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Review

MicroRNAs in parasitic diseases: Potential for diagnosis and targeting

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ABSTRACT

MicroRNAs (miRNAs) are a recently discovered class of small non-coding RNAs that can down-regulate protein expression by specific mRNA recognition. Evidence is accumulating that the miRNAs are implicated in the course and outcome of infectious and non-infectious diseases. Both parasites specific miRNA sequences and the phenomenon of the alteration of host miRNA levels after parasite infection are known, although detailed information about the direct intervention of parasites in the alteration of host miRNA levels and how this is regulated by parasites at molecular level is still lacking. Circulating miRNAs can be detected in biological fluids as serum, saliva and others, exhibiting a good potential as non-invasive biomarkers. Their ability to function as master regulators of the gene expression and the possibility for a relative easy manipulation of the miRNA machinery and related events, coupled with their apparent lack of adverse events when administered, place miRNAs as promising targets for the treatment of diseases. Moreover, the dependence of parasites over the host cellular machinery to accomplish infection and complete their biological cycles, together with the potential manipulation of host's responses through parasite miRNAs, point out that the miRNA machinery is particularly interesting to seek for alternative therapeutic approaches against parasites. Additionally, the studies about parasitic manipulation of the host immune responses thought miRNAs could broaden our knowledge about basic aspects of the host–parasite relationships.

Contents

1. Introduction

The majority of RNA transcripts in animals are small non-coding RNAs (sncRNAs). MicroRNAs (miRNAs) are sncRNAs generated from hairpin structures constituted by the complementary sequences within a given mRNA transcript from which they usually derive a diverse variety of isomers. Most miRNAs are encoded in intergenic regions and are transcribed by RNA polymerase II as primary nuclear long miRNAs (pri-miRNAs). One pri-miRNA typically contains a single or several miRNA precursors (pre-miRNAs) structured in a stem-loop hairpin manner, flanked by unstructured, single stranded RNA sequences thus showing a peculiar biogenesis [1]. Since the discovery of the first miRNA in the free-living nematode *Caenorhabditis elegans*, and the description of its link with the silencing pathway leaded by the key proteins Argonaute (AGO) and Dicer, important regulatory roles for miRNAs have been described in this organism [2]. Binding of miRNAs to partially or fully complementary sequences in the mRNAs leads to inhibition of the expression of the corresponding protein by degradation of the mRNA or by suppression of its translation. Thus, the primary function of miRNAs is the regulation of gene expression at post-transcriptional level. Potentially, each miRNA can regulate the

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expression of several different transcripts [3]. It is predicted that miRNAs can control the expression of up to 30% of the mRNAs in eukaryotes, being signaling pathways the main candidates for regulation mainly due to the common principles of both, their dose-sensitive nature and cell specific repertoire which seems ideal for activating point required responses to extracellular signals, and the fine-tuning role of miRNAs leading to different kinds of target modulation (inhibition, destabilization, degradation) – depending on the degree of sequence complementarity – finally allowing plasticity and robustness in the responses to different cell environments and conditions through complex miRNA-signaling network relationships [4–6].

The miRNAs have emerged in the last few years as key regulatory elements in the life cycle of many other organisms, including plants and animals, and thus have been designated, together with transcription factors, as the main family of regulatory elements of gene expression showing combinatorial regulatory patterns among them [7]. The identification and characterization of miRNAs in several organisms has provided new opportunities to understand the biology of a number of species [8–10].

A repository of the miRNA sequences known to date can be found in the miRBase server, which provides miRNA nomenclature, information about predicted precursor hairpin sequences and experimentally identified mature miRNA sequences [11]. Of those miRNAs that have been identified in various multicellular organisms, some show evolutionarily conserved sequences, while others exhibit species-specific characteristics.

Due to their wide and intricate regulatory roles and to its ubiquity, as well as to their sequence specificity, miRNAs arise as potential alternative diagnostic and therapeutic targets for parasitic diseases. The sequences of parasites miRNAs could provide a new platform to study gene regulation, development and evolutionary processes in parasites and at the same time decisively help to decipher host–parasite interactions and relationships. Nevertheless, and although the silencing-related AGO and Dicer-like proteins have been identified in many parasites, the identification and characterization of parasite miRNA sequences has been limited to date. The same can be said about specific miRNA sequences from the host regulated by parasites and to miRNAs from parasites potentially exerting direct actions in the regulation of host cell mRNA profiles, aspects that has been only slightly reported to date.

2. MicroRNAs in parasites: a growing collection of sequence information

MiRNAs in parasites were first identified in parasitic nematodes. Later, a number of miRNA sequences have been characterized in protozoa, other helminthes and arthropod parasites. A recent review includes the information on the specific sequences found in parasites and their homologies with miRNA sequences of other organisms [2]. After this review, the number of identified miRNA sequences in parasites in which miRNAs were already known has increased due to large scale sequencing experiments, and new miRNA sequences have been also described in parasites in which miRNAs had not been evidenced before (see http://www.mirbase.org/search.shtml; [12–15]; Fig. 1).

In protozoans, miRNAs have been identified in parasites displaying AGO and Dicer proteins, while in unicellular parasites lacking the RNA interference machinery – *Plasmodium* spp., some *Leishmania* species, *Trypanosoma cruzi* and *Cryptosporidium* spp. – miRNAs have not been found as expected [12]. In *Trypanosoma brucei* and *Giardia lamblia*, miRNAs are potentially involved in the mechanism regulating surface protein variation [16,17]. Specifically, in *G. lamblia* two identified miRNAs designated miR6 and miR10 have been predicted using bioinformatics to bind an elevated number (>150) of open reading frames in variant surface proteins (VSP). This prediction was experimentally confirmed by the construction of a defined mRNA tagged with the sequence of the VSP that was recognized by both miRNAs, resulting in its transcription repression [17]. Similarly, a computational approach in *T. brucei* resulted in the identification of several miRNAs that could target mRNAs encoding for different VSPs of this parasite [16]. In *Trichomonas vaginalis*, a series of miRNAs were reported [2], and later the specific interaction of the parasite tva-miR-1 miRNA with the malate dehydrogenase (MDH) mRNA from the parasite was shown [18]. This finding allowed the elucidation of the MDH down-regulation mechanism in the amoebid stage of the parasite, a low ATP consumer compared with the trophozoite stage, thus showing the role of the tva-miR-1 miRNA on the differences in the regulation of the glucose metabolism between the two parasite stages. Additionally, a recent work has focused on the phylogenetic study of *G. lamblia* and *T. vaginalis*, among others, through the analysis of several new miRNA sequences detected in these parasites, providing further information about miRNA sequences in both protozoans [19]. The authors identified 14 ancient animal miRNA families and 13 plant-specific miRNA families in the above-mentioned flagellates. Other protozoan parasites like *Entamoeba histolytica* and *Toxoplasma gondii* have been also investigated searching for miRNA sequences [20,21]. Although the identification and sequencing of miRNAs in protozoan parasites have been relatively numerous, the definition of their functions needs further experimental evaluation.

In helminths, schistosomes (*Schistosoma mansoni* and *Schistosoma japonicum*) have been the most frequently used models for miRNA investigations, due to the availability of a large amount of sequence information in respective databases that has facilitated the bioinformatic prediction of small RNA sequences. The studies of miRNA populations in both schistosome species have resulted in the generation of new data that would facilitate a deep understanding of the parasites' biology, specially regarding those miRNAs that have shown stage-specific patterns [22,23]. In the last five years, parasite nematodes have also been candidates of miRNA studies, *e.g. Trichinella spiralis* and *Haemonchus contortus* [24,25,15]. These studies have shown interesting relationships between changes in the miRNA microenvironment linked to specific parasite life-cycle stages, and between the promotion of drug resistance and the presence of defined miRNAs, among others. Similar relationships have been detected during the study of miRNAs in cestodes. Specifically, several stage-specific miRNAs have been cloned and identified in *Echinococcus granulosus* and *Echinococcus multilocularis*, providing new avenues to study the relationship of each parasite stage with its host and contributing to a better understanding of their complex biology [26,27].

In arthropods, mainly miRNAs from vector insects have been studied. These studies have evidenced that a large number of miRNAs are conserved among insects and between those and other animals [28]. The characterized miRNAs have been mainly related with insect female reproduction, showing sequences and functions conserved among different species inside mosquitoes and also differentially expressed miRNAs at various developmental stages [2]. Although RNA interference and silencing studies in other arthropods like ticks are developed to the same extent than for insects [12], studies on miRNAs are less numerous in these other arthropods than in insects (Fig. 1). The small RNA transcriptomes derived from various life stages and selected organs of the hard tick *Rhipicephalus microplus* have been sequenced, and anciently acquired miRNAs show ubiquitous expression profiles throughout tick life-cycle stages and organs, contrasting with the restricted expression profiles of novel tick-specific miRNAs [29]. These and other above-mentioned results show the presence of conserved miRNAs whose functions seem to be essential for parasite survival regardless