The Biology of MicroRNA

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Abstract MicroRNAs (miRNAs) are small non-coding RNA molecules involved in mRNA regulation at a post-transcriptional level. The first miRNA was discovered in 1993, and since then many branches of research have been explored to fully understand the miRNA world. Studies regarding the biogenesis process have highlighted different pathways according to miRNA gene localization, although the biochemical mechanism is not completely clear yet. In animals, miRNAs act mainly as negative regulators through translation inhibition, but recent evidence has shown their ability to stimulate mRNA degradation through recruiting decapping enzymes and nucleases. The "canonical" binding site of miRNAs is located within the 3′UTR of the mRNA target, but the coding sequence and the 5′UTR can also be bound by miRNAs. Although they mainly play a negative role at a post-transcriptional level, a few miRNAs have been reported to actually enhance mRNA expression. Altered patterns of miRNAs, due to genetic alterations, defects in the biogenesis process, epigenetic modification or aberrant expression of miRNA genes, are associated with many pathological contexts, including cancer and inflammatory diseases. Although the wider world of small RNAs has to be further explored, these regulators have already been shown to play a crucial role in all biological processes.

1 Introduction

MicroRNAs are small non-coding RNA molecules, 20–25 nucleotides long, highly conserved in the plant and animal world. They play a major role in post-transcriptional regulation processes, mainly silencing target mRNAs and thus decreasing their corresponding protein levels. Physiological and pathological mechanisms are affected by these small molecules, and the scientific community is showing more and more interest in non-coding RNA in general and microRNAs

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in particular. In this chapter, we aim to explain the basic biology of microRNAs, from the first miRNA discovered in the early 1990s to the last update in terms of biological mechanisms underlying miRNA biogenesis, function and altered expression.

2 The Discovery of MicroRNAs

Before 1993, small RNA molecules were known to be involved in essential cellular processes such as messenger RNA (mRNA) splicing [1] and RNA editing [2]. In 1993, the first microRNA (miRNA) was characterised as an important player in post-transcriptional regulation of mRNA in nematodes by Lee and collaborators [3]. The authors, focusing on *C. elegans* post-embryonic development, found that *lin-4* produced two non-coding RNAs of approximately 61 (*lin-4*L) and 22 (*lin-4*S) nucleotides with complementary sequences to the 3′UTR (untranslated region) of *lin-14* mRNA. These small transcripts were able to bind the 3′UTR of *lin-14* mRNA and to decrease protein levels without affecting the mRNA stability. The authors suggested that the inhibition of protein synthesis was operated by *lin-4* small RNAs either indirectly (via modification of *lin-14* mRNA or its localization in a sub-cellular compartment not accessible to ribosomes) or directly (via steric hindrance towards the translation machinery). This phenomenon was considered strictly correlated to the larval development of the nematode and only in 2000 was the second miRNA characterised, again in *C. elegans* [4]. Reinhart and collaborators showed that *let-7* encoded a temporally regulated small RNA (21 nucleotides) complementary to sequences in the 3′UTRs of numerous genes (i.e. *lin-14*, *lin-28*, *lin-41*, *lin-42* and *daf-12*) involved in the larval development of *C. elegans*. *Let-7* could directly regulate the protein level of *lin-41*, whereas *lin-4* was involved in *lin-14* and *lin-28* post-transcriptional regulation. These two small RNAs were suggested to be part of a pathway that regulates a temporal sequence of events in *C. elegans* larval development. The official name "microRNA" was used for the first time in 2001, when three papers, all published in the same issue of *Science*, showed the existence of small RNAs involved in post-transcriptional regulation of target mRNAs in both vertebrates and invertebrates [5–7]. Since then, miRNAs have been studied in many organisms and the latest version of the miRNA database (*miRBase*, http://www.mirbase.org/) contains 24,521 microRNA loci from 206 species, corresponding at about 30,000 mature miRNAs [8]. This list is ever expanding and contains at the moment ~2,000 human mature microRNAs, representing 1–3 % of all genes in *Homo sapiens* [9].

3 Localization, Biogenesis and Expression of miRNAs

MicroRNAs can be classified into five groups according to their genomic locations relative to introns and exons (Fig. 1) [10]:

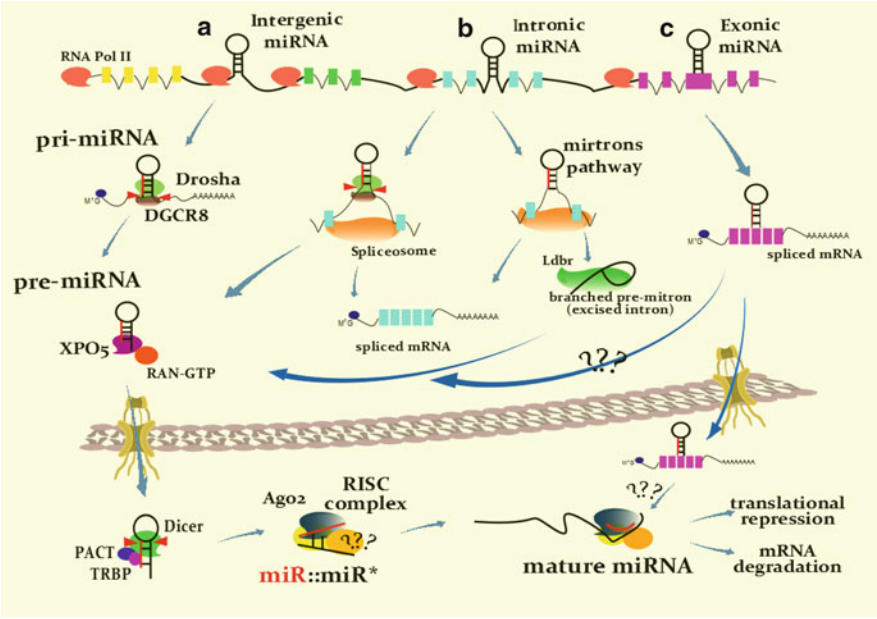

Fig. 1 Biogenesis of microRNAs. MicroRNA biogenesis depends on the genomic localisation of miRNA genes. (A) Intergenic miRNA genes are transcribed by RNA Pol II in pri-miRNAs and then cropped by Drosha-DGCR8 complex in pre-miRNAs and moved by XPO5 in the cytoplasm. They are then cleaved by Dicer and the double strand miR::miR* is loaded in the RISC complex. The mature miRNA is selected and can guide the catalytic machine to a target mRNA and act as a negative regulator. (B) Intronic miRNAs are processed before (or during) splicing. Drosha cleaves the miRNA hairpin that enters into the miRNA pathway. Mirtrons are non-canonical intronic small RNAs produced from spliced introns and debranching. They bypass the Drosha-processing step because Lariat debranching enzyme (Ldbr) resolves the secondary structure of the excised intron into a hairpin structure resembling pre-miRNAs. (C) Exonic miRNA biogenesis is poorly understood. The unspliced nuclear transcript is mainly processed to pre-miRNA. The spliced transcript (still containing the miRNA hairpin) can be exported to the cytoplasm, but it is not clear if miRNA hairpin is processed to a mature form or not

1. "Intergenic miRNAs"—term used when miRNA genes are located between two consecutive protein-coding genes.

2a. "Intronic miRNAs in non-coding transcripts"—e.g. the miR-15a~16-1 cluster found in one intron of a non-coding RNA gene, *Leu-2* [11].

2b. "Intronic miRNAs in protein-coding transcripts"—e.g. miR-126 located within the fifth intron of the *EGFL-7* gene on chromosome 9 and suggested to regulate the innate immune response in cystic fibrosis [12].

3a. "Exonic miRNAs in non-coding transcripts"—e.g. miR-155, one of the most important miRNAs in the immune response [13], located in the third exon of a non-coding gene, BIC.

3b. "Exonic miRNAs in protein-coding transcripts"—e.g. miR-198 located in the 11th exon of the *FLST1* gene, their co-regulation was studied in wound healing [14].

Some miRNAs are defined as "mixed" when, depending on alternative splicing, they can be located either in exons or introns [10]. Intergenic miRNAs are transcribed from their own promoters, whereas intragenic (i.e. exonic and intronic) miRNAs are preferentially located in the same orientation as the host genes [15] and frequently co-expressed with them [16]. About 50 % of total miRNAs are located in close proximity to others [10], and they usually produce long polycistronic transcripts (clusters) that are then processed into mature miRNAs [15].

Biogenesis of canonical miRNAs Canonical miRNA biogenesis in animals undergoes a stepwise process: (1) miRNA genes are transcribed in the nucleus by RNA polymerase II into primary nascent miRNA transcripts called "pri-miRNAs". These are characterised by stem-loop structures (containing the future mature miRNAs), a 3′ poly-A-tail and a 5′ methylated cap and they can be up to several hundred nucleotides long, particularly when a miRNA cluster is transcribed [17]. Some miRNA genes are transcribed by RNA polymerase III, in particular those that are close to *Alu* repeats (transposable elements in the human genome reviewed by Deininger [18]) [19]. (2) Pri-miRNAs are then recognised by a multiprotein complex called the "microprocessor complex" that cleaves them in 60–100 nt products named "pre-miRNAs" [20]. The two main core components of the microprocessor are Drosha and Di George syndrome Critical Region 8 (DGCR8). The double-stranded RNA-binding protein DGCR8 binds to the base of the stem-loop structure and guides the positioning of the RNase type-III enzyme Drosha, which constitutes the catalytic centre of the complex. Drosha cleaves the double-stranded stem about 11 bp from the base and generates a 2-base 3′ overhang [21]. (3) This particular structure is recognised by Exportin 5 (XPO5) that, together with its "energetic" partner Ran-GTP, moves pre-miRNAs from the nucleus into the cytoplasm through nuclear pore complexes [22]. In addition to its transport role, XPO5 also stabilises pre-miRNAs by preventing degradation by exonucleases [23]. (4) Once in the cytoplasm, the hairpin precursors are recognised and cleaved near to the terminal loop by another RNAse type-III enzyme, Dicer, generating miRNA duplexes ~22 nt long with 3′ and 5′ overhangs [15]. These dsRNA duplexes are referred to as miRNA:miRNA* and contain both the mature miRNA strand and its complementary passenger strand (*). Dicer was first characterised as a key protein of the RNA interference (RNAi) machinery [24] and then investigated as a miRNA processing factor [25]; it is highly conserved and is reported to be associated with dsRNA binding proteins such as trans-activation response RNA-binding protein (TRBP) [26] and protein activator of PKR (PACT) [27]. (5) When miRNA duplexes are produced, they associate with Argonaute (Ago) proteins (in particular, Ago2), are recruited by TRBP and form the RNA-induced silencing complex (RISC) [26]. In detail, one of the strands, the one that will become the mature miRNA, is loaded into RISC, whereas the miRNA* strand is degraded or expressed at a lower level. Generally, the strand

having the less stable base pairing at the 5′-end is chosen as the guide strand; the ability to assess the thermodynamic properties of the duplex is mediated by RISC [28]. Ago2 represents the engine of the RISC complex and its structure has been studied in depth in order to understand the RNAi biochemical mechanism. Ago2 has three major domains [29]: *PAZ* (N-terminal, ~20 kDa) which specifically recognises the two base 3′ overhangs of miRNA duplexes generated after Dicer cleavage; *MID* which contains a highly specific binding pocket for the 5′ end of the miRNA and *PIWI* (C-terminal, ~40 kDa) which shows nuclease activity and is involved in the mechanism of action of miRNAs. As part of the RISC complex, the mature miRNA can guide the catalytic machine to a target mRNA and act as a negative regulator at a post-transcriptional level.

Biogenesis of intronic miRNAs The majority of miRNA loci are located in intronic regions [30]. Intronic miRNAs share the same promoter with their host genes, so unspliced pre-mRNAs contain miRNA sequences folded as hairpin structures in intron(s) [31]. It was generally assumed that this class of miRNA was processed from excised introns, in parallel to what happens to intronic small nucleolar RNAs [32], but Kim and Kim showed that Drosha cleavage of miRNA hairpins might take place after the assembly of the early spliceosome complex but before or at the same time as proper intron excision [30]. Thus, the pre-miRNA enters the miRNA pathway and the rest of the transcript undergoes the splicing process to produce a mature mRNA for protein synthesis. “Mirtrons”, a class of non-canonical intronic miRNAs, were first identified in *D. melanogaster* and *C. elegans* in 2007 and their processing bypasses Drosha cleavage [33]. In their case the spliced intron product is not linear but adopts a lariat between the 5′ end and the 3′ branchpoint. Following the resolution of this structure by the lariat debranching enzyme Ldbr, the resulting hairpin structure, resembling a pre-miRNA, can be transferred to the cytoplasm by XPO5, cleaved by Dicer, loaded into RISC and act as a canonical microRNA. Some mirtrons have short extensions at either the 3′ or 5′ end of the hairpin ([33] and [34], respectively) that require a further exonucleolytic trimming in order to be recognised by XPO5 and enter the miRNA pathway. Mitrons have also been described in mammals [35] and are reported to be involved in acute lymphoblastic leukaemia [36] and inflammatory lymphangiogenesis [37].

Biogenesis of exonic miRNAs The biological mechanism underlying exonic miRNA biogenesis is poorly understood. Recently Slezak-Prochazka and collaborators used miR-155 and its host gene *BIC* as a model of exonic miRNA biogenesis and evaluated whether unspliced and/or spliced transcripts were used as template for miRNA processing [38]. The authors suggested that the unspliced nuclear transcript is mainly processed to pre-miRNA, and is then transported in the cytoplasm where it follows classical miRNA biogenesis. Moreover, they showed that alternatively, the spliced transcript still containing the miRNA hairpin can be exported to the cytoplasm; however in this case the miRNA hairpin is not fully available for processing to its mature form. Thus the biochemical details of this process are not clear yet.

4 miRNA Function on Target mRNAs

In mammals microRNAs are predicted to regulate the expression of about 60 % of protein-coding genes [39] and are involved in almost all the physiological and pathological processes investigated to date [40]. Generally, miRNAs regulate gene expression at a post-transcriptional level by base pairing to miRNA recognition elements (MRE) located in the 3′UTR of target mRNAs. The miRNA::mRNA base pairing can be perfect or limited to the so-called "seed region", i.e. the 5′ miRNA sequence that provides most of the pairing specificity [41].

Perfect complementarity The consequence of perfect miRNA::mRNA pairing, mainly present in plants [42], is target degradation (or "slicing"), which leads to a decrease in both mRNA and protein levels. Specifically, Ago2 cleaves the target mRNA between nucleotides complementary to the 10th and 11th bases of the miRNA guide strand [43] then 3′ deadenylation and 5′ decapping occur before complete mRNA degradation by exonucleases, e.g. XRN4, in *Arabidopsis* [44].

Partial complementarity In this case, only the seed region is required for miRNA::mRNA binding. The mRNA region complementary to the miRNA seed sequence is called the "seed match". The most common seed types are the following [45]:

1. 6mer site: this is considered the minimal region required (6 nt) for miRNA::mRNA interaction; there is perfect pairing of the region from the second to the seventh nucleotide of miRNA.
2. 7mer-A1 site: perfect pairing between bases 2–7 and the first nucleotide is an adenine.
3. 7mer-m8 site: perfect pairing from the second to the eighth nucleotide of the miRNA.
4. 8mer site: perfect pairing for bases 2–8 and the first nucleotide is an adenine.

Knowledge of the details about miRNA::mRNA interaction sites is crucial for bioinformatic tools for target prediction that are based on both the binding free energy and conservation between different species of miRNA::mRNA pairing.

In the case of partial complementarity, miRNAs act as negative regulators inhibiting the translation of an mRNA target through two proposed mechanisms [46]: (1) miRNAs can stop the initiation of translation by repressing m^7G cap recognition by eIF4E, thus preventing the recruitment of the 40S ribosomal subunit or by interfering with the 60S joining; (2) miRNAs can interfere with translation after the very initial steps by repressing ribosome elongation or inducing ribosome drop-off and nascent protein chain degradation. These mechanisms lead to a decrease of protein but not mRNA levels. Recent papers also showed that miRNAs are involved in target mRNA degradation in animals but differently than in plants. Indeed, while in plants an endonucleolytic cleavage by Ago2 occurs within the region complementary to the miRNA, in animals miRNAs might direct mRNA targets towards the degradation machinery [46]. GW182 is a protein component of the RISC complex that interacts with the Ago2 *MID*/*PIWI* domain, recruits CCR4-NOT1 (carbon catabolite repression 4—negative on Tata-less) deadenylase and

binds poly-A binding proteins (PABP), promoting the deadenylation process by putting in contact the poly-A tail of an mRNA with the deadenylation machine. Then the mRNA is either degraded by 3′–5′ exonucleases or, first, the decapping enzyme DCP1-DPC2 complex removes the m^7G cap, and then degradation is completed by the 5′–3′ exonuclease Xrn1 [46]. Recent evidence supports the idea that miRNA machinery components and proteins involved in the repression process might not be located in the cytoplasm but organised in structures that promote the efficiency of this mechanism. P-bodies ("processing bodies") were first discovered in 1997 by Bashkirov and collaborators and described as specialised loci for mRNA degradation, since the exonuclease Xrn1 was found to be localised there [47]. Nowadays, most of the components of both miRNA and repression machinery are found to be localised in these cytoplasmatic structures (for instance, Ago2, GW182, DPC1-2, Xrn1, CCR4, NOT1) [48]. Thus, P-bodies are considered as the sites of the final step of mRNA degradation triggered by miRNAs, although their precise role in miRNA-mediated repression needs to be further investigated since it is not clear whether they are essential or not for the efficacy of this mechanism [46].

As mentioned, the main miRNA binding sites are localised in the 3′ UTR of target mRNAs. Despite this, binding sites in the coding sequence (CDS) or in the 5′ UTR have been predicted and experimentally validated [49–51], although their efficacy in negatively regulating gene expression is lower than 3′UTR binding sites [52].

Collectively, miRNAs are principally negative regulators of gene expression. Nonetheless, some have been shown to activate translation of their target mRNAs in particular cellular conditions including quiescence [53] or stress [54] by binding to the 3′ or 5′ UTR of target genes, respectively. Tsai and collaborators reported miR-346 binding to the 5′UTR of *RIP140* mRNA and enhancing its translation by promoting the association between mRNA and polysome fractions [55]. Surprisingly, miR-373 was shown to promote *E-Cadherin* and *CSDC2* transcription by binding to complementary sequences located in the promoters of these genes [56]. This finding raises the question: how can a miRNA act in a nuclear context? In vitro studies suggested that the "AGUGUU" sequence at the 5′ end of miR-29b might direct its nuclear import [57]; a very similar sequence (AGUGCUU) is also present in miR-373 [58]. Little information exists regarding miRNA nuclear import (only Ago2 was shown to be able to transport small RNA from the cytoplasm to the nucleus [59]), their ability to contact with DNA and their role in the regulation of gene transcription.

5 Altered miRNA Expression

MicroRNAs are expressed in a spatio-temporal manner and are sensitive to metabolic or physiological conditions. Since they are involved in the regulation of almost all aspects of cellular biology, it is not surprising that their expression is finely controlled and that alterations in miRNA expression patterns can lead to

various diseases. Here we describe the main mechanisms that can affect miRNA expression, and that are thus deregulated in pathological contexts, with a main focus on inflammatory diseases and alterations of the immune system.

Genetic alterations Genomic amplifications or deletions and chromosomal rearrangements or mutations can affect miRNA genes to the same extent they alter protein-coding genes. Actually, the first paper proving an association between miRNA and cancer showed a deletion of the 13q14 region in chronic lymphocytic leukaemia (CLL), where miR-15 and miR-16 are located, and thus defined them as tumour suppressors [11]. Interestingly, the miR-15/-16 locus is also affected by genetic duplication in the 3q25.33 region (to prevent the loss of expression of these two microRNAs) and by germline mutations in about 15% of CLL patients [60]. No genetic or chromosomal alterations have yet been linked with inflammation or the immune system; however single nucleotide polymorphisms (SNPs) in miRNA genes or target sites are involved in these processes (discussed later).

MiRNA machinery defects Altered expression of or the presence of mutations in genes involved in the miRNA biogenesis machinery leads to a general deregulation of the miRNA pathway. In cancer, many papers have reported down-regulation of the miRNA machinery genes, such as Drosha [61] or Dicer [62], and global repression of miRNA maturation is known to enhance transformation and tumorigenesis in animal models [63]. Nonetheless, other reports show up-regulation of these genes [64, 65]; thus the effect of altered miRNA machinery might be tissue specific. In the immune system, the role of some miRNA machinery proteins was studied through disruptions of their genes in animal models. For instance, deletion of Ago2 in hematopoietic cells affects early development of lymphoid and erythroid cells; Dicer-deficient B lymphocytes show decreased cell survival and antibody production; and conditional knock out of Dicer or Drosha in T_{reg} cells leads to the development of autoimmune inflammatory disease [66]. Moreover, a mouse model of lupus erythematosus is characterised by decreased levels of Dicer mRNA in T_{reg} cells [67]. PACT, a protein associated with Dicer, was found to be up-regulated in chronic rhinosinusitis [68], but this represents nearly the only report about human inflammatory diseases and deregulation of miRNA machinery; thus more studies are needed to assess their role in this pathological context.

RNA editing RNA editing is a cellular mechanism where specific nucleotide sequences within a transcribed RNA undergo different modifications. In animals, A-to-I editing is the most common and involves hydrolytic deamination of adenosine to inosine mediated by adenosine deaminases acting on RNA (ADARs) [69]. A-to-I editing was reported to affect different points of miRNA biogenesis and function: (1) the processing of pri-miRNAs by the Drosha/DGCR8 complex is altered, and edited pri-miRNA can be degraded by Tudor-SN, a nuclease specific to I-dsRNA; (2) the Dicer cleavage step of edited pre-miRNA is suppressed; (3) when an edited mature miRNA is expressed and its seed sequence is modified, it can target a different set of mRNAs compared to its unmodified partner; thus gene expression might be strongly affected in tissues where a substantial proportion of

pri-miRNAs is edited [69]. The effects of edited miRNAs on disease outcome or progression need further investigations. In cancer, unedited miR-376a* stimulates glioma cell migration and invasion, whereas the edited miR-376a* can suppress these features, mainly due to the different sets of target genes bound by both miRNAs [70]. ADAR1 was found to be up-regulated in a mouse model of inflammation [71], but to date no edited miRNAs have been reported to be involved in any human inflammatory disease.

Epigenetic modifications Epigenetics is a term that refers to inheritable modifications in gene activity that are not caused by a change in DNA sequence but by several other alterations such as DNA methylation or histone modification. About half of miRNA genes are located in CpG islands and thus are likely to be affected by the DNA methylation machinery [72]. Epigenetic mechanisms controlling miRNA expression have been described in inflammatory diseases, for instance, the miR-199a promoter was found to be hyper-methylated in chronic obstructive pulmonary disease and this affects the unfolded protein response (UPR) in monocytes [73]. Another example involves increased methylation of miR-124a in colitis-associated cancer patients compared to healthy volunteers and was suggested as a promising marker for individual risk of developing colitis-associated cancer [74]. No data are available on epigenetic histone modification in inflammatory diseases, but it is suggested to be another important regulatory pathway in cancer growth [75], chemoresistance [76] and stem-like properties [77].

Alteration of transcription factors binding miRNA gene promoters Extracellular and intracellular stimuli can lead to alterations in transcription factor binding to the promoters of miRNA genes. Thus, pathological contexts often show deregulated miRNA expression compared to normal physiological conditions, and this affects the expression of target genes that are regulated at a post-transcriptional level. This phenomenon is so important that the term "oncomir" was coined in 2006 to define a primary transcript (OncomiR-1) highly expressed in many cancer cell lines [78] and used since then to describe miRNAs playing an important role in cancer. Some oncomirs act as oncogenes targeting tumour suppressor genes and leading to malignant transformation when over expressed. In contrast, some of them target oncogenes and, to the same extent as protein-coding tumour suppressor genes, they are reported to be down-regulated in cancer.

Various inflammatory diseases are reported to be associated with altered miRNA expression and have been investigated through high-throughput analyses [79–81]. Many other papers focus on only one or a few altered miRNAs in an attempt to explain the biological mechanism underlying the alteration and its effects on disease pathology. miR-155 is considered a master regulator of the immune response as a pro-inflammatory agent, and is reported to be deregulated in numerous inflammatory diseases including multiple sclerosis, rheumatoid arthritis and endotoxemia, via its ability to target various negative regulators of inflammation including *SHIP1* and *SOCS1* [82]. miR-146a represents its negative counterpart. Its expression is altered in lupus erythematosus, rheumatoid arthritis and Sjogren's

syndrome [82], and it can mute the immune response by targeting *IRAK1* and *TRAF6* involved in the NF-κB pathway [13]. Recently, less well-characterised miRNAs were reported to be altered in other diseases and to play an active role in the pathological condition. For instance, miR-145, miR-223 and miR-494, up-regulated in the bronchial epithelium of cystic fibrosis patients [12], were reported to target *CFTR* through luciferase assays and western blots and to contribute, together with inflammatory processes, to the regulation of ΔF508 *CFTR* [83]. MiR-149 was shown to be down-regulated in osteoarthritis and to target many cytokine genes involved in the pathology such as *IL1β*, *TNFα* and *IL6* [84].

6 miRSNP

Single nucleotide polymorphisms (SNPs) are the most common genetic variation in the human genome and can affect introns, intergenic regions or coding regions of genes (leading to altered protein sequence). A single nucleotide polymorphism falling in a miRNA target site commonly called "miRSNP" [85] can affect the strength of miRNA::mRNA binding and thus the post-transcriptional regulation of that mRNA. Several elements have to converge in order for a miRSNP to be considered functional: (1) the SNP must have an association with a physiological or pathological process, (2) both the miRNA and its predicted target must be expressed in the tissue, and (3) the allelic changes must result in differential binding of the miRNA and affect expression of the target gene. Nowadays databases recording polymorphisms in putative miRNA binding sites, e.g. PolymiRTs [86], are available online, although experimental validation is required to test the three previous characteristics. Increasingly, papers suggest that miRSNPs can have important phenotypic consequences. In the past studies reporting their association with the risk of various types of cancer, such as breast [87], ovarian [88], colorectal [89] and lung [90], have been published. Moreover, miRSNPs were found to be involved in chemoresistance [85], risk of myocardial infarction [91] and the performance of diagnostic biomarkers [92]. Concerning inflammatory diseases, Tan and collaborators showed altered binding of miR-148a, miR-148b and miR-152 to the *HLA-G* mRNA in asthma due to the presence of a polymorphism in its 3′UTR [93]. In inflammatory bowel diseases, miRNA-mediated regulation was reported to occur for *IL-23R* since the rs10889677 variant in its 3′UTR could enhance *IL-23R* mRNA and protein levels due to loss of binding capability for Let-7e and Let-7f [94]. A miRSNP located in the 3′UTR of the *BSG* gene and affecting miR-492 binding site was found to be associated with risk of psoriasis [95].

SNPs can also occur within miRNA gene sequences and are again reported to be associated with risk of tumours, such as in childhood leukaemia [96], breast cancer [97] and cervical carcinoma [98]. In inflammatory or autoimmune disorders, miR-146a rs2910164 has been associated with asthma [99] whilst miR-196a-2

rs11614913 plays a functional role in vitiligo through the differential allele modulation of TYRP1 target gene [100].

7 Conclusions/Perspective

Despite the acquired knowledge about miRNA regulation, further investigations are needed to fully understand their role in the physiology and pathology of inflammation and the immune system. Although they have been proposed as diagnostic biomarkers and possible therapeutic targets in cancer [101–103], their clinical use is less well known in immune disorders. A recent review by Zeng and co-authors summarised the available knowledge about circulating miRNAs that could be used as biomarkers in autoimmune diseases, but suggested that more studies with larger number of recruited subjects should be carried out [104]. In conclusion, findings about the fundamental biological role played by miRNAs suggest the potential use of these small molecules in the clinic; collaborative and interdisciplinary efforts together with novel approaches are required to succeed in this goal.

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