

REVIEW

MicroRNAs: An epigenetic tool to study celiac disease

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ABSTRACT

This article summarizes recent findings on the role of microRNAs (miRNAs) in biological processes associated with the regulation of chronic inflammation and autoimmunity. miRNAs are small non-coding RNA molecules that have been recently emerged as a new class of modulators of gene expression at the post-transcriptional level. MiRNAs bind to complementary sequences of specific targets of messengers RNA, which can interfere with protein synthesis. We reviewed studies that evaluated the expression patterns of miRNAs in different autoimmune diseases, especially in celiac disease (CD). CD is a chronic enteropathy triggered by gluten proteins, characterized by altered immune responses in genetically susceptible individuals that results in damage to the bowel mucosa. CD has a high prevalence and an effective treatment by a specific diet ("gluten free diet"). Genetic factors confer susceptibility but do not explain the whole disease, suggesting that environmental factors do play a relevant role in the development of the condition.

The evaluation of the potential role of miRNA is of particular interest in CD given that these epigenetic mechanisms in the pathogenesis of autoimmune and inflammatory diseases have been recently described. Improving our understanding of miRNAs in CD will contribute to clarify the role of altered epigenetic regulation in the development and course of this disease.

Key words: Celiac disease. miRNAs. Epigenetics. Autoimmunity.

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ABBREVIATIONS

ADN: Deoxyribonucleic acid.
AID: Autoimmune diseases.
CD: Celiac disease.
DM1: Diabetes mellitus type 1.
GWAS: Genome-wide association study.
HLA: Human leucocyte antigen.
IBD: Inflammatory bowel disease.
IgA: Immunoglobulin A.
IL-1 β : Interleukin 1 β .
INF γ : Interferon gamma.
ME: Multiple sclerosis.
miRNAs: MicroRNAs.
mRNA: Messenger RNA.
NF κ B: Nuclear factor kappa B.
Pre-miRNA: Premature miRNA.
Pri-miRNA: Primary miRNA.
RNA: Ribonucleic acid.
RNAase: RNA polymerase.
SLE: Systemic lupus erythematosus.
SNP: Single nucleotide polymorphism.
T reg: Regulatory T cells.
TG2: Tissue transglutaminase 2.
TNF α : Tumor necrosis factor- α .
tTG: Tissue anti-transglutaminase.
UC: Ulcerative colitis.
UTR: Untranslated region.

INTRODUCTION

Celiac disease (CD) is a gluten-sensitive enteropathy mediated by the immune system and considered one of the more complex genetic diseases. The rate of concordance in monozygotic twins is about 75 % (1,2). The haplotypes HLA (human leucocyte antigen) DQ2/DQ8 confer the highest estimated heritability reported so far (close

to 35 %) (3). Exposure to gliadin, a constitutive gluten protein is the main factor associated with the onset of symptoms of CD, triggering an altered immune response in patients carrying the risk HLA haplotypes. However, presence of these HLA haplotypes is a necessary but not sufficient condition for the appearance of CD. In fact, approximately 30 to 40 % of healthy subjects carry HLA alleles of risk and only about 1 % of the population develops the disease (4,5). As for genetic factors not associated with HLA, several genome-wide association studies (GWAS) have described several susceptibility loci, each of which is associated with risk of developing CD (6), but as a whole they explain only a small proportion of the risk of CD. An interesting field related to this pathology is the role of epigenetic regulation mechanisms, an area that has been scarcely investigated until now.

The microRNAs (miRNAs) are small (between 20 to 25 nucleotides) non-coding ribonucleic acid (RNAs) that regulate gene expression through the canonical base pairing of complementary sequences in the 3'-untranslated region (UTR) of the target messenger RNA (mRNA) (7). Since its identification in 1993 (8) it has been demonstrated miRNAs are important in both physiological and pathological conditions (9). They are involved in the regulation of gene expression in a variety of biological processes, such as autoimmune disorders (10) and the development and function of mature immune cells (11,12). In this article we will discuss the basic concepts of CD and review the current evidence on the participation that miRNAs may have in the modulation of gene expression in autoimmunity, with special emphasis on CD.

CELIAC DISEASE

In the last few decades the concept of CD has substantially changed. The use of plasma anti-endomysial and anti-tissue transglutaminase (tTG) antibodies allowed an active search of affected individuals among less symptomatic individuals. Historically, the disease was diagnosed on the basis of small bowel biopsies showing intense mucosal damage. It was considered a gastrointestinal disease mainly affecting children, with a low frequency in adults. However, studies in the last few decades have revealed that CD affects equally to adults, being even more frequent than in pediatric population (13,14).

Clinical pediatric presentations of CD typically appear during the first years of life, after the introduction of gluten in the diet. Among these, the most frequent is the so-called "classic" clinical presentation, characterized by prominent digestive disorders, especially diarrhea with malabsorption syndrome, weight loss, and nutritional deficiencies (15). In the adult, CD frequently appears with less gastrointestinal symptoms, absence of malabsorption syndrome and less intense flattening of the villi in the small intestinal epithelium. In "atypical" presentations extra-intestinal symptoms and associated diseases (usually autoimmune diseases) are

the more prominent features leading to diagnosis, a fact that for a long time made difficult their identification and diagnosis (16,17). A study that evaluated differences in clinical manifestations at the time of diagnosis in a group of patients diagnosed during childhood *versus* adulthood, showed that in the former classic forms of the disease clearly predominated, with a high positivity of serological markers and flattening of the mucosa, which helped reaching diagnosis in shorter times. In contrast, in patients diagnosed at adult ages, atypical forms were more frequent and histological lesions more variable and less intense; also, associated autoimmune diseases (AID) were frequent and diagnosis was delayed (18). Currently, several prevalence data come from studies based on the serum levels of antibodies IgA- anti-tTG (IgA-tTG). When CD diagnosis is confirmed by small intestinal biopsy in seropositive individuals, the frequency of CD decreases, but is still much greater than previously known, ranging from 1:70 to 1:500 inhabitants (19). This may be due to the fact that these antibodies identify individuals affected mainly by the autoimmune components of the disease, which often coincides with less intense digestive symptoms.

Serological diagnosis of CD, i.e., diagnosis based solely on positive serological tests, is not currently accepted. An exceptional situation is recognized by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (20); they indicate that in small children, shown the classical presentation, are intensely symptomatic and their IgA-tTG levels is greater than 100 U/ml, the small intestinal biopsy is not necessary to diagnose the condition. In all other cases, the small bowel biopsy continues being required to confirm the diagnosis of CD (21).

Thus, today we understand that CD is a frequent disease affecting approximately 1 % of the population (22), both children and adults, that can appear in any time in life, and that has a relevant genetic and autoimmune component (22). Currently, one of the main challenges for physicians and researchers is to understand the interactions between genetic factors, the environment and the immune system, such that we can understand the huge clinical variability. In relation to genetics, the failure to explain the disease completely has led to study the entire genome and, on the other hand, epigenetic is receiving increasing attention as an interesting field that may shed light in this complex situation. It is for this reason we will review this topic, giving especial attention to miRNAs, an interesting epigenetic mechanism appears as an important tool for understanding gene regulation and their alterations, which are proving to be involved in several autoimmune diseases and might be involved in the onset and course of CD.

Physiopathology

CD is a chronic autoimmune-mediated enteropathy which develops in the small intestine triggered by gluten

proteins present in wheat, rye, and barley in genetically susceptible individuals. As a result, damage develops in the small intestinal mucosa. Their typical histological features are an increase in intraepithelial lymphocytes, flattening of the intestinal villi, crypt hyperplasia and an important infiltration of inflammatory cells in the lamina propria. A gluten-free diet usually results in a rapid recovery of the intestinal mucosa which is accompanied by a significant improvement in the absorption of several nutrients. To date, this dietary treatment is an efficacious treatment for a large majority of patients, who show evident clinical improvement within a few weeks (23).

In CD, CD4 T cells play a major role in the initiation and organization of the altered immune response (24). A model proposed to understand the pathogenesis of CD involves luminal, epithelial and mucosal events, including the activation of T lymphocytes (25). Among luminal events, gluten digestion is the primary feature. Gluten is digested to peptides in the intestinal lumen; due to lack of prolyl endopeptidases in the intestinal villi, gastric and pancreatic secretions, after initial digestion residual (relatively large) gluten peptides rich in proline and glutamine may remain for long periods of time in the lumen, favoring the contact of these peptides and the epithelium. However, in 99 % of individuals, including those that carry HLA-DQ2 and HLA-DQ8 (the susceptibility alleles for CD), this does not represent a problem or an increased risk for CD (25).

Routes through which gluten passes through the epithelium are not fully understood yet, but there is some evidence suggesting that it could occur via paracellular transport, passage through transepithelial transport (transcytosis) (26), or by protrusions of dendritic cells that sense the luminal content. Partially-digested gluten peptides reach then the antigen-presenting cells present in the sub-epithelial region of the small intestine. An acute infection or inflammatory states of the small intestinal mucosa of different origins would set the conditions for mucosal T cells response to gluten peptides. With a Th1 type response established in the mucosa, gluten peptides preferentially binds to HLA-DQ2 or HLA-DQ8 and activates T-cells, starting the production of Th1 cytokines. Then, release of IFN- γ and other cytokines perpetuate this response, altering intestinal permeability and leading to the activation and release of metalloproteinases that will be responsible for the collapse of the small intestinal architecture (27).

Genetic

CD is a polygenic disorder that involves HLA and non-HLA genes. The best known risk factors are encoded in the HLA class II molecules, HLA DQ2 and HLA DQ8, known as susceptibility factors. About 90 and 10 % of individuals with CD are carriers of these heterodimers, respectively (28). Deamidated gliadin peptides have a

high binding affinity for HLA DQ2 and DQ8 molecules, explaining the immunogenicity of gluten in susceptible individuals (29). Tissue transglutaminase (TG2) is a highly ubiquitous enzyme that deamidates gluten peptides after crossing the intestinal epithelium (30). This process introduces a negative charge in a favorable position, eliciting the attachment of gluten peptides to HLA DQ2 and DQ8 molecules and as a consequence, increasing gluten-specific CD4 T cells.

In order to improve our understanding of the genetics of non-HLA genes in CD, genes have been extensively assessed through genome-wide association studies (GWAS). This technique provides a general approach that permits identifying genes and pathways involved in a particular phenotype. The first GWAS on CD was carried out in England (2007) and included 778 patients with CD and 1,422 controls (31). Results showed that, in addition to HLA regions, 13 regions in the genome were associated with CD. The majority of the identified regions contained genes that control immune responses; of them it is worth mentioning locus IL-2 and IL-21 in chromosome 4q27. IL-2 is a critical cytokine for T cells homeostasis and function, and IL-21 is a new member of the superfamily of type 1 cytokines that regulates many other immune cells. These findings suggested, for the first time the potential role of IL-2 and IL-21 in the pathogenesis of CD (31). The same GWAS study also revealed the phenomenon of pleiotropy, indicating that genetic variants associated with CD are also involved with other immunity-related diseases (32). A second GWAS study, which included more than 4,500 patients with CD and near 11000 controls from four different populations (England, Italy, Finland and the Netherlands) identified 13 new genome regions associated with CD; the majority of the identified genes were found to have immune functions and an important regulatory role in the selection of the T-cells in the thymus, for example THEMIS/PTPRK (33,34).

Studies on genetics of AID have been focused on genes encoding proteins, aimed at finding a single nucleotide polymorphism (SNP) that alters both the sequence of proteins and their function. However, one of the more surprising findings indicates that only three (MMEL1, SH2B3 and IRAQ1) of the 57 SNPs identified affect protein sequences in celiac individuals (34). Several SNPs have been identified in intronic and intergenic regions of the genome, suggesting that the transcriptional regulation of these genes could be affected by SNPs of risk for CD.

There are several ways through which epigenetic mechanisms could exert an effect on gene transcription; for example, by altering or creating binding sites for transcription factors or by modifying binding sites of protein complexes that regulate chromatin, translating into an epigenetic effect on the methylation of DNA and histones modification (35,36). Also, the association of CD with genes that affect 3' UTR sequences could lead to a decreased stability or increased degradation of the respec-

tive mRNA. On the other hand, they could promote the inhibition of protein translation to alter binding sites of RNAs or affect binding sites to miRNAs (6).

EPIGENETICS

Epigenetics refers to inheritable changes of genes function, which do not imply a change in the deoxyribonucleic acid (DNA) sequence (37). Heritability implies that an epigenetic marker has the ability to persist during development and that is potentially transmitted from generation to generation (38). The main role of epigenetics is gene regulation. This is a key process in: a) Modulation of individual genes and their activity; b) regulation of groups of genes that are functional in each specific cell type and in the development and differentiation of cell types; and c) the cellular metabolic plasticity, a characteristic that confers an adaptation capacity to exogenous changes. There are different epigenetic mechanisms regulating gene expression, either activating or suppressing it. Examples include DNA methylation, histone modification, the positioning of nucleosomes and miRNAs. It is important to note that these mechanisms may act jointly in the regulation of gene expression (39). Epigenetic is rising as a powerful tool to the study of AIDs, conditions in which there is a strong genetic component but where genetics does not fully explain the disease.

CD has a high prevalence and genetic factors explain many but not all cases. HLA-DQ2/DQ8 risk alleles are the major and best known susceptibility factors. Research on non-HLA genes has not been successful and continues under study. In this sense, epigenetics appears as an important tool that may contribute to the understanding of the pathogenesis of CD. In the following sections, we reviewed the current evidence regarding miRNAs, one of the four best known mechanisms used in epigenetics. New evidence is appearing referred mostly to AID others than CD, such as rheumatoid arthritis, psoriasis, type 1 diabetes (DM1), systemic lupus erythematosus (SLE), multiple sclerosis (MS) and inflammatory bowel disease (IBD). In all them gene expression of several miRNAs has been assessed and changes have been demonstrated.

microRNAs

miRNAs are a class of endogenous single-stranded RNAs, small and non-coding RNA that regulate gene expression through the control of stability and translation of the mRNA (40-42). Some of the biological processes regulated by miRNAs include cell differentiation, proliferation, apoptosis and cell cycle control (42-44). The transcription of genes of a primary miRNA (pri-miRNA) is carried out initially by the RNA polymerase

(RNAase) II or III in the nucleus. Then, a microprocessor complex, composed of the protein Drosha and their associated proteins (DGCR8/Pasha) process the pri-miRNA precursor to a mother strand (of approximately 70 nucleotides) generating a premature miRNA (pre-miRNA) (45-47). Pre-miRNAs are exported to the cytoplasm where they are split into a 21 nucleotide miRNA double strand by the enzyme RNase III Dicer, together with its associated protein TRBP (transactivator RNA binding protein). Then, one strand is included within the RNA-induced silencing complex (RSIC) guiding this complex to the non-translatable 3' region of the sequences of the target mRNA, inducing the degradation of the mRNA by suppressing the expression of the protein (48,49) (Fig. 1).

MiRNAs bind to their mRNA targets through matching bases of RNA-RNA. The sequence of union of an miRNA to the target mRNA is generally known as the Element of Recognition of miRNA (ERM) (50). The mechanism used by miRNAs to silence the mRNA remains unclear. However, it is now known that a complementary strand of miRNA of at least 6 nucleotides is sufficient to carry out the post-translational regulation associated with the miRNAs. A single miRNA could act on several hundreds of target mRNAs and each mRNA can be the target for many miRNAs. For this reason, several degrees of interactions have been proposed, including degradation of proteins, inhibition of the elongation of translation, the early termination of translation, or inhibition of the initiation of translation (51). If the matching degree between miRNA and the target mRNA is partial, the translation of the target mRNA is repressed, without affecting the level of mRNA. However, in cases where there is a perfect or extensive matching degree, the target mRNA is deadenylated and destabilized by endonucleotidic excision and therefore proteins synthesis stops by degradation of the target mRNA in the cell (52).

miRNAs IN THE CONTROL OF AUTOIMMUNITY

Since the discovery of the miRNAs, several studies have shown that they could play a role in AIDs (10,53). It is well known that miRNAs are involved in the development of immune cells and in controlling of their functions. To date, miRNA have been associated with various pathological conditions of the immune system. Recent studies reveal that the miRNAs regulate not only the development of innate or adaptive immunity cells but also the critical balance of this response (54).

The altered miRNAs expression in some inflammatory diseases and AID has been extensively studied, as they could affect key transcripts in the development of these conditions (55). It has been described that miR-155 and miR-146 are overexpressed in synovial fibroblasts of

patients with rheumatoid arthritis compared with healthy subjects. The expression of miR-155 is increased by pro-inflammatory molecules such as tumor necrosis factor- α (TNF α) and interleukin-1 β (IL-1 β), determining an inhibitory effect on the expression of metalloproteins in synovial fibroblasts (56). On the other hand, miR-146, whose function is inhibiting the nuclear factor Kappa B (NF- κ B), is up regulated by pro-inflammatory cytokines (57,58).

In patients with psoriasis, miR-21 and miR-146a have been reported to be overexpressed in skin samples while the expression of miR-125b was diminished in comparison with controls (59). There are a few studies of miRNAs expression in DM1; they suggest that regulatory T cells (T regs) function is influenced by changes in the expression of specific miRNAs. In T regs of diabetic patients there was increased expression of miR-510 and decreased expression of miR-342 and miR-191, miRNAs with a still unknown function. Other studies have shown that miRNAs could have a cytokines mediated cytotoxic effect on pancreatic β cells. When IL-1 β and TNF- α induce miR-21, miR-34a and miR-146a expression in pancreatic islet cells, the increased production of pro-inflammatory cytokines would lead to β -cell failure (60,61).

The majority of genes associated with SLE contain at least one target site for miRNAs. For example, miR-146a

is a negative regulator of Toll-like receptors and its expression is decreased in patients with SLE. Also miR-146a, a negative regulator of the signaling pathway of INF type I, acts regulating the interferon regulatory factor-5 (IRF-5) and signal transducers and activators of transcription protein (STAT-1). Therefore, a decreased expression of miR-146a in peripheral blood mononuclear cells could contribute to the increase in the production of INF type 1 in SLE (62). Another miRNA regulating T and B cell function is miR-155, whose up-regulation in T and B lymphocytes could lead to abnormal activation of B cells and to the abnormal development of inflammatory T cells in patients with SLE (63,64).

MS is another autoimmune disorder in which miRNAs are thought to be involved in pathogenesis. A recent study showed that miR-326 plays a key role regulating the differentiation of Th-17 cells, because of its role inhibiting Est-1, a negative regulator of differentiation in these cells; miR-326 was found to be significantly up regulated in patients with MS in remission or relapsed, causing an increase in the number of Th-17 cells and severe symptoms of the disease (65). Other miRNAs reported in MS are miR-34a and miR-155; it is proposed that they could be involved in the active phase of the disease because of their action on CD47 cells; miR-155 is a membrane protein that allows recognition of proteins and avoids phagocytosis by spe-

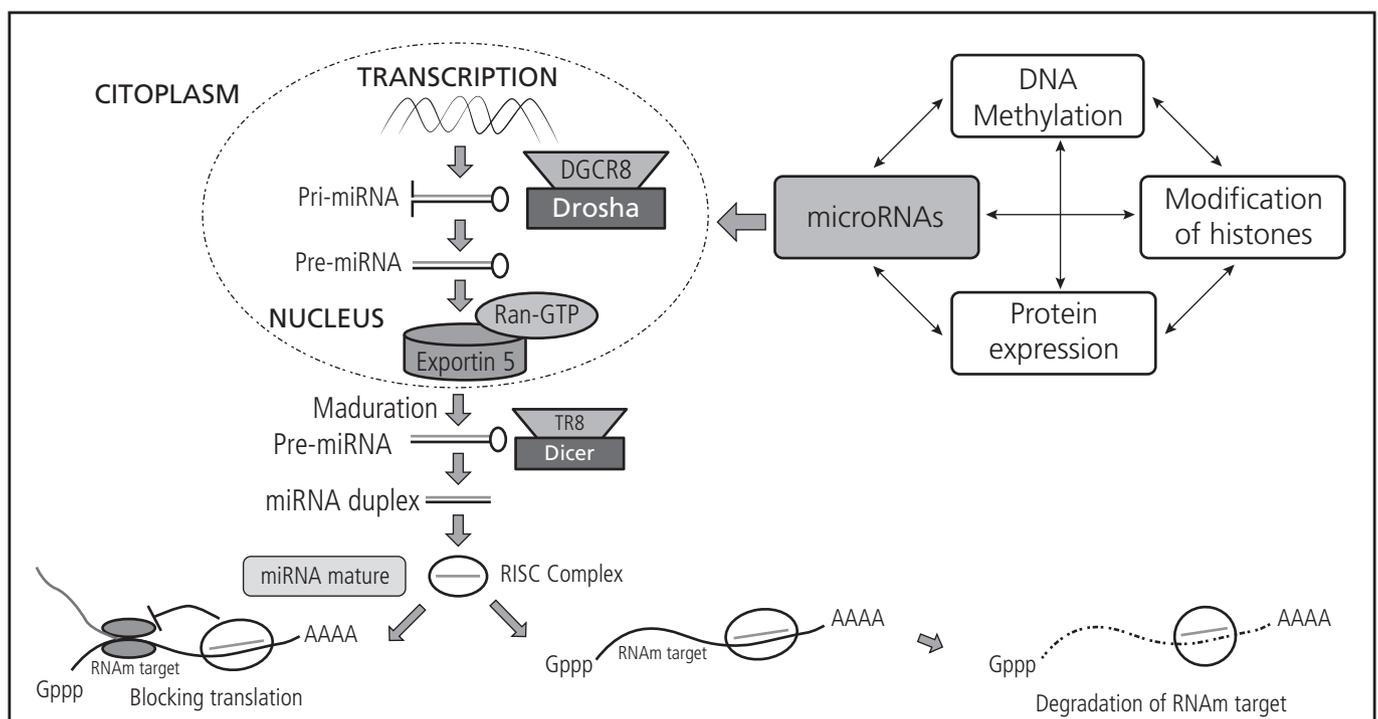


Fig. 1. Epigenetics and biogenesis of miRNAs. The epigenetic regulation of gene expression can be conducted through different mechanisms, activating or repressing gene expression. The expression of protein resulting from the action of these mechanisms would ultimately translate into in the observed phenotype. miRNAs are translated from the genome, being processed in the nucleus and exported to the cytoplasm. miRNA are loaded into the RISC complex and can bind to specific sequences of mRNA to repress the protein translation.

cialized cells. Macrophages with low levels of CD47 act independently from this inhibitory control signal, causing increased phagocytosis of myelin in MS. Mir-155 also promotes the development of Th-1 and Th-17 inflammatory cells (66).

In IBD, Wu et al. (67) in 2008 were the first to report the miRNAs expression in samples of colonic mucosa of IBD patients. They identified 11 miRNAs differentially expressed in ulcerative colitis (UC) in comparison with controls subjects, showing an inverse relationship between the chemokine called macrophage inflammatory protein (MIP 2) –previously implicated in IBD (68)– and miR-192. Similarly, Bian et al. (69) found a significant overexpression of miR-150 in inflamed colonic mucosa of patients with UC; they established an inverse correlation between miR150 and its mRNA target, c-Myb, a proto-oncogene that is involved in apoptotic processes (70). On the other hand, a recent study on Crohn's disease, showed that a silent mutation in the reading frame of the IRGM gene (a gene required for autophagy of intracellular bacteria), acts as a functional variant by altering the site target of union for miR196. This finding showed that the IRGM protective variant possesses a target site for mir196, union that usually results in a decrease in the amount of IRGM proteins in cellular inflamed epithelial cells. This observation provides the first evidence that a silent mutation may be clinically significant (71).

miRNAs IN CELIAC DISEASE

Despite the growing number of studies about the role of miRNAs in AIDs, data about CD are scarce. A recent study highlighted the importance of the miRNAs in the differentiation and function of intestinal epithelium of mice. Authors quantified the expression profile of the total miRNAs present in the intestinal mucosa and determined the contribution of miRNAs to intestinal homeostasis. The study identified 453 families of miRNAs, mmu-miR-192 being the most highly expressed in both the small and large intestine. In Dicer-deficient mice, the intestinal epithelium was disorganized with a decreased numbers of goblet cells and a significant increase of crypts apoptosis in jejunum and colon, in addition with accelerated cell migration in jejunum. Moreover, the function of intestinal barrier was altered resulting in bowel inflammation with infiltration of lymphocytes and neutrophils; the authors concluded that Dicer protein possesses a vital role in the differentiation and function of the intestinal epithelium (43).

Capuano et al., studied small intestinal biopsies of celiac children and found that about 20 % of the miRNAs expression tested were different when compared with control children, regardless of whether the disease was active or not. The study of celiac patients showed high levels of miR-449 expression and an inverse association between the overexpression of miR449a, NOTCH1 sign-

aling (crucial in maintaining homeostasis of the intestine by controlling cell proliferation and differentiation) and the production of goblet cells, two features considered characteristic of the small intestine of celiac patients (72). A more recent report analyzed the expression of miRNA in duodenal mucosa of a group of untreated adult celiac patients (with classic presentation or iron-deficiency anemia), a group of treated patients (with or without remission of the duodenal mucosal lesion) and control subjects without CD. Authors reported a deregulation of seven miRNAs (miR-31-5p, miR-192-3p, miR-194-5p, miR-551a, miR-551b-5p, miR-638, and miR-1290) in patients with different clinical phenotypes compared to subjects without CD. These 7 miRNAs were then measured in duodenal fibroblasts obtained from patients and incubated with gliadin peptides (13 and 33mer). The group of miR-192/194 (involved in remodeling of the extra-cellular matrix) was found deregulated in CD, with variations among the different clinical presentations. After stimulation with gliadin peptides, expression of miR-192-3p was found decreased in fibroblasts derived from celiac patients, whereas its expression remained unchanged in fibroblasts of control subjects. The authors concluded that analysis of miRNAs deserves special consideration, especially as a potential tool to control treatment and management of CD (73).

It is important to note that studies of miRNAs in CD have been conducted mainly in intestinal epithelial cells. The regulation of gene expression in the intestinal epithelium is complex and controlled by different signaling pathways that regulate the balance between proliferation and differentiation, processes altered in pathologies such as CD (74). It would be important to determine the regulation of miRNAs in cells of the immune system, either locally or systemically, to understand how these molecules would be participating in the pathogenesis of the disease. Studies in this area are needed to determine the relevance of miRNAs expression in the functioning of the immune system in CD (Fig. 2).

CONCLUSIONS

miRNAs are important factors in the differentiation and function of the intestinal epithelium, and they have a relevant role in regulating gene expression in physiological and pathological conditions, including inflammatory and autoimmune disorders. Given that at present the molecular determinants underlying the pathogenesis of CD remain unclear, is interesting to investigate miRNA profiles and function in these patients. Current evidence on the interaction between the miRNAs and the spectrum of autoimmune pathologies opens a new approach to the study of CD. At present, the available evidence in celiac patients, with different clinical characteristics, shows an altered expression pattern of miRNA when compared with healthy controls. This suggests that miRNAs deserve further study, as they

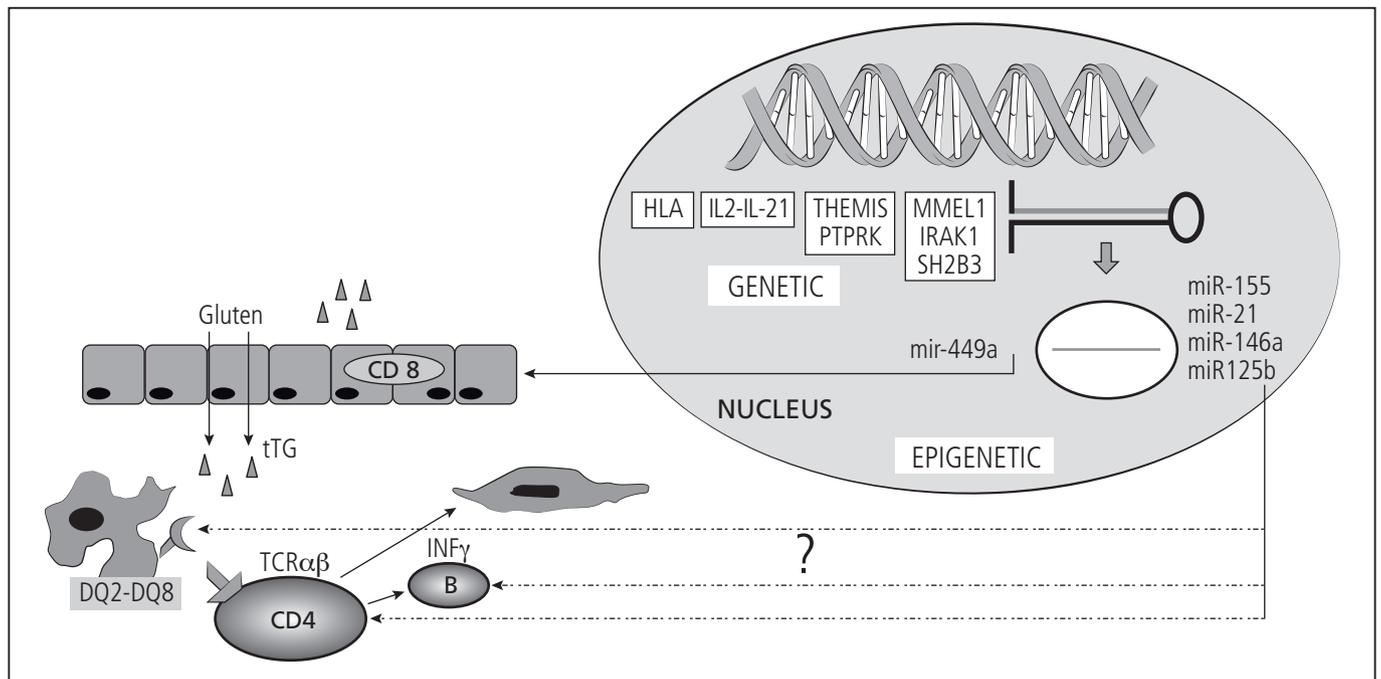


Fig. 2. Genetics and epigenetics in celiac disease. Celiac disease has a recognized genetic susceptibility given by the molecules HLA DQ2-DQ8. After the deamidation of gluten peptides by the tissue transglutaminase (tTG), those peptides bind to HLA DQ2-DQ8 and presented to the CD4 T lymphocytes. CD4 T lymphocytes are responsible of amplify the response through the release of cytokines and/or activation of other immune system cells, accounting for the vast majority of the signs and symptoms of CD. There are other loci that would give susceptibility to CD, each of which is associated with a low risk for developing the disease. miRNAs regulate the proliferation and differentiation of epithelial cells. The expression of miRNAs associated to inflammation and AIDs, may modulate protein expression or target molecules involved in this pathology.

may represent potential biomarkers that could help distinguishing patients with different clinical profiles. Moreover, specific miRNAs profiles could contribute to the individualized management of a patient, transforming the widespread clinical approach to a custom one. Finally, miRNAs could contribute to elucidate how epigenetic alterations participate in the development and course of CD.

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