenetic markers		
MD-2	Demethylation	Vamadevan *et al*[39] 2010
IFN-γ	Methylation	Gonsky *et al*[40] 2011
TH1	????	Gonsky *et al*[40] 2011
miR-200b	Increase	Chen *et al*[41] 2012
miR-29a	Decrease	Nijhuis *et al*[42] 2014
Serological markers		
ASCA	High concentration	Vasiliauskas *et al*[17] 2000, Forcione *et al*[18] 2004, Mow *et al*[20] 2004, Ferrante *et al*[21] 2007, Rieder *et al*[22] 2010, Seow *et al*[23] 2009, Arnott *et al*[25] 2004, Papp *et al*[26] 2008, Simondi *et al*[24] 2008
Anti-OmpC	High concentration	Mow *et al*[20] 2004, Arnott *et al*[25] 2004, Ferrante *et al*[21] 2007, Papp *et al*[26] 2008
Anti-I2	High concentration	Mow *et al*[20] 2004, Arnott *et al*[25] 2004
Anti-CBir1	High concentration	Targan *et al*[19] 2005
ALCA	High concentration	Ferrante *et al*[21] 2007, Rieder *et al*[22] 2010, Seow *et al*[23] 2009, Papp *et al*[26] 2008, Simondi *et al*[24] 2008
AMCA	High concentration	Ferrante *et al*[21] 2007, Rieder *et al*[22] 2010, Seow *et al*[23] 2009, Papp *et al*[26] 2008
ACCA	High concentration	Ferrante *et al*[21] 2007, Seow *et al*[23] 2009, Papp *et al*[26] 2008
Anti-C	High concentration	Rieder *et al*[22] 2010, Seow *et al*[23] 2009
Anti-L	High concentration	Rieder *et al*[22] 2010, Seow *et al*[23] 2009
CRP	Increase	Henriksen *et al*[29] 2008
Laminin	Increase	Koutroubakis *et al*[30] 2003a
Collagen IV	Decrease	Koutroubakis *et al*[30] 2003a
Fibronectin	Decrease	Allan *et al*[28] 1989
YKL-40	Increase	Koutroubakis *et al*[33] 2003b
bFGF	Increase	Di Sabatino *et al*[34] 2004
Radiological markers		
MRI	Enhancement patterns	Rimola *et al*[46] 2015
PET-MRI	Quantitative-qualitative analysis	Catalano *et al*[53] 2016, Pellino *et al*[49] 2016

ASCA: Anti-*Saccaromyces cerevisiae* antibody; anti-OmpC: Anti-*Escherichia coli* outer membrane porine C antibody; Anti-I2: Anti-*Pseudomonas fluorescens* associated sequence I2 antibody; Anti-CBir1: Anti-bacterial flagellin antibody; ALCA: Anti-laminaribioside carbohydrate antibody; AMCA: Anti-mannobioside carbohydrate antibody; ACCA: Anti-chitobioside carbohydrate antibody; Anti-C: Anti-chitin antibody; Anti-L: Anti-laminarin antibody; CRP: C-reactive protein; YKL-40: Chitinase-like glycoprotein; bFGF: Basic fibroblast growth factor; MRI: Magnetic resonance imaging; PET: Positron emission tomography.

Serological markers of fibrosis

Probably one of the first and best-characterized class of serological biomarkers of fibrosis in CD pathology is antibody molecules directed against microbial proteic products of the intestinal tract. These mainly comprise the following antibodies: Anti-*Saccaromyces-cerevisiae* (ASCA), anti-*Escherichia-coli* outer membrane porine C (anti-OmpC), anti-*Pseudomonas*-associated sequence-I2 antibodies (anti-I2), antibacterial flagellin antibodies, antilaminaribioside carbohydrate antibodies (ALCA), antimannobioside carbohydrate antibodies, antichitobioside carbohydrate antibodies, antichitin antibody, and antilaminarin antibody. In detail, high levels of ASCA have been found to be associated with fibrostenosis and penetrating disease and, more generally, with a greater need for surgery within 3 years from diagnosis compared with ASCA-negative patients[17-20].

On the other hand, with the exception of ALCA, glycan markers have been associated with complicated CD manifestations (fistulae and strictures) and surgery[21-24]. However, none of these markers has been specifically associated with fibrostenosis or penetrating disease[21-24].

In addition, it has been inferred that the intensity of immune responses influences the CD manifestations and need for surgery[19-22]. However, despite high levels of these markers being found in serum, none of them is currently recommended for use in following the course of the disease[19,20,22].

The antibodies directed against products of *Escherichia coli* (anti-OmpC and anti-flagellin) and *Pseudomonas fluorescens* (anti-I2) have been also successfully associated with the presence of fibrosis in CD[19-21,25,26]. It has been also reported that positivity for these three markers is associated with a higher frequency of fibrostenosis or penetrating disease[27].

Other markers have been also described, but diagnostically they are less useful than gut microbial markers. C-reactive protein (CRP) and proteins of the extracellular matrix (ECM; fibronectin) have been associated with fibrostenosis[28-30]. In particular, a prospective study found that the CRP level at diagnosis is closely associated with the subsequent risk of surgery in patients with CD[29].

The ECM plays a key role in disease processes, which has led to fibrinogenesis products related to its accumulation being considered as possible markers.
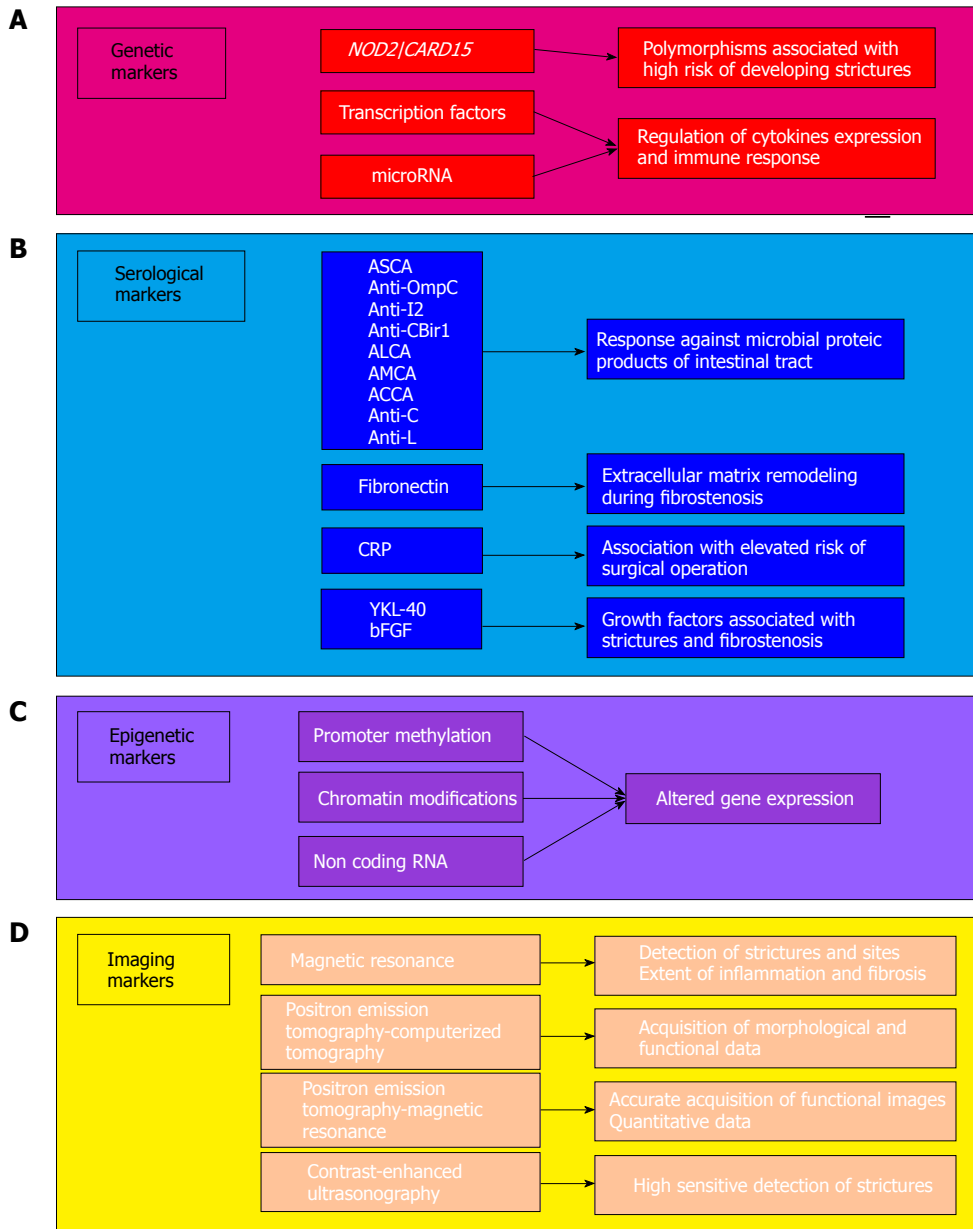
Figure 1 Biomarkers for assessment of fibrosis in Crohn's disease. The assessment of fibrosis currently relies on several biomarkers. Genetic markers (A) such as polymorphisms of the *NOD2/CARD15* gene and transcription factors/microRNAs are currently associated with the presence of strictures and altered immune response. Serological markers (B) are evaluated to assess the presence of fibrosis in a minimally invasive fashion. Epigenetic markers (C) are useful to understand the mechanism underlying development of fibrosis. Markers evaluated through sophisticated imaging technologies (D) allow to acquire and elaborate precise course of the disease.

While collagen types Ⅰ and Ⅲ are important in the intestinal fibrinogenesis process, the serum levels of direct procollagen precursors were not elevated in CD patients[1,5,16,31,32]. One study of the basement membrane found that while levels of laminin were increased, levels of collagen Ⅳ were decreased in CD patients relative to controls, and there was no association with fibrostenosis[30]. Lastly, like collagen Ⅳ, the levels of fibronectin were decreased in CD patients relative to controls, but higher levels of fibronectin were associated with the presence of stricture formations in the intestine[28].

Another class of markers is growth factor molecules, the best representatives of which are chitinase-like glycoprotein (YKL-40) and basic fibroblast growth factor (bFGF). YKL-40 has been associated with CD patients affected by strictures[33], and bFGF serum levels are strictly associated with a fibrostenosis phenotype[34].

Epigenetic markers of fibrosis

Biological information is encoded in the genetic pool of each individual, but gene expression can be altered by the environment, making variations inheritable *via* a mechanism called epigenetics[35]. These epigenetic variations that culminate in the modulation of gene expression are mainly due to a tightly regulated and complex mechanism based on the methylation of DNA,

modification of chromatin, and regulation on noncoding RNA, with the last mechanism mainly involving micro-RNAs. This complex mechanism is also observed in IBD, but its functioning relative to inflammation is not well understood. Nevertheless, it is known that it generally relies on the suppression of gene transcription through methylation of the promoter region. Epigenetic alteration during inflammation could be directly reflected in the activation, tolerance regulation, and regulation of T-cells[36].

Methylation of the promoter DNA and modification of histone proteins represent the main alterations observed in IBD. Several types of DNA methylation typical of intestinal disease that are observed in IBD are likely to be responsible for the altered expression of crucial genes that, in turn, induce the onset and progression of IBD. In this view, an altered methylation profile could be associated with CD. It has also been reported that at least seven CpG islands are differentially methylated in IBD patients[37] and that the pattern of DNA methylation affecting the IL-12 and IL-23 pathways in IBD is subtype-specific[38].

However, little is known about the mechanism through which methylation alters the gene expression and progression of IBD. It has been reported that demethylation of the MD-2 promoter is sufficient to induce the expression of this gene, which is crucial for the Toll-like receptor 4/MD-2 complex[39]. Additionally, it has been observed that methylation alters the levels of interferon-γ in IBD patients, resulting in them being correlated with immune responses[40]. Moreover, the expression profiles of cytokine TH1 have been associated with the epigenome setup of IBD patients[40]. It is therefore clear that genetic factors are involved in the etiology of IBD.

There is compelling evidence for the involvement of microRNAs in the regulation of immune responses in autoimmune pathologies, including CD[7]. This single-stranded noncoding class of RNA is able - *via* base complementarity - to regulate mRNA translation and stability. In particular, microRNAs belonging to the miR-200 family have been associated with different pathologies *via* their ability to modulate the key genes involved in the epithelial-to-mesenchymal transition (EMT).

More-promising candidate biomarkers for the pathology of fibrosis are currently represented by the class of noncoding molecules of circulating microRNAs. However, few studies have exploited microRNAs as fibrosis biomarkers. One study found that miR-200b levels were higher in CD patients with fibrosis than in their counterparts without fibrosis, and that TGF-β1 was able to induce the expression of miR-200b[41]. On the other hand, another study found that miR-29a was down-regulated in the serum of CD patients with stricture formations[42]. These two studies are valuable since they demonstrate that some microRNAs can be used to precisely discriminate between inflammatory and fibrotic disease, and thereby aid decisions about the use of therapy or surgery. It would therefore be very useful to find an accurate micro-RNA biomarker for monitoring the outcome during the course of therapy. Five microRNAs were found to be up-regulated in CD patients after 6 wk of treatment with infliximab, and two of them (let-7d and let-7e) were particularly elevated in patients exhibiting complete clinical remission[43]. These findings clearly suggest the usefulness of microRNA monitoring as a biomarker of the response to therapy in CD patients with intestinal fibrosis.

Because of the complexity of the disease and the continuous characterization of novel biomarkers, it is necessary to create structured collaboration networks for collecting and cataloging the findings of biopsies (including liquid biopsies), and to allocate appropriate resources for translational research. The results obtained on the laboratory bench could rapidly be applied to patients in hospital beds. Networks of this type have already been realized, and new ones focusing on colorectal cancer are being implemented.

Imaging markers of fibrosis

Almost all IBD patients need cross-sectional imaging for guiding their clinical management. Magnetic resonance imaging (MRI) is currently considered the ideal tool for identifying fibrosis in CD[44-47]. Studies have found significant variations in the sensitivity and specificity of MRI in detecting strictures. The clinical relevance of detecting stricture is intuitive, but it might be even more important for (1) detecting pathological sites where the stricture of the lumen is too minor to be detected using current MRI technologies; and (2) assessing the extent of inflammation and fibrosis at each detected stricture.

Since there is currently no treatment method for reversing fibrosis once it has settled in the bowel wall in humans, it would be useful to be able to detect asymptomatic sites. This would allow the physician to start medical treatment or modulate an existing treatment based on other indexes of CD activity and clinical parameters.

Inflammation and fibrosis represent two sides of the same coin in CD, and most strictures show both features but to different extents[1,47]. A certain degree of inflammation can be observed even in strictures with an extensive fibrotic component, and *vice versa*[1]. It is consequently more likely that strictures are predominantly fibrotic or inflammatory, rather than showing features of only inflammation or fibrosis. The latter can occur in CD, but this is extremely rare in practice. This observation has significant clinical implications, because it is now well known that strictures with active inflammation - irrespective of a fibrotic component - can be effectively managed with anti-inflammatory medications[1,48,49]. Knowing the exact proportions of these two components could delay or even avoid surgery in selected patients. At the same time this could identify those patients who are very likely to not respond to medical treatment because they have no active inflammation, and should instead be treated immediately with surgery to avoid unnecessary exposure to drugs.

The focus of recent studies has moved toward quantifying fibrosis in CD using imaging, with MRI

showing promise[46]. Such quantification requires both morphological and functional data to be obtained during cross-sectional imaging, which means combining conventional cross-sectional imaging with examinations that are able to detect sites of active inflammation. The most-used cross-sectional hybrid test is the [18]fluoro-deoxiglucose positron-emission tomography (PET) combined with computed tomography (CT). The method can provide both functional images, provided by PET, and anatomic images, provided by CT. Studies have shown that PET-CT offers advantages over PET alone in CD[50,51]. However, CT involves exposure to ionizing radiation, and most of the information provided by PET-CT can be obtained with current MRI scanning technologies in experienced hands.

PET-MRI is a new hybrid tool that was recently tested in patients with cancers[52,53]. It was shown that a machine that can perform PET and MRI scans simultaneously changed the management of cancer patients[52]. This has led to PET-MRI being used to assess patients with CD. PET-MRI is superior to both MRI alone and PET-CT because it provides functional images that are not available with conventional MRI, and the quality of images is significantly higher for PET-MRI than for PET-CT[49]. PET-MRI is more accurate than PET-CT at detecting extraluminal disease, and may be used to identify patients who are more likely to need fecal diversion during surgery. Moreover, PET-MRI can more reliably identify distant CD sites, hence aiding the selection of patients in whom surgery should either start open or with hand-assisted laparoscopy, reducing the intraoperative time that would be associated with starting with minimally invasive surgery that would eventually need to be converted to open surgery[49]. PET-MRI has been reported to detect fibrosis more accurately than both PET-CT and MRI alone. The use of PET-MRI to select patients suitable for a trial with rescue medical treatment before surgery found that over 70% of them did not require surgery[49]. Even more importantly, PET-MRI can produce quantitative data[53]. A direct correlation has been observed between PET standard uptake values and the degree of inflammation, by testing simultaneously each stricture detected with PET-MRI. Furthermore, some variables can be used to grade the extent of fibrosis quantitatively, ultimately resulting in a more reliable and reproducible way to diagnose CD patients[53]. This results in better patient management, based on agreed criteria, thereby also reducing interobserver and intraobserver variability.

Questions could be raised about the safety of PET-MRI, due to it involving exposure to radioactive nuclides, especially in young patients. However, a PET-MRI examination can be effective even at low radiation doses, and MRI alone can involve exposing patients to higher radiation levels during the reconstruction phase[54].

Shortcomings of PET-MRI are the high costs of individual tests, the requirement for a hybrid machine, and the long acquisition times, which make this technique unsuitable for patients needing immediate treatment and those who cannot tolerate long examinations[49,51,52].

A novel tool that is now being used frequently is contrast-enhanced ultrasonography (US), which has been associated with good sensitivity in detecting strictures[55,56]. However, concerns exist about the ability of US to discriminate between inflammation and fibrosis, and the implementation of a US scan with elastography has not yet been validated. Moreover, the physical shape of a patient can significantly influence the efficacy of US, which may be relevant given that many CD patients show mesenteric obesity (often drug-related), and the capabilities of US are affected by the operator's ability.

Fibrosis in UC

While fibrotic strictures are key features of CD, recent studies have suggested that UC patients can also develop fibrosis. This occurs in the large bowel and raises the concern of malignancy, justifying a surgical approach. In addition to concerns about cancer, thickening and increased stiffness of the large bowel cause several types of dysfunction since they affect intestinal motility[57,58].

Fibrosis in UC was neglected until very recently[57,58], and investigating it further would improve our understanding of the mechanisms underlying fibrosis development. The fibrosis that occurs in UC is particularly intriguing since it might occur without mucosal inflammation[58]. It is known that fibrosis results from chronic inflammation, but cases with a fibrostenosis pattern of CD are often observed. This could reflect a link between CD and UC fibrogenesis. Serological markers of fibrosis in the diagnosis of these patients could also be applied to medical treatments, assessing responses, and the following up of these patients. As an example, mucosal healing is currently used to assess the effectiveness of treatment in IBD, but this pathway might not be involved in patients who progress to fibrosis without significant mucosal involvement.

In addition, investigating UC-related fibrosis could highlight possible differences among CD and UC patients who develop fibrosis and facilitate the identification of biomarkers of fibrosis specific to each of these two entities. In UC patients it could be important to look beyond the mucosal surface[58], and this represents a further challenge in IBD patients that researchers involved in intestinal fibrosis development should take into account.

Wnt signaling and fibrosis: Any link with bowel cancer in CD?

The aberrant activation of the Wnt pathway has recently been associated with the pathogenesis of IBD[59]. It has also been reported that several molecules involved in Wnt signaling are down-regulated in CD tissues: Wnt2, Wnt5a, Wnt5b, Fzd2, Fzd4, Fzd6, LRP6, Dvl, and SFRP1[60,61]. In addition, it has been reported that attenuation of the Wnt pathway by alteration of TCF4 and LRP6 is directly responsible for the diminished production of alpha-defensins by Paneth cells[62] and the dysfunction of the barrier[63]. This represents a hallmark of CD, and hence
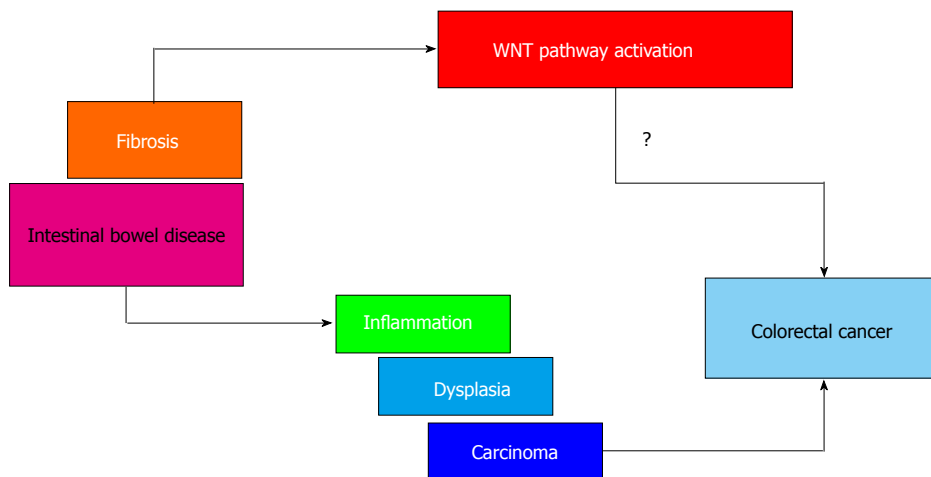
Figure 2 The involvement of Wnt pathway in the progression from fibrosis to cancer. Patients affected by intestinal bowel disease are highly susceptible to a specific type of colorectal carcinogenesis characterized by the inflammation-dysplasia-carcinoma progression, without the appearance of adenoma. The activation of Wnt pathway in colitis could represent a possible link between fibrosis and the development of colorectal cancer.

could potentially represent a therapeutic target[64]. Furthermore, activation of the Wnt/β-catenin pathway is able to induce the EMT and is important for fibrogenesis mediated by TGF-β[65]. In particular, TGF-β is able to stimulate Wnt signaling by suppressing the expression of DKK-1, a Wnt inhibitor. In addition, activation of the Wnt/β-catenin pathway is able to increase ECM synthesis and regulate several MMP genes[66]. It is also reported that blocking the Wnt pathway can reverse the fibrosis, thus representing a useful therapeutic target[67].

The Wnt pathway represents an important signaling pathway during development[68]. Wnt ligands are able to activate either canonical or noncanonical pathways, with the former based on the crucial role of the β-catenin protein[69]. The Wnt pathway is of course also important in adult tissue homeostasis, and it is down-regulated in cancer. In particular, about 80% of colorectal cancer patients carry mutations in key components of the pathway, and in particular activating mutations in β-catenin and inactivating mutations in the adenomatous polyposis coli gene[70]. The Wnt pathway could be activated either directly through mutations of its components, or indirectly through the secretion of triggering ligands or the depletion of inhibitors.

It is well known that the lifetime susceptibility to colorectal cancer is higher among IBD patients than the rest of the population[71,72]. This specific type of carcinogenesis is characterized by the well-defined progression from inflammation to dysplasia to carcinoma, without the appearance of adenoma, as observed in sporadic cancer[72-74], and with several differences in the pathogenesis with respect to the sporadic counterpart. p53 mutations are frequently found in colitis-associated carcinogenesis, whereas in sporadic cases they are only found in advanced disease[75,76]. Moreover, these kinds of mutations are also found in the colonic mucosa adjacent to the area with dysplastic colitis[77,78]. It has also been found that the Wnt pathway is frequently activated very

early in colitis-associated carcinogenesis[59], as well in the adjacent nondysplastic mucosa. This clearly suggests that early activation of the Wnt pathway in the area surrounding a dysplastic or malignant lesion in colitis could represent a link between fibrosis and the future development of colorectal cancer (Figure 2).

CONCLUSION

In recent years several biomarkers of fibrosis have been proposed, tested, and verified in patients with CD. However, validating studies are still needed to confirm the reliability of these markers. This would eventually allow for the development of noninvasive tools to detect them and perform early diagnoses of incipient fibrosis, and hence implement prompt treatment. Moreover, such researches could lead to a better understanding of the mechanisms underlying intestinal fibrogenesis and clarify the potential links between CD fibrosis and cancer.

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