Micro-RNAs as regulators and possible diagnostic bio-markers in inflammatory bowel disease

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Received 27 April 2011; received in revised form 20 May 2011; accepted 21 May 2011

KEYWORDS:
Micro-RNAs; IBD; Crohn's disease; Ulcerative colitis

Abstract
Not fully defined pathophysiological mechanisms of inflammatory bowel disease (IBD) involve an array of genetic, epigenetic, infectious, physiological and immunological factors. Nowadays, an inadequate activation of the innate immune system to a luminal factor occurring in genetically predisposed subjects is the most widely accepted today. Micro-autoimmune diseases, a group of small, single-stranded, non-coding RNA molecules act as potent negative gene regulators. Beyond cancer and various autoimmune diseases, their impact on IBD has recently been the focus of research. Differential expression of various micro-RNAs has been documented in active and inactive ulcerative colitis, while micro-RNA profile appears to differ between ileal and colonic Crohn's disease. Except for tissue samples, attempts have been made to estimate similar differences at patients' blood samples. Apart from offering new directions in related research, these molecules arise as useful diagnostic tools and potential therapeutic targets. This review focuses on micro-RNA alterations in IBD and their potential implication on immunologic deregulation.

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doi:10.1016/j.crohns.2011.05.007
1. Introduction

Inflammatory bowel disease (IBD) is mainly represented by two subtypes: ulcerative colitis (UC), which is a chronic and recurrent inflammatory disorder of the colon and the rectum, and Crohn’s disease (CD), the other major autoimmune, inflammatory disorder that affects more frequently the last part of the ileum, the rectum and the sigmoid colon. These two notions are standing in the two opposite ends of an imaginary line, a fact that explains the inability to classify up to 10% of IBD patients into either group and the diagnosis of indeterminate colitis. The pathophysiology of it is not well defined yet, but it seems that an array of genetic, epigenetic, infectious, physiological and immunological factors plays an important role in the expression and evolution of these diseases. What is striking is that different pathophysiologic mechanisms are involved and IBD has distinct gene and protein expression signatures. Due to the complex etiology, the theory that IBD and its gastrointestinal inflammation are the results of an inadequate activation of the innate immune system to a luminal factor (i.e. intestinal flora) occurring in genetically predisposed subjects is the most widely accepted today. CD and UC are associated with expression differences in genes, involved in immune function, inflammation and tissue remodeling, including cytokines, growth factors, inflammatory mediators, extracellular matrix proteins antimicrobial factors and cell cycle regulators.

2. Micro-RNAs

Micro-RNAs (Mi-RNAs) are a group of small (about 18–24 nucleotides in length), single-stranded, non-coding RNA molecules that act as potent negative regulators in genes. The biogenesis of miRNA remains still unknown but it is quite clear that it is carried out in a multi-step process, from longer pre-miRNAs in the nucleus, to mature mi-RNAs in the cytoplasm. The maturation of miRNA occurs via a series of steps and two main RNase III endonucleases (Drosha and Dicer) are involved in this process. The inhibitory function of mature miRNA is mediated by two main pathways: the degradation of mRNA by binding of miRNA to the 3’ untranslated region of mRNA, or direct repression of the transcriptional process (Fig. 1). A number of biological processes are regulated by miRNA, including cell survival, differentiation and homeostasis; furthermore, specific miRNAs regulate the differentiation of intestinal epithelial cells. While the majority of studies are focused on the role of these molecules in the development of cancer, their impact on autoimmune diseases and especially IBD is much less studied. It has been suggested that the critical function of these small RNAs is to contribute to the establishment of immunological homeostasis at mucosal sites. Recently, Biton et al. reports on how intestinal epithelial miRNAs might regulate the differentiation of goblet cells, the associated development of antiparasitic T helper type 2 (Th2) immunity and the expression of cytokines that generate crosstalk between gut epithelial cells and the mucosal immune system. Additionally, Tili et al. reported that specific miRNA, miR-155) is induced by lipopolysaccharide stimulation to promote the differentiation of native helper T cells into Th1 cells. However, the role of miRNAs in the differentiation of epithelial cells and regulation of mucosa immune system is only just beginning to be explored. This review focuses on whether and in which mi-RNAs, the expression is altered (up- or down-regulation) in immune function, and their
implication in IBD. To better comprehend the involvement of mi-RNAs in the immunological derangement of these diseases, an attempt will be made to clarify their role in the development and function of immune cells.

3. Mi-RNAs and immune response

Given the results of multiple studies and experiments in the understanding of the immune response, it's not surprising that mi-RNAs build another step in the development and physiology of the immune system. There are several reports, where genetic ablations in the algorithm of mi-RNA maturing and incorporation in the human genome, play an important role in the deregulation of the function of immune system. For example, a deletion of Dicer (ribonuclease III enzyme) in the mi-RNA processing machine in immature double negative thymocytes leads to a reduction of the total number of thymocytes. Very few peripheral T cells are detectable when Dicer is deleted at the double-positive stage of thymocyte differentiation, and the peripheral CD4+ T cell population is reduced.7 The expression of miR-150 increases during both B-cell maturation in the bone marrow and T-cell maturation in the thymus, but decreases rapidly when naive T-cells differentiate into Th1 or Th2 cells. Similarly when Dicer is deleted in the B-cell lineage from the earliest stage of B-cell development, the pro-B to pre-B transition is almost completely blocked. This is, at least in part, due to the apoptosis of Dicer-deficient pro-B cells.8 According to another recent study9 the miR-150 regulates the transition from the pro- to pre-B-cell stage, and over-expression of this miRNA prevents B-cell development.9 Interestingly, miR-155 is required for T-cell differentiation and, also, the deletion of miR-155 results in a diminished germinal center response and impaired B-cell memory formation.10 Additionally, the same study shows that miR-146 is also important for the toll-receptor (TLR) signaling in the innate immune system. In human monocytes, expression of miR-146a and miR-146b is induced by exposure to TLR ligands, lipopolysaccharide (LPS), peptidoglycan, and flagellin.11 Micro-RNA, miR-146a reduces expression of 2 components of the TLR signaling cascade: i. TNF-receptor-associated-factor (TRAF)-6 and ii. IL-1-receptor-associated kinase (IRAK)-1. Thus, increased miR-146 expression leads to a negative feedback that attenuates the TLR response. While miR-155 and miR-146 expression is increased in macrophages in LPS stimulation, miR-125b expression is decreased. MiR-125b targets TNF-α, and thus, decreased expression of miR-125b leads to an increased inflammatory response due to an elevated expression of TNF-α.6

4. Mi-RNAs in UC

The growing understanding of the importance of mi-RNAs in innate and adaptive immunity has been followed by several studies identifying the differential expression of mi-RNAs in immune-mediated disorders such as psoriasis, atopic eczema, rheumatoid arthritis (RA), asthma, and systemic lupus erythematosus (SLE).9 As the specific differential expression of mi-RNAs has been confirmed for many immune-mediated disorders, UC and CD may uniquely demonstrate some differentially expressed mi-RNAs. Recently, mi-RNA microarray analysis and reverse transcription polymerase chain reaction (RT-PCR) validation of the active UC patient group compared to the inactive group revealed distinct mi-RNA expression patterns in patients with active versus inactive UC.9 Of the 3 mi-RNAs that were decreased in active UC, miR-192 was unchanged in inactive UC compared to healthy patients, while miR-27b and miR-422b were increased in inactive UC. Of the 8 mi-RNAs with increased expression in active UC, 4 had similar expression levels in inactive UC (miR-23a, miR-16, miR-24, and miR-29a), while the expression of miR-21, miR-126, miR-195, and let-7f was more consistent with the levels seen in control individuals. This difference suggests that some mi-RNAs may be involved in the chronic, dysregulated immune response, while other mi-RNAs are involved only in acute inflammation.9 The alteration of miRNA expression, though, was not confined to inflamed, but pre-existing non-inflamed mucosa.13 It was identified that 14 and 23 mi-RNAs were respectively altered in UC and CD, of which 8 were similarly dysregulated both in non-inflamed UC and CD biopsies.13 Wu et al.14 have also confirmed such an observation; mir-26a and mir-29a are up regulated in quiescent UC mucosa.14 Altogether, these results support the notion that dysregulation of miRNA expression pre-exists in the quiescent-colonic mucosa of UC and CD patients and may play a key role in the sensitization of the quiescent mucosa to environmental factors and/or IBD inducers.13 The study by Wu et al.14 concerning the altered expression of mi-RNAs in UC tissues shows that 3 mi-RNAs (miR-192, miR-375 and miR-422b) were significantly decreased in active UC tissues whereas 8 mi-RNAs (miR-16, miR-21, miR-23a, miR-24, miR-29a, miR-126, miR-195, and let-7f) were significantly increased in active UC tissues, as compared to healthy control tissues.14 After the mi-RNA microarray analysis and qRT-PCR the miRNAs: miR-192 and miR-21 were identified as the most highly expressed of the active UC-associated miRNAs in human colon tissues. Furthermore the expression of miR-192 was also found to be predominately expressed in epithelial cells in the normal colon (non-inflamed colon). The same study demonstrates an inverse correlation between the expression of miR-192 and MIP-2a, an epithelial cell-expressed cytokine produced by colonic epithelial cells and macrophages.14 In addition, in the same colonic epithelial cells, TNF-α-induced MIP-2a expression was also influenced by miR-192, and previous studies have shown that miR-192 was induced by transforming growth factor (TGF)-β.14,15 Its regulation by TGF-β and TNF-α and its regulation of collagen and chemokine expression indicate that miR-192 may play a key role in inflammation and fibrosis. Concerning the other active-UC associated mi-RNAs, it was indicated that only three could have a potential role in inflammation: miR-21, miR-16, and let-7f.14,15

Another study brings into light further information concerning the potential role of another miRNA with altered expression in UC.16 Differentially expressed miRNAs were observed in patients with active UC (pinched biopsies from the sigmoid colon). Using miRNA microarray and real time PCR, it was demonstrated that miR-21 and miR-155 were up regulated in the inflamed colonic mucosa.16 The miR-21 had already been reported in Wu et al.’s14 study, but this was the first report for the possible role of miR-155. Recently, miR-155 was identified and characterized as a component of the...
primary macrophage response to different types of inflammatory mediators. In the immune system, miR-155 expression is increased in activated B and T cells, and in dendritic cells (DC).17–20 Micro-RNA miR-155 plays an important role in the differentiation of B and T cells,21 and contributes to the development of regulatory T cells. In mature human DC, miR-155 is part of a negative feedback loop that down-modulates inflammatory cytokines production in response to microbial stimuli.20 Thus, miR-155 is a component of the innate immune response to a broad range of inflammatory mediators, both viral and bacterial.

5. Mi-RNAs in CD

Another study by Wu et al. has shown that mi-RNA expression is not only altered in biopsies of patients with active CD, but there is also an intestinal region-specific miRNA expression, which is altered in ileal CD (Crohn’s ileitis) and colonic CD (Crohn’s colitis).15 More specifically, in Crohn’s ileitis, the initial mi-RNA microarray profiling and, later, the validation by mature mi-RNA qPCR confirms that 3 micro-RNAs are increased in active CD tissues; miR-23b, miR-106, and miR-191.15 Additionally, it is important that these mi-RNAs were not previously identified as UC-associated miRNAs and the lack of their altered expression in active UC was confirmed. Furthermore, the initial microarray profiling and validation by miRNA qPCR has identified two other miRNAs to be under-expressed: miR-19b, and miR-629. The latter had neither been previously identified in UC.15 To confirm the initial hypothesis of miRNAs differential expression in Crohn’s ileitis, Wu et al.15 conducted a similar assessment comparing the miRNA expression in terminal ileal biopsies from patients with chronically active terminal ileal CD. Results demonstrate four miRNAs to be significantly over-expressed; miR-16, miR-21, miR-223, and miR-594.15 It can be suggested that IBD-associated mi-RNAs and intestinal tissue region specific mi-RNAs are involved in the maintenance of intestinal homeostasis and in differences in the pathogenesis of IBD subtypes. In particular, it is noteworthy that there was very little overlap between the expression levels of specific miRNAs in active UC, Crohn’s colitis, and Crohn’s ileitis. It has been demonstrated in this study that none of the active UC-related miRNAs, including miRs-16, -21, -23a, -24, -29b, -126, -192, -195, -375, -422b, and let-7f, is altered in Crohn’s colitis tissues. Similarly, none of the Crohn’s colitis-associated miRNAs has been previously found to be altered in UC tissues.15 Among the miRNAs altered in Crohn’s ileitis, only miR-16 and miR-21 have been identified to be altered in UC, but not in Crohn’s colitis.15 Table 1 is a summary of the several miRNAs involved and their differential expression in UC and CD.

6. Mi-RNAs in peripheral blood of IBD patients

Differential mi-RNA expression in UC and CD has been demonstrated in experiments that included mostly human tissues collected by colonoscopic biopsies, which automatically render mi-RNA expression profile analysis that makes this an invasive method. Recent research has been expanded in detection of differential miRNA expression in blood samples.9 According to the derived data, CD and UC differ not only in their tissue miRNA but also in their peripheral blood miRNA profiles.9 More specifically, mi-RNA microarray analysis was performed in patients with active and inactive CD and UC.9 Four miRNAs; miR-199a5p, miR-362-3p, miR-532-3p, and miRplus-E1271 were increased and one mi-RNA (miRplus-F1065) was decreased in the peripheral blood of patients with active CD, but not in the blood of patients with inactive CD, compared to healthy controls.9 Both patients with active and inactive CD had increased expression of miR-340 and decreased expression of miR-149.9 Similarly, in active UC patients’ blood, 9 miRNAs were found to have an increased expression.9 Three other miRNAs (miR-103-2, miR-262-3p, and miR-532-3p) were increased in the blood of both active and

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<td>Chronically active CD (compared to healthy controls)</td>
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inactive UC patients. Additionally, 11 miRNAs could distinguish active CD from active UC (Table 2). But in the same time, in the study of Taganov et al. the miRNA expression among the patients with active CD didn’t differ significantly between Crohn’s ileitis and Crohn’s colitis sub-groups. Thus, differences in peripheral blood miRNA expression didn’t appear to be related to the distribution of disease activity. It has to be mentioned that in this study no miRNAs common between the tissue biopsies and the blood samples in patients with Crohn’s colitis, Crohn’s ileitis and active UC were found to be differentially expressed. Since miRNAs in peripheral blood likely reflect expression in circulating white blood cells, this miRNA expression would not be expected to be the same as that seen in ileal and colonic epithelial cells. In contrast, differential whole blood miRNA expression is expected between CD and UC, since the two diseases differ in their associated T- and B-cell subtypes.

7. Conclusion
All these studies and the growing interest of scientific community, concerning the role of micro-RNAs in the presentation and evolution of the diseases associated with disorders in the immunity system, certify their fundamental role. These molecules may be influenced by inflammatory factors, before the presence of the phenotypic and clinical expression of an autoimmune, or/and inflammatory bowel syndrome. They may likely act as genetic signatures for these entities, as they vary according to the type and the evolutionary stage of IBD. Contemporary studies have demonstrated not only the existence of alterations in numerous mi-RNAs, but also confirmed the sensitivity and the specificity of certain miRNAs expressions for each one of the different subtypes in IBDs. Apart from offering new directions in related research, data extracted from similar future studies may expand the role of these molecules as useful, less invasive diagnostic tools and potential therapeutic targets.

References