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Chapter

Taenia solium microRNAs: Potential Biomarkers and Drug Targets in Neurocysticercosis

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Abstract

MicroRNAs (miRNAs) found in animals, plants, and some viruses belongs to the heterogeneous class of non-coding RNAs (ncRNAs), which posttranscriptional regulates gene expression. They are linked to various cellular activities such as cell growth, differentiation, development and apoptosis. Also, they have been involved in cancer, metabolic diseases, viral infections and clinical trials targeting miRNAs has shown promising results. This chapter provides an overview on *Taenia solium* and *Taenia crassiceps* miRNAs, their possible biological functions, their role in host–parasite communication and their potential role as biomarkers and drug targets.

Keywords: miRNAs, small noncoding RNAs, neurocysticercosis, biomarkers, drug target

1. Introduction

1.1 Overview of miRNAs: definition, biogenesis, and functions

MicroRNAs (miRNAs) are a major class of small noncoding RNAs (ncRNAs) found in animals, plants, and some viruses, which negatively regulate gene expression at the messenger RNA (mRNA) level [1]. The first known miRNA (lin-4) was found in the free-living nematode *Caenorhabditis elegans* [2]. Seven years later let-7 was identified, and together with lin-4 were found to regulate developmental timing of *C. elegans* larvae [3]. By definition, miRNAs are small RNA molecules incapable of encoding proteins, but possessing important structural, catalytic, and regulatory functions comprise one of the more abundant classes of gene regulatory molecules in multicellular organisms and likely influence the output of many protein-coding genes. According to the latest miRBase release (v22.1, October 2018, http://www.mirbase.org/), there are miRNAs from 271 species, expressing 38589 mature miRNAs.

It is well established that miRNA biogenesis is a complex process classified into canonical and non-canonical pathways. The canonical biogenesis is the dominant pathway by which miRNAs are processed [4]. This pathway includes three main steps: (i) In the nucleus, miRNA genes are transcript by RNA polymerase II as part of much longer RNAs called pri-miRNAs which contain one or a few stem-loop structures composed of approximately 70 nucleotides each (**Figure 1**). Sometimes miRNAs are transcribed as one long transcript called clusters, which may have similar seed regions, and in which case they are considered a family [5]. About half of all currently identified miRNAs are intragenic and processed

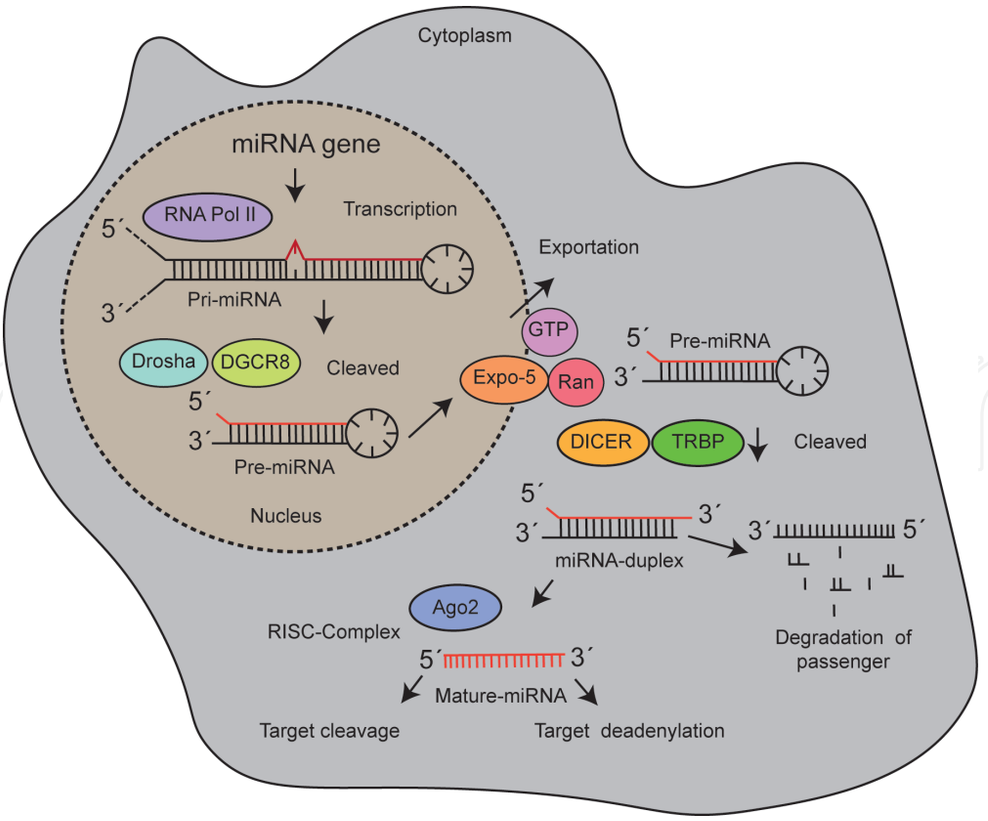

Figure 1.
Canonical microRNA biogenesis and mechanism of action. The biogenesis begins with the generation of the pri-miRNA transcript by RNA polymerase II in the nucleus. The microprocessor complex, composed of Drosha and DiGeorge syndrome critical region 8 (DGCR8), cleaves the pri-miRNA to produce the precursor-miRNA (pre-miRNA). After translocation into the cytoplasm by exportin 5, pre-miRNAs are processed by Dicer to form the mature miRNA/miRNA duplex. Following processing, miRNAs are assembled into the RISC complex. Only one strand of the duplex is stably associated with the RISC complex. The mature miRNA directs repression of mRNA containing complementary miRNA binding sites within the 3'UTR.*

mostly from introns and relatively few exons of protein coding genes, while the remaining are intergenic, transcribed independently of a host gene and regulated by their own promoters [6] (ii) Then, the microprocessor complex, consisting of an RNA binding protein DiGeorge Syndrome Critical Region 8 (DGCR8) and a ribonuclease III enzyme, Drosha trims the pri-miRNA to liberate a pre-miRNA hairpin which is actively transported to the cytoplasm by 5 (XPO5)/RanGTP complex (**Figure 1**) (iii) Its final maturation is processed in the cytoplasm where Dicer RNase III endonuclease cleaves the pre-miRNA into a single-stranded mature miRNA, removing the terminal loop (**Figure 1**) [4]. The directionality of the miRNA strand determines the name of the mature miRNA form [5]. Subsequently, with assistance from chaperone proteins (HSC70/HSP90) the mature miRNA is loaded into proteins of the Argonaute (Ago) family and assembles the RNA induced silencing complex (RISC) together to exert its further physiological functions (**Figure 1**). We defined the unloaded strand as passenger or star strand. The start strand that contains no mismatches are cleaved by AGO2 and degraded (**Figure 1**). Also, miRNA duplexes with central mismatches or non-AGO2 loaded miRNA are unwound and degraded. After being incorporated into the RISC, the mature miRNA induces posttranscriptional gene silencing by tethering RISC to be partially complementary to the target mRNA predominantly found within the 3′-untranslated regions (UTR). Targeting can also be facilitated by additional sequence elements, such as an unpaired Adenosine in the mRNA target sequence, corresponding to the nucleotide 1 in 5′ end of the mature miRNA.
On the other hand, non-canonical miRNA biogenesis pathways are grouped into

Drosha/DGCR8-independent and Dicer-independent pathways. In general, these pathways make use of different combinations of the proteins involved in the canonical pathway, mainly Drosha, Dicer, exportin 5, and AGO2 [4].

It has been estimated that miRNAs regulate the expression of approximately one-third of the protein-coding genes [1]. Each miRNA can have many target mRNAs and a single mRNA can be regulated by multiple miRNAs [7]. Given this vast majority of mRNA targets regulated by miRNAs, aberrant miRNA expression profoundly influences a wide variety of cell regulation pathways important to cell proliferation, apoptosis, and stress responses.

Single miRNA gene can generate multiple miRNA isoforms (isomiRs). For example, the inconsistent choice of the strand loaded into AGO can generate two different functional miRNAs from both strands of the pre-miRNA duplex [8]. Imprecise cleavage of pri-miRNA by Drosha or pre-miRNA by Dicer can generate heterogeneous 5′ or 3′ ends. Another way to generate isomiRs is by RNA editing which may have a functional impact when the seed nucleotide is altered [4]. Exonuclease activity could remove nucleotides from miRNA 3′ end, and terminal nucleotide-transferase could add nucleotides to the miRNA 3′ end generating different isomiRs.

For almost a decade, some of the miRNA genes have been categorized into different groups, named miRNA families, based on the mature miRNA sequence and/or structure of pre-miRNAs [9]. Thus, means that microRNAs are grouped into families based on their targeting properties, which depend primarily on the identity of their extended seed region (miRNA nucleotides 2–8) [10]. Interestingly, it has been observed that miRNA genes in the same miRNA family are non-randomly co-localized and well organized around genes involved in infectious, immune system, sensory system and neurodegenerative diseases, development and cancer [11]. As with paralogous proteins, members of the same seed families often have at least partially redundant functions, with severe loss-of-function phenotypes apparent only after multiple family members are disrupted [12].

Biological functions of individual miRNAs have been extensively explored and have revealed the important role of miRNAs in many biological functions such as developmental timings, cell differentiation, embryogenesis, metabolism, organogenesis, and apoptosis [13]. Thus, miRNAs have been introduced as therapeutics or as targets of therapeutics for the treatment of disease [14]. Also, the existence of extracellular miRNAs has been widely reported these molecules as potential biomarkers for a variety of diseases. At present, miRNAs-mediated therapies for treatment of cancer and chronic hepatitis C virus (HCV) infection have shown promising results in human Phase I clinical trial [15].

2. miRNAs in *Taenia solium* and *Taenia crassiceps*

The presence of homologs to Drosha, Dicer, and Pasha (as identified in the *T. solium* Genome Project) [16] and the differences in miRNAs profiles between the *Taenia solium* cysticerci and adults suggest that the process for the synthesis of miRNAs is similar to that described for mammals. Identification and sequencing of miRNAs have been largely facilitated by the newly available high-throughput tools that have generated a growing set of miRNA sequences from parasites. Our group report for the first time the high confidence miRNA repertoire from *T. crassiceps* and *T. solium* and show that miRNAs account for most small RNA expression in *T. crassiceps* cysticerci by small RNA-seq experiments [17]. Since our miRNA identification strategy required the matching of *T. crassiceps* small RNA sequences to the *T. solium* genome, the identified miRNAs are considered common to both species, as was previously considered for other helminth parasites [18, 19]. The percentage of miRNAs in *T. crassiceps* cysticerci

reaches 83% of the total small RNA expression, suggesting important functions of this type of RNA in the biology of taenias [17]. Also, miRNAs were identified and validated by northern blot experiments [17]. This validation is especially important in the case that genomic data from other species is used. Additionally, we experimentally detected pre-miRNAs for the first time in cestodes adding confidence to the miRNA identification procedure performed [17]. The *T. solium* and *T. crassiceps* miRNA catalog includes 41 conserved miRNAs grouped into 30 families [17]. The number of conserved miRNA families is similar to that of *Echinococcus canadensis* [20] and *Mesocestoides vogae* (syn: *M. corti*) [21] (28 conserved miRNA families), providing further evidence for the loss of conserved miRNA families in cestodes [22]. In *T. solium* genome it was reported two miRNA precursors (pre-mir-new-1a and pre-mir-new-1b) arranged in a cluster [17] that were only reported before for the *Echinococcus granulosus* s. s. G1 genotype [23]. The first of these precursors shows expression from both arms in *T. crassiceps*, unlike the *E. granulosus* s. s. G1 genotype that only expressed the 3p arm [17]. Differences in the genome organization of miRNA precursors among cestodes was reported. For example, the cluster miR-7b and miR-3479a found in *T. solium* are not clustered in *Echinococcus* or *M. vogae* [17]. The cluster miR-71/2c/2b, it was found only once in the genome of *T. solium*, as observed in other cestodes analyzed to date [20, 21, 24]. The presence of only one miR-71/miR-2 cluster seems to be a common feature of cestode genomes. The uneven expression found among miRNAs of this cluster was also observed in *Echinococcus* spp. [20, 24] and *M. vogae* [21]. Cluster miRNAs are evolutionally and functionally related and may co-regulate multiple biological processes. Additionally, cluster miRNAs have shown to evolve more rapidly than individual miRNAs [25]. Current evidence in humans suggests that, various genetic events (deletion, insertion, duplication and base substitution) within a cluster, followed by adaption and neofunctionalization, is the underlying mechanism responsible for the evolution of miRNA clusters [26]. To date, the importance of these differences in genomic arrangement of cestodes is unknown but could potentially influence the expression of the corresponding mature miRNAs.

The expression profile of *T. crassiceps* cysticerci showed that miRNA expression is highly biased to a few miRNAs: miR-10, let-7, miR-71, bantam and miR-61 [17]. These five miRNAs account for ~90% of miRNA expression [17]. These miRNAs and miR-4989 were also highly expresses in *Taenia solium* cysticerci [27]. Coincidentally, in other reports of small RNAs from cestodes that used the same methodology for miRNA discovery, miR-10, let-7, miR-71 and bantam were the most highly expressed, suggesting important functions in cestode biology [20, 21, 23, 24]. The repertoire of miRNAs in *T. solium* genome included protostomian miRNAs, such as miR-4989 and bantam that are absent in human host [17]. MiR-4989 is a divergent member of the miR-277 family. This protostomian-specific family is known to be involved in amino acid catabolism in *Drosophila*. Recently, miR-4989 was shown to be involved in development of juvenile worms in *S. mansoni*. In the *T. solium* genome miR-4989 target a Cationic amino acid transporter and a basic leucine zipper bZIP transcription factor without orthologs in any model species. Additionally, in the genome of *T. solium* and expressed in *T. crassiceps* cysticerci we found bilaterian miRNAs that are absent in human host, such as miR-71 or divergent from their host ortholog, such as let-7 [17].

The identification and characterization of miRNA targets is essential for understanding the function of these ncRNAs at molecular level. MiR-10 is the most expressed miRNA in *T. crassiceps* and *T. solium* cysticerci. This miRNA is highly conserved across metazoan organisms and is implicated in Hox gene regulation, embryonic development, and cancer [28–30]. Tow ANTP class homeobox genes were found among predicted miR-10 targets in *T. solium* genome [17] as in many others bilaterians [31]. Let-7, a conserved miRNA across evolution, was shown to regulate the developmental timing in *C. elegans* [32] and was shown to be a central

regulator of mammalian glucose metabolism by targeting several genes of the insulin-PI3K-mTor pathway, including the insulin receptor [33]. MiR-9 is a deeply conserved miRNA across evolution known to be involved in neural development. One possible target for miR-9 is a Slit 2 protein, the ortholog of *C. elegans* slt-1 that is expressed in muscle cells and neurons and is involved in generation of neurons and axon guidance during embryonic and larval development. Also, a Carbonic anhydrase was reported as a putative target gene for miR-9, which is the ortholog of *C. elegans* cah-1 that is expressed in different neurons and head ganglion and is predicted to have a carbonate dehydratase activity. Other relevant target for miR-9 was Peregrin, the ortholog of *C. elegans* lin-49 that is involved in normal larval development. MiR-71 is bilaterian miRNA absent in vertebrates and involved in the promotion of longevity and neuronal asymmetry in *C. elegans,* is the miRNA with more targets predicted in the genome of *Taenia solium*. Some interesting targets are shown in **Table 1**.

Parasite miRNAs that are absent in the host, such as miR-71 or highly divergent (e.g let-7) from their host orthologs may be considered as selective therapeutic targets for treatment and control of helminth parasite infections. In addition, miR-71 is highly expressed in *T. solium* adults stage suggesting that it could be involved in important biological functions in the life cycle of *Taenia* genus. Nevertheless, the characterization of the physiological effects of the presence or absence for each identified miRNA needs more complex approaches in *T. solium*. In this respect, in vitro and in vivo optimization strategies for efficient and long-lasting loss-of-function, such miR-71 silencing reported in *E. multilocularis* [34] are still required for meaningful silencing studies in other metacestodes.

2.1 miRNAs and immune response

Helminth's parasites modulate immune responses in their host to prevent their elimination and establish chronic infections. Neurocysticercosis (NCC) implicates chronic parasitic disease with different variety of host and parasite interactions [35]. Clinical manifestations are mainly the result of inflammatory response to degeneration of parenchymal cysticercus [36].

Taenia solium cysticerci actively prevents this inflammatory response [37], which prolongs its survival in the host. The intensity of NCC symptoms depends primarily on the inflammatory response, which is associated with the Th1 response with high levels of TNF, IFN-γ, IL-17, and IL-23 whereas the Th2 response (anti-inflammatory response) is associated with asymptomatic NCC with high level of IL-10, IL- 4, IL5, and IL-13 [38]. In *T. crassiceps* model, the cysticercus growth is controlled by macrophages and the promoting of Th1 and Th2 responses. The production of inflammatory cytokines by macrophages and dendritic cells are blocked by excreted/secreted antigens (E-S antigens). Also, toll-like receptor (TLR) are blocked facilitating cysticercus growth [38]. The miRNA signature of T regulatory (Treg) cells has been characterized and among the miRNAs expressed are mir-21 and mir-31, which have opposing effects on the Treg TF FOXP3 [38]. Also, it was demonstrated that the E-S antigens of *T. crassiceps* cysticerci can modulate proinflammatory responses in macrophages by inducing regulatory posttranscriptional mechanisms, while E-S antigens reduced the production of inflammatory cytokines (IL-6, IL-12, and TNFα), they increased the release of IL-10 in LPS-induced bone marrow-derived macrophages [39]. microRNAs are a key component of macrophage posttranscriptional regulation [40] and it was shown that E-S antigens of *T. crassiceps* cysticerci induced upregulation of miR-125a-5p, miR-762, and miR-484, which are predicted to target canonical inflammatory molecules and pathways in LPS-induced bone marrow-derived macrophages.

Gene ID	Gene description	Molecular funtion (GO)	Other miRNAs
TsM_001055500	Zinc finger, type RING / FYVE / PHD	—	bantam; miR-4989
TsM_001107400	Ubiquitin carboxyl terminal hydrolase (inferred by orthology to a protein from *S. mansoni*)	Thiol-dependent ubiquitinyl hydrolase activity	miR-36a; miR-36b
TsM_000718200	Ceramide glucosyltransferase (inferred by orthology to a human protein)	Transferase activity, transfer of glycosyl groups	let-7
TsM_000983300	Casp, putative (inferred by orthology to a protein from S. mansoni)	—	miR-71
TsM_000787600	Protein kinase-like domain. Tyrosine-protein kinase, active site.	Protein kinase activity, protein serine / threonine kinase activity, ATP binding, phosphotransferase activity, alcohol group as acceptor	miR-9
TsM_000964500	IUAA Family Transporter	—	miR-10
TsM_000502500	Fibroblast growth factor receptor homolog 1 (inferred by orthology to a *D. melanogaster* protein)	Protein kinase activity, protein tyrosine kinase activity, ATP binding	miR-124a; miR-124b
TsM_000692000	Mitochondrial coenzyme transporter A SLC25A42 (inferred by orthology to a human protein)	—	miR-61

Table 1.
Interesting miR-71 targets in Taenia solium *genome.*

Taenia solium E-S antigens have been implicated in immune modulation and it is also known that the intimate association between host and parasite and the immune response is highly controlled at the post transcriptional level [41]. Target prediction of miR-10 and miR-125 found in *T. solium* cysticerci are potentially involved in macrophage IRF/STAT pathways, such as CD69 and TNF. Also, miR-9 was found to be related in the classical activation of macrophages [38]. Mir-10 and miR-125 were also implicated in expression of cytokine receptors, cell activation markers and cell adhesion molecules that activate macrophages to secrete TNF involved in IFN-signaling pathway. Furthermore, potential miR-10 targets such as IL12 and IL23, could interfere with the IL-12 family signaling pathway with a probably Tregs induction. In addition, let-7 showed predicted targets, such as IL10 that encodes cytokines involved in M2 polarization [42]. These suggest an important role in the polarization of macrophages. Macrophages in cysticercosis promote a transient Th1 protective response with classical activated macrophages that is changed by parasite products to a Th2 permissive response with alternatively activated macrophages [43].

Already knowing that the more abundant miRNAs (miR-10-5p, let-7-5p) putatively have target genes of immune response and that macrophages in murine cysticercosis promote Th1 or Th2 responses it was demonstrated that synthetic miR-10-5p and let-7-5p were internalized into the cytoplasm of murine peritoneal macrophages in vitro [27]. Interestingly, the down regulation of the expression of pro-inflammatory cytokines, such as Il6, Il1b, and TNF, IL-12, was reported when activated macrophages were incubated with IFN-γ and miR-10-5p or let-7-5p.

Moreover, in macrophages activated with IL-4 these miRNAs reduced the expression of cytokines involved in M2/Th2 differentiation. These results were important, because murine resistant to cysticercosis display high levels of TNF, IL-12, IL1-β, and NO during early infection (Th1 response), which is associated with the elimination of larvae [44]. On the other hand, high levels of pro-inflammatory cytokines (IL-6 and TNF) cause damage to the microglia promoting autoimmune and neurodegenerative diseases [45, 46]. This tissue damage is also observed in human NCC at the beginning of larvae degeneration and in pig NCC when they are treated with praziquantel [47]. In contrast, viable larvae are associated with a long initial asymptomatic phase that correlate with undetectable inflammation in the SNC, presumably due to *T. solium* larvae factors prevent inflammation [48].

The striking ability of helminth parasites in conferring protection from diseases of immune dysregulation has increased the attention into the immunomodulatory mechanisms evoked by these parasites. Administration of E-S antigens of *T. crassiceps* in experimental ulcerative colitis, autoimmune encephalomyelitis and type 1 diabetes shown positive results [49, 50]. The ability of *T. crassiceps* to prevent inflammatory responses was demonstrated to be dependent on a population of macrophages that produced markers of alternative activation (M2) [51]. Excreted/secreted *T. crassiceps* products decreased the production of inflammatory cytokines (IL-12, TNFα, and IL-6) in LPS-induced macrophages but has a limited role in inducing directly the production of M1 and/or M2-associated molecules. The immune-modulatory ability of these E-S antigens was further associated with increased levels of specific microRNAs, which are predicted to target numerous inflammatory mRNAs involved in the TNF and NF-κB signaling pathways [39].

3. miRNAs in drug response

In 2010 Devaney and collaborators [52] speculated that the link between changes in miRNA levels and drug resistance in cancer cells may also be a feature of drug resistance in parasitic nematodes. On the other hand, few data have been published in connection with drug resistance in cestodes [53]. Our group study the miRNA expression profile of *T. cr*assiceps cysticerci incubated for 24 h with sublethal doses of praziquantel (PZQ), one of the main antiparasitic agents used for cysticercosis and taeniasis [17].

The experiments showed that the overall miRNA profile remained unchanged under PZQ treatment, except for miR-7b that showed a sixfold enhanced expression [17] under PZQ treatment. One of the predicted miR- 7b targets was calponin, a calcium binding protein that inhibits myosin. This may be related to the expected alteration of intracellular calcium concentration produced by PZQ, a drug binding and inhibiting voltage-gated calcium channels, a key molecule for the regulation of calcium level inside the cell. Also, other targets of miR-7b are involved in several pathways such as amino acid and nucleotide metabolism, vesicular transport, signaling pathways, cell adhesion, cell growth, cell death and interaction with neuroactive ligands, suggesting the importance of this miRNA in parasite biology [17]. Calponin is one of miR- 7b predicted targets linked to calcium binding protein that inhibits myosin and may be related to the intracellular calcium concentration produced by PZQ. Other predicted targets of miR-7b are involved in several pathways such vesicular transport, signaling pathways, cell adhesion, cell growth, cell death and interaction with neuroactive ligands, suggesting the importance of this miRNA in parasite biology [17]. In these experiments other miRNAs showed differences in expression levels during treatment with PZQ, such as miR-31. This miRNA showed a decrease in the level of expression in cysticerci treated with PZQ and therefore the

Pathway	Gene ID	Description	miRNAs
Ion transporters	TsM_000896200	Voltage-gated calcium channel 2C	miR-307
	TsM_000896200	alpha-2 / delta	miR-36a
	TsM_000025300		miR-36b
	TsM_000025300		miR-124b
	TsM_000783500		miR-124a
	TsM_000896200		miR-133
	TsM_000436700		miR-745
	TsM_000598200		miR-31
	TsM_000845300		miR-2c
Drug metabolism / enzyme conjugation	TsM_000648500	Cytochrome b5-heme type /	miR-124a
	TsM_000648500	steroid binding	miR-124b
	TsM_000191900	Selenoprotein type 2C Rdx	miR-31
	TsM_000625200	Thioredoxin-like fold	miR-2a
	TsM_000625200	Glutathione S-transferase 2C	miR-2b
	TsM_000685100	class S	miR-9
	TsM_000979200	Thiolase-like	miR-281
	TsM_000610200	Glutathione S-transferase 2C	miR-307
	TsM_000860300	similar to C-terminal	miR-2162
	TsM_000219500		miR-96
ABC transporters	TsM_001160500	ABC type conveyors	miR-2b
	TsM_000989600	Type 2 ABC conveyors	miR-2c
	TsM_000971500	Type 1 2C ABC conveyors	miR-31
	TsM_001225200		miR-61
	TsM_000459700		miR-71
	TsM_000740200		miR-125
	TsM_000006800		miR-184
	TsM_000720400		miR-219
	TsM_000882500		miR-307
	TsM_000575300		miR-3479b
	TsM_000726600		

Table 2.
Interesting drug response miRNAs and targets in Taenia solium *genome.*

genes regulated by the miRNA would be overexpressed compared to the cysticerci that did not receive treatment with PZQ. Predicted target genes for this miRNA include: ABC transporters (transporters responsible for expelling different drugs out of the cell), thioredoxins (involved in drug metabolism), and the voltage-gated L-type calcium channel subunit alpha-1D, which is a probable site of action for praziquantel (PZQ) [54] **Table 2**. Additionally, other *T. solium* miRNAs were found to have targets related to flow, metabolism, and drug action [17].

These results prepare the way for continue with more studies in order to understand the response of miRNAs to drug treatment and the influence that these ncRNAs may have on drug action and/or drug resistance.

4. miRNAs as potential biomarkers

A biomarker is described as a feature that is objectively measured and evaluated as an indicator of many biological processes. Hunting for helminths biomarkers capable of providing diagnostic, prognostic, or therapeutic information has become a necessary but challenging work in cestodes research. MiRNAs were reported in blood – plasma, serum and other fluids like urine and saliva. This attribute has raised the interest of their use as potential biomarkers and diagnostic tools [55]. In the case of cestodes diseases the use of pathogen miRNAs as biomarkers promises the advent of highly specific and non-invasive diagnostic tools, since the miRNA

repertoire of *T. solium* present a set of unique or divergent miRNAs with respect to the corresponding host homologs.

The small size and the stability of miRNAs are two important features that permit the circulation of these molecules in biological fluids. The formation of protein–miRNA allows circulating miRNAs escape of degradation [5]. Also, the majority of miRNAs detectable in serum and saliva are found inside extracellular vesicles (EV) that could avoid miRNA degradation and serve as transport particles to facilitate miRNA actions in neighboring cells [56]. The term EV groups includes several types of vesicles among which microvesicles and exosomes are the most thoroughly characterized. In helminths parasites EVs are the preferred extracellular compartment under study and miRNAs as the most thoroughly characterized RNA biotype [57]. The identification and sequencing of *T. solium* miRNAs is a must for their use as diagnostic tools. Among these contexts, the potential of miRNAs being involved in cestodes diseases as biomarkers has been investigated. It was demonstrated that the miR-10 and let-7 families are present in the EV from cestodes [57–61].
These two miRNAs are highly conserved throughout evolution and are present in bilaterians where they play fundamental roles in regulating stem-cell division and differentiation and embryonic development. The metacestode larval stage of *E. multilocularis* presents a morphological barrier to the secretion of EV towards the extra-parasite milieu and hence, ex-RNAs secreted in vitro are mostly detected in the EV-depleted fractions [57, 61]. Interestingly, the opposite is observed in the mestacestodes of *T crassiceps* and *M. vagae*, a parasites models of *T. solium*, that do not have such a structure [61] but parasite ex-RNA detection in patient biofluids is still in a very early phase of study.

The extensive use of next generation of technologies such as miRNA microarrays and high-throughput deep sequencing techniques, translating biomarker into practice with increased diagnostic and therapeutic sensitivity and specificity would be less of a problem [62]. With respect to the use of ex-RNAs as biomarkers in NCC, to date, no laboratory assay from plasma, serum or cerebrospinal fluid has been performed. Furthermore, patient samples from different geographic regions together with specificity assessment with samples of patients would also provide a more realistic view of the potential of ex-RNAs as biomarkers of NCC.

5. miRNAs as potential drug target

The hypothesis that many *T. solium* miRNAs have crucial roles in development, host–parasite interaction and immune response, and also the absent of some miRNAs in the host has led to considerable interest in the therapeutic targeting of miRNAs in NCC. The main approaches commonly taken are: i) miRNA inhibition by antisense oligonucleotides, miRNA sponges or small-molecule inhibitors ii) miRNA upregulation with miRNA mimics [63]. Mirna sponges' strategies rely on the expression of mRNAs containing multiple artificial miRNA-binding sites, which act as decoys. The overexpression of mRNA- sponges selectively sequesters endogenous miRNAs and thus allows expression of the target mRNAs [64]. Approaches that are based on small molecules generally rely on reporter-based assay systems for compound library screening and have identified small molecules that could specifically inhibit miRNA expression, such as azobenzene (which affects human miR-21 expression) and several diverse compounds that inhibit human miR-122 [65]. Considerably more attention has been paid to antimiRs, particularly to those that target miRNAs directly to specifically inhibit miRNA function and upregulate miRNA targets. In practice chemical modification of oligonucleotides is required to increase resistance to serum nucleases, to enhance binding affinity for targeted

miRNAs and to improve the pharmacokinetics and pharmacodynamics profile in vivo. Other limitations are associated with rapid clearance, immunotoxicity an low tissue permeability. The delivery of artificial miRNAs or of blocking counterparts that could interfere with key processes in parasites has been already postulated by several authors, and some potential targets are already characterized. MiRNA manipulation in parasites has been also proposed as a new strategy for control against schistosomiasis and cystic echinococcosis [38, 66].

6. Conclusions

For the better understand of the pathophysiology of parasitic diseases at the molecular level is crucial identify and characterize parasite-specific miRNAs and their targets in hosts. The significant advance in biomedical research of miRNAs as target drugs and biomarkers is expected to be widely translated in the field of parasitology in the coming years. Why not think about miRNAs as a profitable approach to better diagnose and properly treat NNC? There is an increasing number of studies that are being done in *T. solium* and cestodes miRNAs, however translational research of miRNA still remains a challenge.

Regarding the neglected diseases, researchers have dedicated decades to the development of new drugs and identifying new biomarkers of disease progression but most researches are limited to academia indicating a gap between basic science and clinical application. Also, the use of miRNAs as a biomarker or potential drug target are poorly explored compared with cancer, neurological disorders, metabolic, cardiac and circulatory diseases.

It is expected that in future years the biological knowledge acquired on miRNAs, especially in biomedical research, could be widely translated into NNC since miRNAs could hold great potential as therapeutic and diagnosis targets for the control of diseases.

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