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Current roles of microRNAs in infectious diseases – advancing into healthcare

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Review article

Among all new emerging RNA species, microRNAs (miRNAs) have attracted the interest of the scientific community due to their implications as biomarkers of prognostic value, disease progression, or diagnosis, because of defining features as robust association with the disease, or stable presence in easily accessible human biofluids. This field of research has been established twenty years ago, and the development has been considerable. The regulatory nature of miRNAs makes them great candidates for the treatment of infectious diseases, and a successful example in the field is currently being translated to clinical practice. This review will present a general outline of miRNA molecules, as well as successful stories of translational significance which are getting us closer from the basic bench studies into clinical practice.

Uloga mikroRNA u infektivnim bolestima – napredak u zdravstvu

Pregledni rad

Među svim novootkrivenim vrstama RNA, mikroRNA (miRNA) su privukle interes znanstvene zajednice zbog svoje moguće upotrebe u obliku biomarkera, njihove prognostičke vrijednosti za progresiju bolesti ili u dijagnostici, zbog snažne povezanosti s bolestima i stabilnom prisutnošću u lako dostupnim ljudskim biološkim tekućinama. Ovo polje istraživanja je utvrđeno prije dvadeset godina, a njegov razvoj je zapažen. Regulatorna priroda miRNA ih čini značajnim kandidatima za liječenje infektivnih bolesti, a uspješan primjer u tom području trenutno se prenosi u kliničku praksu. Ovdje će biti predstavljen opći pregled miRNA molekula, kao i uspješne translacijske priče prenesene iz temeljnih istraživanja u kliničku praksu.

1 Introduction

Since the last 20 years, we have witnessed how the universe of RNA species has broadened with the discovery of several non protein-coding subtypes (ncRNAs) [1, 2]: small interfering RNAs (siRNAs) [3], microRNAs (miRNAs) [4, 5], long non-coding RNAs (lncRNAs) [6, 7], RNAi [8], piwiRNA [9], or circular RNAs (circRNAs) [10]. This review will focus on miRNAs as a class of ncRNAs with potential clinical applicability due to several characteristics. Examples of these interesting features for clinical practice are their higher stability in circulating biofluids compared with other coding RNAs, and their close association to a given clinical condition, making them strong candidates for biomarker discovery. Additionally, they have been studied in the immune system, and were found to have key roles in signaling, differentiation, or in response to pathogen. The role of miRNAs in this response has been especially addressed, due to the potential implications for the treatment of infectious diseases.

1.1 General biology of miRNAs

For the reader that comes new to the field, as ubiquitously described and reviewed in literature, small mature miRNAs molecules range from 18 – 25 nucleotides in

length [11 – 16], and as described above, they don't encode for proteins [17, 18]. A key point in their biology that have attracted the attention of the research community is their ability to regulate and tune gene [18–20] or protein expression [21–25], what leads into changes in the target protein levels [26]. These molecules represent a form of epigenetic regulation and control of the genome [27], and their mechanisms of action include: blocking of the messenger RNA (mRNA) [18, 28], directing degradation through a protein complex [13], or by destabilization of the messenger [29]. There are cases in which a miRNA can up-regulate the expression levels of its target as shown by Vasudevan and colleagues [30]. Whichever the way they use to alter the outcome, it has been reported that the recognition of the target occurs through base complementarity between their 5' end region (seed region), which contains nucleotides 2 to 8, and the mRNA sequence [31, 32]. However there are studies in which, while this complementarity was not perfect, they still have a regulatory effect over the messenger [32]. This short sequence recognition confers a single miRNA the potential capability to regulate a number of targets [14, 22, 33, 34], *e.g.* hsa-let-7a-5p with several experimentally validated targets [34] such as B--cell CLL/lymphoma 2 (*BCL2*), cell division cycle 25A (*CDC25A*), cyclin-dependent kinase 6 (*CDK6*), dicer 1 ribonuclease type III (*DICER1*), interleukin 6 (IL6), v-myc avian myelocytomatosis viral oncogene homolog (*MYC*), or NFKB inhibitor interacting Ras-like 2 (*NKIRAS2*), among others. In addition to the previous statement, there are studies that have shown that a single target can be regulated by multiple miRNAs [14, 15, 35]. It is important to address that it has been described the existence of a selective pressure over more than half of the human mRNAs, in order to keep the pairing capability with miRNAs [31, 36]. Despite being abundant molecules, their expression can be restricted to a developmental stage [17, 27, 37] or a very specific environmental condition [38]. An example of the latter is the recent study from Sturchio *et al.* [39], carried out in a human Jurkat leukemic T cell line. In this study the authors exposed the cells to a chemical form of arsenic, sodium arsenite, and evaluated their miRNA expression profile through a microarray platform, having found significant differences in expression. Those differences were subjected to further validation through real time quantitative polymerase chain reaction (RT-qPCR), and some of the altered miRNAs were miR-221, miR-222, miR-638, being all up-regulated when compared with the control. Lastly, miRNAs have been described to be produced in the nucleus of the cell as a general rule, although some authors found that mitochondria can also be a source of miRNAs [40, 41].

All of the above can provide an idea of the reasons why miRNAs have attracted attention of the scientific community, due to their key role as important regulators in the biological process/functions game [23].

2 MicroRNAs and diseases

2.1 miRNAs and the immune system

The role of miRNAs in diseases has been addressed in several studies, which are presented in the following section. However, for a good understanding on the impact of miRNAs over the outcome of the disease, it is basic to understand how miRNAs influence and regulate different processes in the immune system. This has been an active area of research with very good examples of their importance in shaping the immune system, affecting the development of cells such as macrophages [66], B cells [67], or NK [68, 69], among others.

Macrophages are one of the key cells of the immune system due to their role during inflammation and posterior resolution of it. A long lasting inflammation after pathogenic clearance can have deleterious effects if maintained in time [70, 71]. Squadrito and collaborators [66] point out that miRNAs can notably impact and modify all stages of the macrophage life, from hematopoietic stem cells (HSC) to macrophage polarization, either to become the proinflammatory M1 or the anti-inflammatory M2, showing evidence through extensive literature review to support their statement. Liu and Abraham [72] discuss further the main miRNAs that act on each of the polarized states, being miR-155 a pro-inflammatory miRNA, observation that is shared in the study of Graff and colleagues [73]. It has been indicated that miR-155 has the ability to inhibit the M2-specifying factor [74, 75]. Another example of M1 promoting miRNA is the case of miR-127 [75], reporting the authors that deletion of this miRNA induced the M2 phenotype. The fact that NK cells are under the influence of miRNA levels, has been reported by Zhang *et al.*, in a study where they show that bioactivity of NK cells is directly related with the circulating serum levels of miR-155 [68], results that are also shared by Sullivan and colleagues [69].

For a coordinated mode of action, any biological system must have a mean of communication between its components [76, 77], especially if we are considering the immune system. Let's not forget that the system functions in a coordinated manner as a whole, and not as isolated cells [76]. This aspect has been covered by Gurwitz in a recent editorial [78], highlighting that until present most of the studies concerning miRNAs are performed on the same cells, what leaves the communication aspect on the roadside. Not only the miRNAs are endogenous molecules that modify the response of a cell on its own according to the environmental or developmental conditions, but also they serve as key means of communication between cells. That for example has been demonstrated in immune cells [79], as well as in other type of tissues or even organisms [80]. The specific referred case of transference in immunity was reported by Mittelbrun *et al.* [79], where they show trans-

fer of miRNAs from T-cells to antigen-presenting cells (APC). They found that the delivery was carried out in exosomes, in a unidirectional manner directed towards the APC. Besides, those miRNAs were functional and able to down-regulate genes in the target cell [80]. This is one of the possible ways, but not restricted to, in which miRNA communication can occur. Another type of delivery of miRNAs is mediated through gap junctions (GJs), topic that is extensively reviewed by Lemcke and co-authors [81]. GJs mediate delivery of miRNAs of clinical relevance, but as indicated in their review or in previous studies [82], so far only mature miRNAs are capable of crossing through the channel. This transference can have a selective nature, regarding the type of transported miRNAs, as it has been shown for human macrophages which were capable of regulating processes in the receptor cell type, in this case cancerogenic one, through the delivery of miRNAs [83]. This represents, according to the authors, a very important mode of action in the immune cells, besides cytotoxicity or cytokine production. Another type of cell communication relevant for the immune system [84] that may aid in the transference of miRNAs between cells, is the existence of nanotubes [85], that mediate cell to cell interaction between relatively long distances, considering the cell size as a reference measurement unit [86].

2.2 Role of miRNAs in infectious diseases

The immune cells act in a coordinated manner, and miRNA are one of important components for communication and control. The presence of a pathogenic agent alters the homeostatic balance of miRNA regulation in several ways, which can be broadly simplified in two mechanisms: pathogen encoded miRNAs, or alteration of the host miRNA expression levels.

2.2.1 Pathogen encoded miRNAs

Pathogens encode miRNAs aimed to counterfeit that ones from the host, thus providing an advantage for the multiplication and survival of the pathogen. They have been described for viruses and bacteria.

2.2.1.1 Pathogen encoded miRNAs: Viruses

Pathogen encoded miRNAs were first described for DNA viruses [87–90], with the first successful report dating from 2004, in a study in which Pfeffer and collaborators [88] (Table 1.) identified five miRNAs in Epstein-Barr virus (EBV). The authors characterized the whole set of small RNAs deriving from a Burkitt's lymphoma cell line latently infected with EBV, finding that four per cent of the small RNAs had a viral origin. Further studies covering miRNAs derived from DNA encoded viruses have been widely reviewed [91].

It has been argued that this feature was exclusive of DNA viruses [91, 92], because miRNA expression would leave RNA genome viruses in a weak position from a biological fitness perspective [87, 93, 94]. Recent findings are changing this view [95 – 97], since Kincaid and collaborators [94] (Table 1.) carried out the first study reporting miRNAs from a virus with a RNA genome, the bovine leukaemia virus (BLV). Among other studies that have succeeded in finding viral encoded miRNAs in RNA genome viruses [98], Hussain *et al.* [97] (Table 1.) described a small regulatory viral RNA (srvRNA), named KUN-miR-1, which features miRNA properties in West Nile virus (WNV). They described how viral replication depends on this miRNA, and that it targets the messenger of a transcription factor, GATA binding protein 4 (GATA4), determining an increase in the gene messenger accumulation in mosquito cells. In another related study these authors [99] (Table 1.) were able to identify a miRNA-resembling-candidate in mosquito cells infected with Dengue virus (DENV). However, this last article raised questions from other authors, as reflected in a response from Skalsky *et al.* [100]. Skalsky and colleagues base their doubts on a previously reported study from their group [101], in which they did not find any DENV virus derived miRNA-like particles through a deep sequencing approach, and especially pointing to the low expression values that Hussain and colleagues report on their study as a major concern for drawing any biologically relevant conclusions. In a posterior letter [102], Hussain and Asgari further addressed those questions from Skalsky, stating that the advancements in sequencing allow for the discovery of new molecules, despite any previously reported negative results, or that low-levels of expression of regulatory RNAs are not in disagreement with a relevant biological function.

Ebola virus (EBOV) has also been described as capable of encoding miRNAs [95] (Table 1.). The authors of this study were able to computationally identify two putative miRNA precursors (pre-miRNAs) and three putative mature miRNAs. In a further experimental step they transfected HEK293T cells with a plasmid harbouring an EBOV pre-miRNA construction, and were able to observe that the host cell machinery was capable to produce the viral miRNAs [95]. The last example that will be presented here is that of Hepatitis A virus (HAV). Shi and colleagues [96], through a combined computational and experimental approach, demonstrated that HAV can encode miRNAs, finding a miRNA-like candidate hav-miR-N1-3p, which can be transcribed in KMB17 and HEK293T cell lines, leading to the production of the mature form, hav-miR-N1-3p.

2.2.1.2 Pathogen encoded miRNAs: Bacteria

In bacteria, the presence of encoded candidate miRNAs was not reported until 2014 [103, 104]. Within these two examples, Furuse and colleagues describe a candidate miRNA encoded by *Mycobacterium marinum*

strain M during the infection of a RAW264.7 murine cell line [103] (Table 1.); while Shmaryahu and collaborators [104] (Table 1.), developed a bioinformatics approach to predict putative miRNAs from 448 different genomes of pathogenic bacteria. They were able to identify a large number of candidates and their targets in human mRNAs, and performed functional studies in transfected human cell lines, with a construction including the predicted miRNAs, finding a significant down-regulation of the target expression [104]. Ren *et al.* [105] (Table 1.) aimed to characterize the miRNA profiles between multidrug resistant (MDR) tuberculosis (TB) and a drug-sensitive TB. Through a deep-sequencing approach for RNA fractions smaller than 50 nucleotides, they found differential profiles between both TB strains after an *in silico* analysis of the sequence data.

2.2.2 Host altered miRNA profiles

The second mechanism, in which pathogens alter the system for its own benefit, is the alteration of the host miRNAs expression levels. It has been described for many types of infections, either viral or bacterial, with an excellent review for several pathogenic bacteria species in [106].

2.2.2.1 Host altered miRNA profiles: Viruses

Within the first group, Japanese encephalitis virus (JEV) is a pathogenic agent that causes acute viral encephalitis. The first report of its infection profile was carried out by Cai *et al.* [107] (Table 1.), using a JEV-infected PK-15 cell line (*Sus scrofa*/pig kidney cell line). In their study they compared the deep sequencing profile of infected versus non-infected cells, using an Illumina platform, having found 1 up-regulated and 6 down-regulated miRNAs specific for the JEV infection. Other study that used JEV as pathogen, but this time performed *in vitro* using an immortalised human microglial cell line, CHME3, was reported by Sharma and colleagues [108] (Table 1.). In their study the authors focused on the profile of miR-146a, a known anti-inflammatory molecule [109, 110] that targets the cytokine signalling system through transcriptional down-regulation of its components *IRAK1* and *TRAF6* [111]. Besides the *in vitro* infection of CHME3 cell line, Sharma and colleagues also tested the effect of the infection with JEV in either miR-146a over-expressing cells, or cells in which miR-146a was blocked [108]. The authors observed an increase in the expression levels of miR-146a upon JEV infection, what indicates that the virus is using the post-translational machinery of the cell for its own benefit in order to escape the negative effect that the increased inflammation would have over its survival. Nevertheless, they also indicate that this effects are highly dependent on the viral strain line, as Pareek *et al.* report opposite results with the same cell type but a different strain of JEV [112]. It has been previously shown that JEV infection induces up-regulation in the levels of inflammatory cytokines [113], such as TNF-α, IL-6, or RANTES [114] among others, what is aimed to clear the infection, but an excessive inflammatory response leads to the neurological damages associated with the disease. In keeping with this observation, Zhu *et al.* [113] report an up-regulation of miR-15b during the infection of JEV in a U251 cell line, with increased viral clearance when the antagonist is used in JEV infected mice, suggesting that control of viral-caused inflammation could be an opportunity for leveraging the clinical symptoms.

Hantavirus (HTV) is another type of virus of clinical relevance. The host miRNA profile during infection has been addressed in two recent studies. Shin *et al.* [115] evaluated the distinct miRNA profiles of four hantavirus strains when infecting three human cell lines (A549, HUVEC, and THP-1), through a microarray based platform [115] (Table 1.). Among their results, the authors highlight the specific miRNA expression profiles observed depending either on the host cell type, or on the HTV viral strain. The following study by Shin *et al.* [116] carried out a set of experiments assessing the response to two types of HTV, Hantaan virus (HTNV) and Imjin virus (MJNV), in a human astrocytic line (A172), obtaining results in line with previous reports and suggesting that innate immune responses occur in brain upon HTV infection.

Enteroviral infections are caused by a group of viruses which belong to the genus *Enterovirus* (EV), as referred previously [117], causing several type of complications such as hand-foot-mouth-disease, sepsis-like disease, myocarditis, or hemorrhagic conjunctivitis among others. Wu *et al.* have extensively reviewed [118] the current development of the field, where it has been reported a central role of miRNAs in the EV-host interaction.

2.2.2.2 Host altered miRNA profiles: Bacteria

Bacteria are also capable of modifying the host miRNA levels, as this has been demonstrated in several studies [119 – 121]. Ma and collaborators evaluated the influence of a miRNA on the course of bacterial infections in mice [122] (Table 1.), being the pathogens *Listeria monocytogenes* or *Mycobacterium bovis* bacillus Calmette-Guérin (BCG). They report a lower expression of miR-29 and an enhanced expression of IFN-γ upon infection. Moreover, they found through further experiments that miR-29 can target IFN-γ and suppress its production by interfering with the mRNA. Tuberculosis (TB) in humans is caused by *Mycobacterium tuberculosis* (MTB), which is related to the previously mentioned BCG. Zhang *et al.* [68] (Table 1.) evaluated the miR-155 expression profiles of TB infected patients compared to healthy ones, as a potential way to measure the activity of NK cells in TB patients, having found a lower expression level in the serum of infected patients. These results could further be used for the

Table 1. Relevant miRNA studies during the course of different infectious diseases. Studies are divided between those studying pathogen encoded miRNAs, and reports of altered host miRNA profiles.

Tablica 1. Relevantna istraživanja o ulozi miRNA tijekom različitih infektivnih bolesti. Istraživanja su podijeljena na ona koja proučavaju kodirajuće miRNA patogenih uzročnika te ona koja izvješćuju o promjenama u miRNA profilu domaćina.

	Pathogen type/Vrsta uzročnika	Specific pathogen/Specifični uzročnik	Material/Materijal	Remark/Opaska	Ref/Lit.
Pathogen encoded miRNAs/ miRNA kodirane patogenom	Virus	Epstein-Barr virus (EBV)	Burkitt's lymphoma cell line (human)	First reported viral encoded miRNAs	Pfeffer *et al.* [88]
		Bovine Leukemia virus (BLV)	BL3.1 cell line (*Bos taurus*), and fetal lamb kidney (FLK) cell line	First reported RNA virus expressing miRNAs	Kincaid *et al.* [94]
		West Nile virus (WNV)	*Aedes albopictus* C6/36 cell line	Viral derived miRNA that controls viral replication and targets a host mRNA	Hussain *et al.* [97]
		Dengue virus (DENV)	*A. aegypti pool*, Aag2 cells, *A. albopictus* RML-12 line	Identification of a miRNA-resembling candidate through a deep sequencing approach	Hussain *et al.* [99]
		Ebola virus (EBOV)	HEK293T	Computational identification of two pre-miRNAs, further functional studies in HEK293T cell lines	Liang *et al.* [95]
	Bacteria	*Mycobacterium marinum* strain M	RAW264.7 murine cell line	Found the first candidate miRNA from bacterial origin, deep sequencing approach	Furuse *et al.* [103]
		448 different genomes of pathogenic bacteria	Transfected human cell lines	Identification of putative bacterial miRNAs, significant regulation of host mRNA levels in functional assays	Shmaryahu *et al.* [104]
		MDR-TB, and drug-sensitive TB	Lung tissues from affected TB patients	Differential profiles between TB strains	Ren *et al.* [105]
Host altered miRNA profile/ promjene u miRNA profilu domaćina	Virus	Japanese encephalitis virus (JEV)	PK-15 cell line (*Sus scrofa*)	Deep sequencing miRNA profile alterations upon infection	Cai *et al.* [107]
		Japanese encephalitis virus (JEV)	Immortalised human microglial cell line, CHME3	Expression changes of the anti-inflammatory miR-146a, which was increased during JEV infection	Sharma *et al.* [108]
		Hantaviruses (HTV))	Human cell lines (A549, HUVEC, and THP-1)	Microarray miRNA based platform; specific profiles depending on the host cell type, or the viral strain	Shin *et al.* [115]
		HTV strains: Hantaan virus (HTNV), Imjin virus (MJNV)	Human astrocytic cells (A172), mice	Dysregulated induction of innate immune genes due to miRNA expression changes	Shin *et al.* [116]
	Bacteria	*Listeria monocytogenes*, *Mycobacterium bovis* bacillus Calmette-Guérin (BCG)	Mice	miR-29 targets IFN-γ, suppression immune response to intracellular pathogen	Ma *et al.* [122]
		Mycobacterium tuberculosis	Serum from human TB infected patients	miR-155 expression profiles in serum of infected patients compared with the one from healthy donors	Zhang *et al.* [68]
		Mycobacterium tuberculosis complex (MTBC), and a panel of pathogenic bacteria	Human derived DCs from healthy donors	Found a core set of 49 miRNAs expressed independently of the pathogenic bacteria, or the time point	Siddle *et al.* [120]
		Streptococcus pneumoniae	Affected patients of pneumonia	Higher circulating levels of miR-21, miR-155, and miR-197	Abd-El-Fattah *et al.* [124]
		Salmonella enterica serovar Typhimurium	Murine RAW 264.7 macrophage-like cell line	General down-regulation on several of the let-7 family members, leading to an increased cytokine production	Schulte *et al.* [125]
	Bacteria and Fungus	Different combinations of bacteria and fungi infections	Sepsis patients and healthy subjects	Microarray miRNA based platform; increased levels of miRNA-155 correlated with severity of sepsis; lower miR-155 expression in surviving patients	Liu *et al.* [129]
	Fungus	*Candida albicans*	Murine macrophages	Up-regulation of miR-146, miR-155, miR-455, and miR-125a, induced by heat inactivated bacteria, or LPS exposition	Monk *et al.* [131]
	Prion	Prion particles	Patients affected by sporadic Creutzfeldt-Jakob disease (sCJD)	Deregulation of normal miR-146a levels	Lukiw *et al.* [134]

treatment of TB infections. Siddle *et al.* [120] (Table 1.) have also studied the differential miRNA profiles upon mycobacteria infection in human derived DCs from healthy donors, using a panel of bacteria that contained among them three members of the *Mycobacterium tuberculosis* complex (MTBC), with two virulent strains as well as the BCG strain used by Ma *et al.* [122]. Siddle and colleagues have found a core set of 49 miRNAs that are expressed in the host cell independently of the bacterial strain, and which is additionally conserved in time.

Pneumonia caused by *Streptococcus pneumoniae* has been studied in mice [123], and it was reported that miR-155 is involved in the clearance of the pathogen. In humans the expression profile of miRNAs in patients affected by pneumonia has been obtained [124] (Table 1.), where the authors report higher circulating levels for miR-21, miR-155, and miR-197.

Schulte *et al.* [125] (Table 1.) researched the miRNA alteration in murine RAW 264.7 macrophage-like cell line, and human HeLa epithelial lines upon infection with *Salmonella enterica* serovar Typhimurium, a pathogen that causes gastroenteritis in humans [126, 127]. Results include the observation of a general down-regulation on several of the let-7 family members upon *Salmonella* infection, independently of the cell type. Through functional assays the authors were able to show that the targeted let-7 family members can alter the expression of important cytokines, IL-6 and IL-10, having them under negative regulation. The infection was capable to alter this control system, leading to an increased cytokine production.

Neisseria meningitidis has been described as a causative agent for meningitis and fatal sepsis [128]. In a sepsis-related study, Liu *et al.* [129] (Table 1.) have used a miRNA microarray platform as a high-throughput approach to identify deregulated miRNAs between a group of healthy controls and sepsis patients, with experimental validation of an increase in the expression levels of miR-155. Furthermore they report that the expression of this miRNA was positively correlated with the severity of the disease, and that surviving patients show a lower miR-155 expression.

2.2.2.3 Host altered miRNA profiles: Fungi

Candida albicans has been appointed as an important infectious pathogen in urinary tract infections (UTI) [130]. Monk *et al.* [131] (Table 1.) described that either heat inactivated *Candida albicans* or LPS, are able to induce up-regulation of miR-146, miR-155, miR-455, and miR-125a in mice macrophages. In the same line of research Li and colleagues studied the effect of candidemia over mice with systemic candidiasis, having found a protective effect of miR-204/miR-211 over the infection associated injuries in the kidneys. Further studies in human cell lines could give interesting results if the same trend is found, what potentially can lead to treatment of the infection by restoring miRNA profiles for these two miRNAs.

2.2.2.4 Host altered miRNA profiles: Prions

Prion diseases [132], have also been studied for miRNAs presence. This disease is different from those that have a bacterial or viral origin, due to the self-replicating nature of the infective particles. It has been addressed by Shapshak [133], that miRNAs can influence several processes important for the development of the disease. Lukiw and collaborators [134] (Table 1.) found a deregulation on the levels of miR-146a, a type of miRNA that is induced in viral infections, in sporadic Creutzfeldt-Jakob disease (sCJD), suggesting that it is a candidate to be a key molecule in the regulation of inflammatory brain responses during prion infections.

3 Potential uses as biomarkers

Biomarker can be defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention", definition that was proposed at the 1998 National Institutes of Health Biomarkers Definitions Working Group [42 – 44]. As defined by Weber and collaborators [12], an ideal biomarker should comply with non-invasiveness, cheap quantification methodology, specificity to the disease, reliable indication of disease at early stages although no clinical symptoms, and additionally quick procedure; being the latter of extreme relevance for the diagnostic services at hospitals, as the physicians must deliver a therapy as soon as possible.

The uses of miRNAs as biomarkers are diverse, as for prognossis, early stage identification, diagnosis and stratification, recurrence, pharmacodynamics (PD), or prediction [12, 45 – 47]. They are good candidates for biomarkers as they can be measured with a higher sensitivity than proteins [48], as reported for miRNA-124 in comparison with alanine aminotransferase (ALT) [49] when evaluating liver necroinflammation in hepatitis B virus (HBV) infected patients. Their ubiquitous presence in various types of fluids as blood, saliva, or urine [14, 39, 48, 50], make them an ideal target in terms of sampling feasibility and non-invasiveness. Besides, when compared with mRNAs they show higher stability and decreased degradation rate in saliva [9, 44, 51], as well as in other biofluids [11]. In these cases, powerful diagnostic techniques such as RT-qPCR are often limited due to the labile nature of RNA in the provided samples [45]. It cannot be forgotten that collection of samples in clinical practice is far from the laboratory research setting, where all environmental variables are under control, and very importantly sample freezing or processing can be done immediately upon col-

lection. That ideal situation is not possible in the clinical setting, and therefore having such a stable molecule as miRNA can offer a significant advantage for the diagnostic divisions. The importance of biomarkers in PD has been addressed in a recent review by Gainor *et al.* [46]. As they highlight, it is not only important to use predictive biomarkers in order to aid in the patient selection for the trial, but also to evaluate the outcome of the drug-target interaction. Indeed, a very good example for that is the previously mentioned study of Wang *et al.* [49]. The authors report a type of miRNA biomarker candidate that can potentially serve as an indicator of the scale of liver damage in HBV infected patients, as an alternative to liver biopsy. Interestingly, the serum miR-124 expression levels varied accordingly with the levels of necroinflammation. At the same time, it is a good methodology to evaluate the potential protective effects of antiviral HBV drugs through monitoring of the tissue damage by the levels of miR-124. Further studies in larger set of patients will be needed for this promising biomarker in order to reach clinical practice.

3.1 Current biomarker or therapeutic uses of miRNAs

In contrast with other examples when the hope in new technologies exceeds the development of treatments [52], there are some successful stories in the use of miRNA as biomarkers or as targets for therapy. This time miRNAs are closer to the clinical practice. Several companies have miRNA-based drugs or biomarker assays at different phases of development. Three examples have been selected to give the reader an idea of the state-of-the-art, and to reflect three different stages of product development and commercialisation. One active player is a Japanese company where they follow NGS approaches for the identification of miRNAs as new biomarkers in non-invasive samples. Another company in USA is actively developing miRNA diagnostic platforms, based on their own previous studies [53 – 55], with a Phase II trial going on for evaluation of a panel to detect early Alzheimer's disease related miRNAs in plasma. The last example of the three is an Israeli company, which is part of a consortium involved in the development of new miRNA based therapeutics. Besides, they commercialize several miRNA diagnostic kits: for cancer origin detection [56], lung cancer subtype stratification [57], kidney tumour classification [58], or a test do differentiate mesothelioma from pleura and lung cancers [59].

Lastly, there is a promising example of miRNA therapeutical use, as shown in the recent works of Janssen *et al.* [60] in subjects affected by chronic HCV infection. This group have administered a locked nucleic acid-modified antisense oligonucleotide [61 – 63], Miravirsen, which targets and binds miR-122 by forming a highly stable heteroduplex, what leads to the inhibition of the downstream functions of this miRNA. By doing so, they report a long-lasting viral suppression in the studied individuals, up to 29 days, what is in accordance with previous studies in chimpanzees. Very importantly, the target drug was subjected to Phase II clinical trials (identifier No. NCT01200420) [47, 60, 64, 65].

4 Concluding remarks and future prospects

MicroRNAs are a widely studied set of ncRNAs with important capabilities over gene and protein expression regulation and control. They affect a number of processes according to specific developmental stages or environmental events, and are also key regulators of the human immune system. Some of their features make them excellent candidates for biomarker discovery, such as a higher stability in circulating biofluids in comparison to mRNA, or a close association to a given disease. Despite the need of all possible advances in different technologies to address current challenges in the treatment of diseases, technological developments are unfortunately not always quickly translated into clinical developments. MicroRNAs are currently available in the clinic for the staging and classification of several cancer diseases due to their consistent expression profiles. For the field of infectious diseases, it is a milestone the current Phase II trials for treatment of HCV. This example will probably encourage other groups to develop miRNA based strategies for the treatment of infections.

Targeting pathogen-encoded miRNAs can be a key point for therapeutic strategies, because a potential inhibition of their expression or production will interfere with essential processes for the multiplication and/or replication of the pathogenic agent. Although it is still quite a polemic field and further studies are needed, advances in research provide evidence of miRNAs encoded by bacteria or RNA-genome viruses. A second type of mechanism is the modification of the host miRNA profiles, as it has been reported for all types of pathogens, with several important examples in which miR-155 and miR-146a have been altered. The current treatment of HCV falls within this second category, what gives an idea of the potential for treatment in controlling the fluctuations of host miRNA expression levels during a process of infection.

In conclusion, miRNA can be used as biomarkers for the classification of infectious diseases, as well as a target for the treatment of disease progression.

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