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REVIEW ARTICLE

miRNAs as attractive diagnostic and therapeutic targets for Familial Mediterranean Fever

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ABSTRACT

Familial Mediterranean Fever (FMF) is a hereditary early-onset disease that causes periodical fever attack, excessive release of IL-1β, serositis, arthritis and peritonitis. Genetic analyses conducted on FMF patients (mutated and non-mutated) have highlighted that additional contributing factors such as epigenetics and environment play a role in clinical manifestations of FMF. Recently researchers report that microRNAs (miRNAs), implicated in epigenetic mechanisms, may contribute to the pathogenesis of FMF. miRNAs, a member of the captivating noncoding RNA family, are the single-strand transcripts that work in physiological and pathophysiological processes by regulating target gene expression. Recent studies have shown that miRNAs are associated with various mechanisms involved in the pathogenesis of FMF, such as apoptosis, inflammation and autophagy. Moreover, these miRNAs molecules might have potential use in treatment, therapeutic response monitoring and the diagnosis of subtypes of the disease in the future. Motivated by these potential benefits (diagnostic and therapeutic) of miRNAs, we focus on recent advances of clinical significances and potential action mechanisms of miRNAs in FMF pathogenesis and discuss their potential use for FMF.

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Introduction

Familial Mediterranean Fever (FMF) is known as a hereditary early-onset illness distinguished by periodical fever attack, excessive release of IL-1β, serositis, arthritis and peritonitis [1]. The prevalence of FMF is prominently high in Mediterranean populations. However, FMF has also been diagnosed in other countries such as Japan and Brazil although less frequently, and this may be the result of immigration [2,3]. Secondary amyloidosis, which mainly affects kidney tissue, is a serious complication of FMF [1]. Furthermore, attacks and chronically inflammation continue to adversely impact the quality of life of FMF patients [4]. For FMF treatment, Colchicine is commonly used to avert attacks, reduce subclinical inflammation and avoid secondary amyloidosis [5]. The MEditerranean FeVer (MEFV) gene is considered to be responsible for FMF, and it encodes the Pyrin protein [6,7]. Pyrin has a crucial part for the immune system and regulates the secretion of Interleukin 1β [8]. The epigenetics and environmental factors are reported to cause heterogeneous clinical conditions in FMF patients who have similar MEFV genotypes [9]. Recent research reports that microRNAs (miRNAs) are implicated in epigenetic mechanisms, may contribute to the FMF pathogenesis [10]. miRNAs are single-strand non-coding small RNA molecules, and they are associated with many physiological and pathophysiological conditions. miRNAs bind to complementary sequences of target messenger RNA (mRNA) as essential regulators for gene-silencing [11]. miRNAs are crucial players in various cellular mechanisms such as proliferation, inflammation, apoptosis, autophagy and metabolism [11,12]. The studies of miRNA continue to increase our understanding of molecular pathogenesis related to inflammatory diseases, including FMF [13–15]. Altered expression levels of miRNAs in serum and plasma make them attractive molecular targets for FMF therapy [16,17]. In this paper, we focus on recent advances of clinical research and potential action mechanisms of miRNAs in FMF pathogenesis and discuss their potential use for FMF.

The pathogenesis of FMF

FMF is one of the diseases associated with the inflammatory system, and the molecular pathogenesis of FMF is mostly explained with Pyrin molecule encoded by the MEFV gene [6]. However, it is not clear whether the genetic defect causing the FMF disease leads to loss or gain of Pyrin function. Papin et al. [18] revealed that while Pyrin normally works

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as a caspase-1 and IL-1β inhibitor, the knockdown of Pyrin increases caspase-1 activation and IL-1β release. The results of this study support hypothesize Pyrin function loss. On the other hand, Booty et al. [9] found that Pyrin protein levels are elevated in FMF patients, suggesting that Pyrin may cause enhanced inflammatory events related to FMF. Pyrin is known as a regulator protein that is implicated in various processes such as apoptosis, inflammation, and signalling transduction through specific protein interactions, and it is prominently expressed in immune blood cells and synovial fibroblasts [8]. Pyrin includes structurally five domains with unique functional features: I) Pyrin is founded at N-terminal end; II) a bZIP is located at the right side of Pyrin domain; III) the B box zinc finger; IV) the a-helical; V) a B30.2 (PRYSPRY) is located at carboxy end [19,20]. These domains of Pyrin interact with specific proteins (Figure 1). Currently, there is no consensus on the action mechanism of Pyrin in FMF. It is believed that Pyrin acts as a suppressor to the response of inflammatory events under normal physiologic conditions, whereas mutant Pyrin cannot inhibit the inflammation, and thereby lead to aggravation of inflammatory response [21]. Fundamentally, Pyrin protein regulates the secretion of IL-1β which has a crucial part in the pathophysiology of FMF through interacting with inflammasome components and caspase-1. Although we have been learning new crucial knowledge about the MEFV gene and Pyrin protein for over 20 years, the molecular pathophysiology of FMF remains still unclear.

Genetic background of FMF

FMF is defined as an autosomal recessive disorder and closely related to the MEFV gene, which is found in chromosome 16 and consisting of 10 exons [6,7]. MEFV was identified in 1997 and as discussed above, encodes a protein known as a Pyrin, which contains 781 amino acids and predominantly expressed in immune cells and fibroblasts (Figure 1). Pyrin is a regulatory molecule that is crucially implicated in several processes such as apoptosis, inflammation and secretion of cytokine [6,7,22]. Hitherto, greater than 370 MEFV sequence variants are recorded at INFEVERS (an online genetic database-https://infevers.umai-montpellier.fr/web/search.php#ancre1468) [23]. However, not all of these variants are directly associated with the clinical features and molecular pathogenesis of FMF, and many of them are considered as variants that have unclear significance. A group of experts, in 2012, reached a consensus on MEFV variants that can be used in the genetic diagnosis of FMF, and they recommend a total of 14 MEFV variants, including nine pathogenic variants and five variants of unclear significance for the purpose of diagnostic (Figure 1) [24]. Some genetic mutations in exon two and exon ten are reported to be responsible for greater

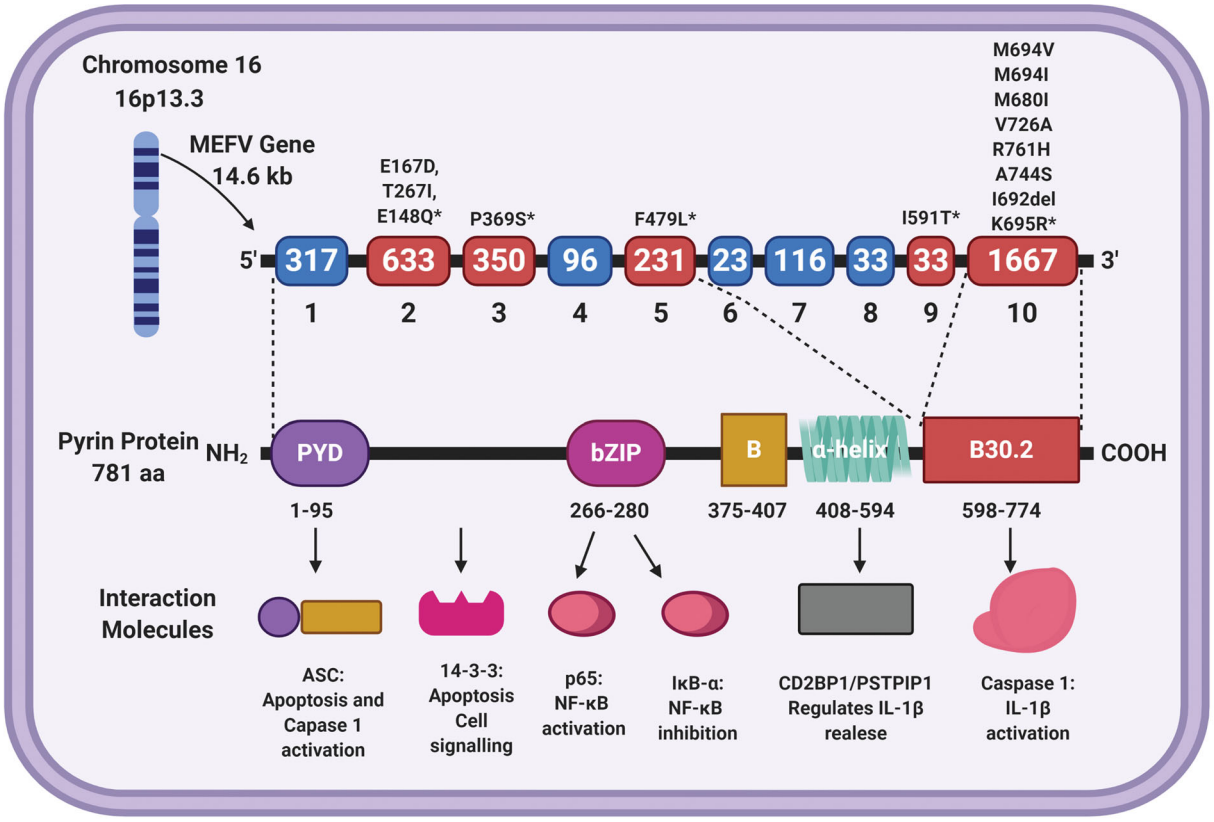

Figure 1. Schematic representation of MEFV gene, Pyrin protein and molecules interacting with Pyrin. MEFV gene, which is consisting of 10 exons, is located in chromosome 16. There are 14 MEFV variants (nine pathogenic variants and five variants of *unclear significance) recommended for diagnostic purposes. Pyrin includes structurally five domains with unique functional features; Pyrin domain, bZIP domain, B box zinc finger domain, α-helical domain and B30.2 (PRYSPRY) domain. These domains of Pyrin interact with specific proteins. ASC: apoptosis-associated speck-like protein containing a CARD, 14-3-3:14-3-3 protein, p65: transcription factor p65, IκB: NF-κB inhibitor, PSTPIP1: proline serine threonine phosphatase.

than 85% of the FMF cases in the Mediterranean region [1]. The E148Q mutations in exon two are seen less frequently in FMF patients, and this variant also has a high carrier rate in general populations [25,26]. However, the role of E148Q variant in FMF is still under debate.

Some studies showed the link between symptoms and genotype of FMF [27–30]. The frequency of MEFV mutations and impact of clinical symptoms may vary among populations. For example, M694V is the most prevalent mutation in the Turkish population and has a relationship with the severity of the disease, adverse prognosis and treatment resistance [31]. Also, patients who have homozygous M694V mutation are more likely to have an early FMF onset, and more incidence of amyloidosis complications were observed in these patients [27–30]. However, the research investigating the correlation between genotype and phenotype is unsettled. Some papers emphasized that there is no relationship between genotype and clinical symptoms [31,32], and for example; Yalcinkaya et al. found that there is no correlation between M694V mutation and the clinical severity of FMF [32].

Mutations in both alleles of FMF patients are expected since FMF is traditionally known as an autosomal recessive disease. However, some studies revealed that there is only one MEFV gene mutation in the considerable majority of FMF patients [9,33,34]. A study by Booty et al. investigated whether a second MEFV mutation exists in FMF patients with clinical symptoms. Their results showed that there is no second MEFV mutation in those patients [9]. The presence of clinical symptoms among FMF patients with heterozygous is prevalent, and this is accepted as a dominant condition with low penetrance [35].

In summary, many people with only one MEFV mutation may be an FMF carrier without symptoms. The clinical features of FMF may occur in heterozygous patients with only one MEFV mutation. Moreover, patients with homozygous mutations may show no clear signs of FMF. As a result, one can speculate that FMF is a multifactorial disease generated by environmental factors and many genes, and these factors affect the clinical expression of FMF [36–39].

miRNAs

miRNAs, a member of the captivating noncoding RNA family, are the single-strand transcripts that have a critical part in cellular mechanisms such as metabolism, apoptosis, autophagy and inflammation by regulating target gene expression [40]. It is estimated that miRNAs suppress the expression of 30–60% of genes that encode a protein by binding to the 3′ end of their complementary target mRNA [41]. However, this binding of miRNAs to target mRNA is usually not perfect [40,42,43]. Recent functional studies performed with knockout models and overexpression experiments have enabled understanding of the physiological functions of specific miRNAs [44]. miRNA knockouts do not frequently cause severe phenotypic changes. The genetic silencing of some miRNAs reduces the suppression impact on their target mRNAs, and this condition mostly can be tolerated by cells. Nevertheless, a light overexpression of various mRNAs may lead to abnormal phenotypical changes. For instance, Tan et al. reported that downregulation of miR-128 may result in fatal epilepsy in mice due to increased mRNAs involved in the MAPK pathway [45]. miRNA biogenesis process starts in the nucleus, and the primary miRNA is synthesized by RNA polymerases II and III from a specific independent miRNA gene or intron of a protein-coding gene, and the maturation and further processes of miRNAs continue and are completed in the cytoplasm [46,47]. After transcription, the forming pri-miRNAs are processed by RNase III enzymes and turned into approximately 70 nucleotides length, and later, these emerging molecules are named as the precursor-miRNA (pre-miRNA) [48]. Subsequently, pre-miRNA, which contains a hairpin-like structure, is transported to the cytoplasm with the action of exportin channel proteins, and a mature miRNA double strand is generated from the pre-miRNA in the cytoplasm with the second cleavage process by RNAse III Dicer [49]. One of the two strands is mature functional miRNA and combined with the RNA-induced silencing complex (RISC), while the other one is known as the passenger strand, which will be degraded [50]. Mature miRNA double strand is loaded into Argonaute (Ago) proteins to create functional miRNA and the RNA-induced silencing complex (RISC). So, the functional silencer miRNA is able to regulate the gene expression by base-pairing to the complementary sequence of target mRNA [43]. miRNAs are regarded as an essential part of epigenetic mechanisms. The expression of some miRNA genes can be regulated by DNA methylation and histone modification. miRNAs can also regulate gene expression and chromatin structure through binding a complementary sequence of gene promoters with a specific protein complex [41].

The secreted miRNA transcripts can function in intercellular communication [51]. These mature miRNAs can be transferred through Gap Junctions and target mRNA in the adjacent cells [52]. In addition, it is speculated that vesicles, exosomes, apoptotic bodies and lipoproteins are involved in the transportation of extracellular secreted miRNAs to target cells [53]. However, the majority of extracellular miRNAs can be transported without vesicles [40]. Furthermore, emerging evidence unveiled that miRNAs can also be carried by high-density lipoprotein (HDL) in peripheral blood [52].

The significant part of miRNAs is mainly expressed as tissue-specific. For instance, Ludwig et al. reported that miR-122, miR-192-5p, miR-7 and miR-124 are expressed as tissue-specific in liver, colon, pituitary gland and brain, respectively [54]. Until now, numerous studies revealed that the dysfunction of miRNAs is a significant disease-causing factor, and the altered expression patterns of them were associated with many human diseases, including cancer and FMF [14,55]. At present, miRNAs have been considered as diagnostic and therapeutic targets for the diseases.

Association between miRNAs and FMF

Genetic analyses performed in mutated and non-mutated patients with FMF have emphasized that additional

causative mechanisms such as epigenetics and environmental factors contribute to clinical manifestations of FMF [9]. So far, studies expanding the knowledge of our genetic and pathogenic mechanisms of FMF suggest that FMF is a complex disease that involves many players such as miRNAs [56,57]. We present a list of recent studies unveiling the association of miRNAs with FMF pathogenesis in Table 1.

Karpuzoglu et al. [58] examined expression levels of miRNAs related to apoptotic mechanisms and the effect of these miRNAs on clinical symptoms in FMF patients with various genotype. They analyzed 33 miRNAs expressions in venous blood of 191 FMF patients using the miRNA profiling method. For the first time, their results found that 19 circulating miRNAs are down-regulated while 7 circulating miRNAs are upregulated, suggesting that these miRNAs might be associated with apoptosis in FMF pathogenesis (Table 1).

In another study, Demir et al. [16] analyzed expression levels of certain miRNAs in pediatric patients's plasma using the quantitative real-time polymerase chain reaction (qRT-PCR) method. Their results study revealed that the expressions of miR-204 in FMF patient's plasma were reduced in the attack-free period, and this decline was more remarkable in the heterozygous individuals with M694V mutations. Also, the expressions of miR-155 were observed to be diminished in attack-free periods when compared with HC (Table 1). We are not aware of any papers examining the functional role of miR-155 and miR-204 in the pathogenesis of FMF, but there are some previous studies suggesting that these miRNAs may be associated with inflammatory events [59–61].

Hortu et al. [15] investigated the expression profile of 15 miRNAs in 51 FMF patients with clinical symptoms and genetically confirmed, using the qRT-PCR. They found that expression levels of certain miRNAs were declined in FMF patients relative to the control group. Also, their results revealed that the treatment and attack status in FMF patients affects the expression levels of miRNAs. Especially, Colchicine treatment elevates some of the miRNAs levels (miR-26a, miR-23b, miR-181a, miR-15a, miR-132) while decreases others (miR-34a, miR-26a, miR-16, miR-15a, miR-146a). In addition, expression levels of a group of miRNAs in non-attack patients were significantly lower compared to levels of those in attack patients (Table 1).

Amarilyo et al. [14] examined 798 functional miRNAs' expression levels in peripheral blood mononuclear cells (PBMC) of M694V homozygous FMF patients using miRNA profiling technology. Their results indicated that expression levels of a group of miRNAs (miR-451a, miR-4454, miR-21-5p and miR-144-3p) were upregulated while others (miR-148b-3p, let-7d-5p and miR-107) were downregulated (Table 1).

Koga et al. [17] analyzed expression levels of serum miRNAs in 9 FMF patients during attack and attack-free episodes using the microarray and qPCR methods. The results revealed that expression levels of miR-204-3p were diminished in patients with different genotypes, and these levels were negatively correlated with ISSF (International Severity Scoring System for FMF). The researchers performed their experiments in macrophages derived from THP-1 cell culture to elucidate the functional role of miR-204-3p and showed that miR-204-3p could act as a repressor for cytokine synthesis in FMF through targeting PI3Kγ signalling. They indicated that miR-204-3p may be a molecular marker or target for FMF therapy (Table 1).

Akkaya-Ulum et al. [57] investigated the expression levels of miRNAs associated with inflammation and clinical severity in homozygote and heterozygote FMF patients. The results indicated that in homozygotes FMF patients' miR-20a-5p expressions were increased while the expressions of miR-197-3p were decreased. In heterozygote FMF patients, the expressions of let-7d-3p and miR-574-3p were elevated. Besides, according to DAVID analyses, these miRNAs were found to be associated with inflammation (Table 1).

Wada et al. [62] studied 26 miRNAs' expression levels in 24 FMF patients composed of three subgroups: exon 10 mutations, exon 3 mutations and without exon 10 or 3 mutations. Their results suggested that miRNAs may have a biomarker role to distinguish FMF subgroups since they found that circulating miRNAs'expression levels differ between the subgroups (Table 1).

Latsoudis et al. [63] examined for the first time the expression profile of miRNAs in 9 FMF patients and detected that 29 miRNAs were expressed differentially using the microarray method. Among these miRNAs, miR-4520a was estimated to be associated with autophagy mechanisms, and miR-4520a expression levels were elevated in these nine FMF patients relatively compared to those in the control group (Table 1).

The abovementioned studies regarding miRNAs in FMF predominantly focuses on biomarker potential, inflammatory and apoptosis mechanisms of miRNAs. Identification of miRNAs that target genes involved in inflammasome forming and the elucidation of molecular mechanisms of these miRNAs in inflammasome regulation could contribute to developing novel therapeutic approaches for effective FMF therapy [64]. Inflammasomes are endogenous cytoplasmic protein complexes that are implicated in tissue damage and abnormal metabolism and act as a crucial regulator in autoinflammatory disorders including FMF through caspase-1 activation and release of proinflammatory cytokines such as IL-1β and IL-18 [65]. Recent emerging evidence suggest that potential agents that directly target upstream or downstream of inflammasome signalling have great significance for the treatment of chronic inflammatory diseases [66]. Moreover, some studies unveil that miRNAs targeting inflammasome activation mechanisms could be promising diagnostic markers or therapeutic targets for inflammatory diseases [64].

Previous *in vivo* and *in vitro* studies revealed that some miRNAs target NLRP3 (NLR family pyrin domain-containing protein 3), a well-known inflammasome component and member of the nucleotide-binding domain-like receptor family and thereby, suppress inflammasome activity [67–69]. Bauernfeind et al. reported that miR-223-3p functions as negative regulators of NLRP3 which contribute to

Table 1. the Recent studies unveiling the association of miRNAs with the pathogenesis of FMF.

Alteration of miRNA expression	Study groups	Genetic background	Medication status	Biological materials	Possibly function	Detection methods	References and year
let-7a-5p↓, let-7c↓, let-7 g-5p↓, miR-15b-5p↓, miR-16-5p↓, miR-17-5p↓, miR-23a-3p↓, miR-24-3p↓, miR-25-3p↓, miR-26a-5p↓, miR-26b-5p↓, miR-27a-3p↓, miR-29c-3p↓, miR-30a-5p↓, miR-30d-5p↓, miR-30e-5p↓, miR-106b-5p↓, miR-146a-5p↓, miR-195-5p↓, miR-365a-3p↑, miR-214-3p↑, miR-181c-5p↑, miR-181b-5p↑, miR-181a-5p↑, miR-29b-3p↑, miR-15a-5p↑	191 P-FMF (86 female, 105 male) 31 HC (15 female, 16 male)	Heterogeneous M694V hom ($n = 33$) Other mutations ($n = 151$) No mutations ($n=7$)	All patients	Venous Blood	Apoptosis	miRNA profiling/ qRT-PCR	[58] 2020 Karpuzoglu et al.
miR-155↓, miR-204↓	30 P-FMF (12 female, 18 male) 30 HC (12 female, 18 male)	Heterogeneous M694V homozygous ($n = 7$) Other mutations ($n = 19$) No mutations ($n=4$)	All patients	Plasma	Inflammatory events	qRT-PCR	[16] 2019 Demir et al.
miR-125a↓, miR-132↓, miR-146a↓, miR-155↓, miR-15a↓, miR-16↓, miR-181a↓, miR-21↓, miR-223↓, miR-26a↓, miR-34a↓ miR-132↑, miR-15a↑, miR- 181a↑, miR-23b↑, miR-26a↑ and miR-146a↓, miR-15a↓, miR-16↓, miR-26a↓, miR-34a↓ (Getting treatment) miR-34a↓, miR-26a↓, miR-223↓, miR-21↓, miR-181a↓, miR-16↓, miR-15a↓, miR-146a↓, miR-132↓ (attack-free)	51 P-FMF (female 29, male 22) 49 HC (Female 28, male 19)	Homogeneous M694V homozygous (n = 16) Other mutations ($n=35$)	Under treatment (31) No treatment (20)	Venous blood	Not determined	qRT-PCR	[15] 2019 Hortu et al.
miR-144-3p↑, miR-21 − 5p↑, miR − 4454↑, miR-451a↑ miR-107↓, let − 7d − 5p↓, miR-148b-3p↓	10 FMF (female 5, male 5) 10 HC (5 female, 5 male)	Homogeneous M694V homozygous (n = 10)	All patients	PBMCs	Biomarker	Microarray	[14] 2018 Amarilyo et al.
miR-204-3p↓	9 A-FMF (female 6, male 3) 19 HC (female 11, male 8)	M694I/M694I homozygous (n = 3) Other mutations ($n= 2$) No mutations ($n=4$)	Not determined	Serum macrophages derived from THP-1 cells	Biomarker Inflammatory cytokine production	Microarray / qPCR	[17] 2018 Koga et al.
miR-20a-5p↑ and miR-197-3p↓ (homozygotes patients) let-7d-3p↑ and miR-574-3p↑ (heterozygote patients)	6 FMF (A + P) 6 FMF carriers 6 HC	Homogeneous M694V homozygous (n = 6) M694V heterozygous (n = 6)	All patients	Venous blood	Inflammatory pathways Severity of disease	Microarray / qRT-PCR	[57] 2017 Akkaya-Ulum et al.
miR-8069↑, miR-7975↑, miR-7704↑, miR-7150↑, miR-7107↑, miR-6891↑, miR-6869↑, miR-6800↑, miR-6510↑, miR-638↑, miR-6125↑, miR-6090↑, miR-6089↑, miR-6088↑, miR-6087↑, miR-451a↑, miR-4516↑, miR-4485↑, miR-4281↑, miR-3960↑, miR-320e↑, miR-320d↑, miR-320c↑, miR-320b↑, miR-2861↑, miR-1225↑	24 P-A-FMF8 PFAPA	exon 3 muations ($n=8$) exon 10 mutations ($n=8$) without exon 3 or 10 mutations ($n=8$)	Not determined	Serum	Biomarker	miRNA profiling / Microarray	[62] 2017 Wada et al.
miR-4520a↑	9 FMF 8 HC	Heterogeneous M694V homozygous (n = 1) Other mutations ($n=7$) No mutations ($n=1$)	No medication for 2 days	THP-1 cells Monocytes from PBMC	Autophagy	Microarray	[63] 2017 Latsouids et al.

↑: Upregulation; ↓: Downregulation; P-FMF: Pediatric FMF; A-FMF: Adult FMF; HC: Healthy Control; PBMCs: Peripheral blood mononuclear cells; qRT-PCR: quantitative real-time polymerase chain reaction.

inflammasome forming in myeloid cells [67]. Xiao et al. revealed that the administration of miR-133b mimics ameliorates the pathogenesis of allergic rhinitis in mice by downregulating NLRP3 expressions, suggesting that miR-133b might have therapeutic potential [69]. Furthermore, the activation of inflammasomes can be regulated by miRNAs which target up/downstream signalling [64,66]. Some miRNAs modulate the inflammasome activity of NLRP3 by targeting NF-κB signalling [64,70]. Lian et al. showed that the upregulation of miR-383-3p which targets the Interleukin −1 receptor diminishes the caspase-1 and cytokines releases such as IL-1β and IL-18 in the atherosclerosis model of rats by suppressing inflammasome signalling [71]. Mitochondrial stress produces the reactive oxygen species (ROS) which involved in inflammasome forming and these ROS levels can be decreased by Superoxide dismutases (SOD) [72]. The upregulation of miR-377-3p causes elevates ROS levels through negatively regulating superoxide dismutases (SOD1-2) protein levels, indicating that miR-377-3p positively regulated inflammasome activity [73].

Recent studies in different research fields show that altered miRNAs expressions cause the dysregulation of the inflammasome signalling pathway associated with some inflammatory diseases such as rheumatoid arthritis and multiple sclerosis [64,74–77]. Studies performed in the blood compartment of patients with autoinflammatory diseases demonstrates that the expression levels of miRNAs are deregulated, suggesting that these miRNAs might have regulator functions in inflammasome signalling [64]. However, the potential mechanisms of miRNAs associated with inflammasome signalling in FMF have not been completely studied to date.

Diagnostic potential of miRNAs as biomarkers in FMF

Numerous studies have suggested that circulating miRNAs may have diagnostic use for a variety of human diseases in clinical settings [78]. miRNAs have many features as great ideal biomarkers in pathological conditions. miRNAs, which are disease and tissue-specific, can easily be analyzed after isolated from body fluids through minimally invasive or non-invasive methods. Moreover, unlike mRNA, miRNAs have considerable stability and durability in body fluids such as plasma, serum and urine against the RNase activity, freeze-thaw cycle and changes in pH [79,80]. Furthermore, the advancement of medical technologies has enabled the detection of miRNAs with high specificity, sensitivity and low cost [81]. The expression profile of miRNAs may contribute to accurate diagnosis, differentiating subtypes of diseases and monitoring the response to treatment in clinical settings.

Only one miRNA molecule may be sufficient as a biomarker to diagnose clinical outcomes in some pathological conditions, whereas miRNA panels including multiple miRNAs may be used in the diagnosis of some diseases such as osteoporosis, thyroid and pancreatic cancer with increased sensitivity and specificity [81,82].

So far, only a few papers presented significant findings that the dysregulated levels of circulating miRNAs are associated with subtypes, genotype, treatment status and a period of attack in FMF patients [14–16,58]. Although miRNAs are considered as promising molecular biomarkers that might contribute to the diagnosis and treatment of many diseases, including FMF [56,78,83], the biomarker research of miRNAs for FMF is still in its early stages. Also, there is currently insufficient evidence for the use of miRNAs in the clinical management of the disease.

Therapeutic potential of miRNAs as targets in FMF

As discussed above, the deregulation of miRNAs is closely related to FMF pathogenesis and thus, restoring biological functions of miRNAs by molecular interventions is vital for the treatment of the disease [84]. miRNAs could serve as the therapeutic targets in the treatment of diseases, and numerous approaches have been developed to improve abnormal expressions of these miRNAs in pathological conditions [84]. Antagomirs, complementary to targeted miRNAs, are synthetic oligonucleotides in length 8–25, and these molecules can be utilized when specific miRNAs are upregulated in pathological conditions. Antagomirs bind to the complementary mature strand of desired miRNAs and suppress the formation of the miRISC complex, and thus miRNA mediated mRNA degradation is suppressed [84,85]. Another approach to make up for decreased miRNA functionality is miRNA replacement therapy, and it is the best choice to restore the loss of miRNA functions in various pathological conditions [86,87]. miRNA mimics, which are exogenously given to diseased cells, can function like endogenous miRNAs. And one of two strands of these mimics miRNAs forms the miRISC complex, and hence this method can be used to restore loss of miRNA function [84,86].

Considering the upregulation or downregulation of miRNA expressions with the approaches mentioned above, such as Antagomirs and mimics use, research laboratories and biotech companies have focused on developing miRNA-based therapeutics in recent years [82]. The studies on miRNA-based therapeutics with some successful trials present promising approaches for treating diseases [82]. However, some miRNA-based therapeutics are now at a clinical development stage, and none are yet used in clinical settings for therapeutic purposes [88]. Some miRNA-based drugs are shown to have substantial efficacy in several pathological conditions such as hepatitis C, cancer, and fibrosis in preclinical, clinical phases 1 and 2 [82,88]. miR-122 increases the stability of the hepatitis C virus (HCV) RNA genome by binding 5′ end of the non-coding region of the viral RNA, thereby promotes the replication of the HCV genome in liver tissue [89]. Previous in vivo studies reported that miR-122-targeted therapy decreased HCV infection's viral capacity [90,91]. Miravirsen (or SPC3649), the first one of the miRNA-based therapeutics candidates developed by Santaris Pharma, is an antagomir targeting miR-122 designed by chemically LNA technology. miR-122

activity and viral titre of the HCV were suppressed after administration of Miravirsen into patients with HCV in the phase II trials (clinicalTrials.gov, NCT01200420), suggesting that Miravirsen have therapeutic antiviral efficiency [92]. miR-155, implicated in the differentiation and proliferation processes, is upregulated in blood cancer such as leukemia and lymphoma [93]. Babar et al. reported that suppression of miR-155 might have therapeutic activity in the mouse model of lymphoma [94]. Cobomarsen, also known as an MRG-106, is an LNA-based antagomiR developed by MiRagen therapeutics to suppress miR-155 and is still involved in phase 1 and phase 2 (respectively, clinicalTrials.gov, NCT02580552 and NCT03713320) [93]. miR-29 acts as a regulator of the genes which contribute to extracellular matrix deposition, and the downregulation of miR-29 in fibroblasts cause pathological fibrosis by enhancing the expressions of the collagens [95]. Montgomery et al. revealed that intravenously administering miR-29 mimics has a protective effect against pulmonary fibrosis by reducing collagen synthesis in a mouse model [96], suggesting that miR-29 could be a potential therapeutic target in fibrosis-related diseases [97]. MRG- 201 mimics developed by miRagen Therapeutics to restore decreased miR-29 expressions in fibrotic diseases is a promising example of miRNA replacement therapy, and phase II trial of MRG-201 is still ongoing (ClinicalTrials.gov, NCT03601052) in patients with systemic sclerosis [98].

Although many studies investigating the potential therapeutic role of miRNAs in pathological conditions have been performed at the preclinical levels in recent years, a limited number of miRNAs entered into clinical trials, and none of them have yet reached clinical phase III [97]. Due to various challenges, such as identifying the best candidate miRNAs, stability, and delivery, miRNA-based drug development has been delayed [97]. The selection of promising candidate miRNAs is the first crucial point in the development of miRNA-based drugs. Current databases providing knowledge about miRNAs in human diseases will help researchers identify more ideal miRNAs to develop therapeutics [99]. The stability issue caused by nucleases in physiological conditions is one of the important challenges in developing miRNAs-based therapeutics [97]. Modifications that alter oligonucleotide structures chemically through locked nucleic acid (LNA) and methylation are commonly utilized in miRNA-based therapeutics to avoid RNA degradation issues and increase the stability of RNAs. Antigomirs have LNA chemical modifications containing a bridge between the 2′O group and the 4′ carbon atom, whereas miRNA mimics involve methylation in their structures for higher stability [97,100]. In addition to these modifications, several approaches such as Liposomes and Dendrimers have been developed to improving *in vivo* delivery. However, the delivery of these miRNAs to targeted tissues in physiological conditions is still a considerable challenge for miRNA-based drugs [97].

Although there are studies that revealed that miRNAs are associated with pathological mechanisms related to FMF, such as inflammation, apoptosis and autophagy, the research on therapeutic uses of miRNAs in FMF is at early stages. Further functional studies are needed for identifying the therapeutic potential of miRNAs in FMF.

Methods of miRNAs detections in clinical settings

miRNAs expression levels analyses contribute to the determination of miRNAs that regulate many pathophysiological processes and their use for diagnostic and therapeutic purposes in clinical practices [101]. However, sample collection and processing, RNA isolation, expression analysis and data interpretation may restrict the use of miRNAs in clinical settings [102]. Northern blotting, Next-generation sequencing, microarray and qRT-PCR are well-known methods used to detect miRNAs expression levels.

Northern Blot Hybridization is a laborious, time-consuming and low-sensitivity method for miRNAs detection. The method includes gel electrophoresis, membrane transfer, cross-linking and probe hybridization steps [102,103]. Next-generation sequencing (NGS) is an advanced technique that enables the quantification of known miRNA and the identification of novel miRNAs. Currently, the use of NGS is limited in clinical settings due to its high cost and bioinformatics analysis requirements [101,104]. Microarrays, based on the nucleic acid hybridization method, are one of the most frequently used methods with low-cost for expression profiling of miRNAs. Microarrays enable the profiling of a large number of miRNAs in a single operation and preferably can be used to compare healthy and patients groups. However, it usually requires validation by another method such as qRT-PCR due to restricted specificity caused by cross-hybridization during microarray [80,101,102]. qRT-PCR is a highly sensitive technique, considered as the gold standard for quantification of gene expression and widely used in genetic laboratories [101]. This method allows the detection of a very small alteration of miRNAs expressions thanks to its high sensitivity, specificity, reproducibility, and accuracy [80]. Also, widely available commercial ready to use kits simplify the use of this technique. The detection of miRNA expression by qRT-PCR includes a reverse transcription of miRNA to cDNA, then, amplification of the target gene with qPCR [102].

Each method has advantages and disadvantages, and therefore researchers should choose a method that appropriates their purpose, taking into account conditions such as specificity, sensitivity, repeatability and cost [105]. As shown in Table 1, microarray and qRT-PCR are commonly used methods for quantification of miRNAs expression levels in FMF patients. Moreover, qRT-PCR appears to be the best choice with high sensitivity and specificity among the methods described above in the analysis of miRNAs in FMF patients.

Concluding remarks

There have been exciting findings that could uncover the possible roles of miRNAs in the FMF pathogenesis in recent years. miRNA studies highlighted above expand the

knowledge of FMF molecular genetics and attempt to understand and explain the complex pathogenesis which cannot be fully elucidated by inheritance of monogenic. It seems that the significance of miRNAs in FMF disease is gradually getting better understood. However, the researches are still at the early stages.

The studies investigating the role of miRNAs in FMF have significant limitations. The first one is a relatively small sample size used. The research of miRNAs should be done with larger groups where and when possible since FMF is a heterogeneous disease. Moreover, pathogenic molecular mechanisms such as inflammation and apoptosis are more active during the attack period of FMF patients. Therefore, more patients with active attack periods should be included in the studies better to understand the action mechanisms of miRNAs during the disease. One another limitation is that most of the FMF patients included in the studies receive Colchicine treatment, and the effect of this therapy on miRNA levels is not known. So studies that control for Colchicine treatment would be important. Another limitation of existing studies is that clinical severity of the disease is affected by genotypes of FMF patients. Therefore, patients should be put into separate groups based on genetic mutations, and such research design could determine the link between miRNAs and FMF genotypes.

In this paper, we have investigated the related literature and presented the association of miRNAs with FMF pathogenesis. Recent studies have revealed that miRNAs are associated with various mechanisms involved in the pathogenesis of FMF, such as apoptosis, inflammation and autophagy. In the near future, these molecules may have potential uses in treatment, therapeutic response monitoring, and diagnosis of differentiating subtypes of the disease. However, many significant points about the functional role of miRNAs in FMF still remain unclear. Until now, a limited number of studies have examined how miRNAs are implicated in the FMF. Especially, there are only a few studies on how various FMF pathogenic mechanisms such as apoptosis, autophagy, inflammation and signal transduction are affected by miRNAs. Furthermore, one miRNA may target more than one gene or cellular event and thus, it is vital to understand the complex action mechanisms of miRNAs. Further studies on the therapeutic use of miRNAs are required since targeting pathological mechanisms provides a promising strategy for FMF therapy.

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Author contributions

H.M.O. and M.A.B. designed the study. H.M.O. and M.A.B. wrote, drafted and edited the manuscript. H.M.O. and M.A.B. prepared tables/figures and revised the manuscript.

Conflicts of interest

None.

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