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miRNAs in the regulation of mTOR signaling and host immune responses: The case of *Leishmania* infections

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ABSTRACT

Micro RNAs (miRNAs), as regulators of gene expression at the post-transcriptional level, can respond to/or interact with cell signaling and affect the pathogenesis of different diseases/infections. The interaction/crosstalk of miRNAs with various cellular signaling networks including mTOR (as a master regulator of signaling relevant to different cellular mechanisms) might lead to the initiation, progression or restriction of certain disease processes. There are numerous studies that have identified the crosstalk between regulatory miRNA expression and the mTOR pathway (or mTOR signaling regulated by miRNAs) in different diseases which has a dual function in pathogenesis. However, the corresponding information in parasitic infections remains scarce. miRNAs have been suggested as specific targets for therapeutic strategies in several disorders such as parasitic infections. Thus, the targeting of miRNAs (as the modulators/regulators of mTOR) by small molecules and RNA-based therapeutics and consequently managing and modulating mTOR signaling and the downstream/related cell signaling/pathways might shed some light on the design of new therapeutic strategies against parasitic diseases, including Leishmaniasis. Accordingly, the present study attempts to highlight the importance of the crosstalk between regulatory miRNAs and mTOR signaling, and to review the relevant insights into parasitic infections by focusing specifically on *Leishmania*.

1. miRNA biogenesis

miRNAs are endogenously expressed small non-coding RNAs 19–25 nucleotides in length, and are synthesized by RNA polymerase II and III enzymes, which can regulate post-transcriptional gene expression. RNA polymerase II or III transcribe miRNA genes into primary miRNAs (pri-miRNAs) in the nucleus. After Pri-miRNA processing by Drosha and DiGeorge syndrome Critical Region 8 (DGCR8), pri-miRNAs change to precursor miRNAs (pre-miRNAs) (Cai et al., 2017; Condrat et al., 2020). Pre-miRNAs are transferred into the cytoplasm by exportin 5 and the Ras-related nuclear protein (Ran) Guanosine-5'-triphosphate (RanGTP) and are then cleaved by Dicer, leading to production of two single-stranded RNAs (ssRNAs). The ssRNAs interact with the RNA-induced silencing complex (RISC) (composed of Argonaute 2, Dicer, and transactivation response RNA binding protein (TRBP)). Gene inhibition (mediated by the miRNA-RISC complex) may occur via site-specific cleavage, or by increasing mRNA degradation or through translational inhibition (Cai et al., 2017; Condrat et al., 2020; Matsuyama and Suzuki, 2020; Treiber et al., 2019). The 3′ untranslated regions (3′ UTR) of target mRNAs are considered the target sites of miRNAs to induce mRNA degradation and translational repression (Ramchandran and Chaluvally-Raghavan, 2017). However, the interaction of miRNAs with other regions, such as the 5′ UTR, coding sequence, and gene promoters, have also been reported.

The expression of miRNAs leads to the regulation of different cellular processes such as proliferation, differentiation and apoptosis.

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Accordingly, they have been implicated in the pathogenicity of several human diseases such as diabetes, cancer and many infectious diseases (Eng et al., 2019; He et al., 2021; Mirzaei et al., 2021).

2. The mechanistic/mammalian target of rapamycin (mTOR) pathway

mTOR, an atypical serine/threonine kinase of the phosphoinositide 3-kinase-(PI3K)-related kinase family, is composed of two intracellular protein complexes: called mTOR complex 1 (mTORC1) and mTORC2 each of which includes different protein components and is regulated by different protein complexes. mTORC1 is involved in the regulation of several crucial cellular processes including ribosomal biogenesis, lipid and nucleotide synthesis, mitochondrial functions, autophagy and mRNA translation (protein synthesis). mTORC1 is activated by nutrients and mostly acts through the phosphorylation of two effectors, eukaryotic translation initiation factor 4E (eIF4E)-binding protein (4EBP) and p70S6 kinase 1 (S6K1). mTORC1 phosphorylates S6K1, which in turn phosphorylates and activates the substrates involved in mRNA translation initiation, such as eIF4B, a positive regulator of the 50 cap-binding eIF4F complex. mTORC1 phosphorylates 4EBP at various sites to induce its isolation from eIF4E, triggering 50cap-dependent mRNA translation by the assembled eIF4F complex. mTORC1 functions are crucially controlled by the two upstream proteins, called tuberous sclerosis complex 1 (TSC1) and TSC2. The TSC1/TSC2 complex is essential for the proper activation of mTORC1 (Popova and Jücker, 2021; Querfurth and Lee, 2021).

Overall, mTOR exerts a pivotal role in integrating different intracellular and extracellular cell signals (via multi-protein complexes) and regulating and orchestrating their physiologic outcomes. Based on the potential functions of the mTOR pathway in vital cellular processes and different disorders, the use of mTOR inhibitors such as rapamycin (mTORC1 and mTOR2; rapamycin sensitive and insensitive, respectively) has been suggested to fight against various diseases especially cancers (Popova and Jücker, 2021).

3. The interplay of miRNAs and the mTOR signaling pathway

In addition to the role of miRNAs as regulators of gene expression at the post-transcriptional level, these biomolecules also respond to/interact with cell signaling to affect cell responses which entail major regulatory effects. Conversely, signaling networks can also control biogenesis, stability, and abundance of miRNAs, by regulating the components of the miRNA biogenesis pathway (Avraham and Yarden, 2012). miRNAs can regulate a number of key molecular pathways including mitogen-activated protein kinase (MAPK), Notch, Hippo, Transforming Growth Factor β (TGF-β), Hedgehog and Wnt signaling pathways (Ichimura et al., 2011). Thus, miRNA crosstalk with such cellular signaling networks could be correlated with the initiation, progression or inhibition of pathogenesis in different diseases such as cancers and viral infections (Fig. 1) (Onyido et al., 2016). mTOR signaling is one the most important pathways among those regulated by miRNAs (Nazari et al., 2021). miRNAs can affect proliferation, cell cycle, metastasis, and apoptosis of cancer cells by regulating mTOR signaling. These non-coding RNAs can be also useful for the development of therapies against cancer (phase I clinical trials) (Ping et al., 2018). Moreover, it has been shown by targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that host miRNAs exert important functions in neutralizing the virus. miRNAs induce all mechanisms relevant to virus neutralization in the infected cells through hijacking or regulating different cell signals such as those of the PI3K-AKT/mTOR axis (Khan et al., 2020). Table 1 further highlights the crosstalk between miRNA expression and the PI3K/AKT/mTOR signaling pathway in different diseases showing their dual function in pathogenesis.

AMP-activated protein kinase (AMPK), Phosphatase and tensin homolog (PTEN), TRPM2 antisense RNA (TRPM2-AS), Non-small cell lung cancer (NSCLC), Epidermal growth factor receptor (EGFR), Ras homolog enriched in brain (Rheb), Sirtuin 1 (SIRT1), Cyclooxygenase-2 (Cox-2), mEAK-7 (mTOR associated protein, eak-7 homolog), Suppressor of cytokine signaling 1 (SOCS1), Insulin-like growth factor 1 receptor (IGF1R), Ten-eleven translocation 2 (TET2), Glycogen synthase kinase 3β (GSK3β), B-cell lymphoma 2 (Bcl-2), Liver kinase b1 (LKB1)

4. The role of miRNAs in regulating mTOR signaling in cell immune responses

Cell responses such as apoptosis and autophagy are affected by the regulatory roles of miRNAs and their crosstalk with the mTOR signaling

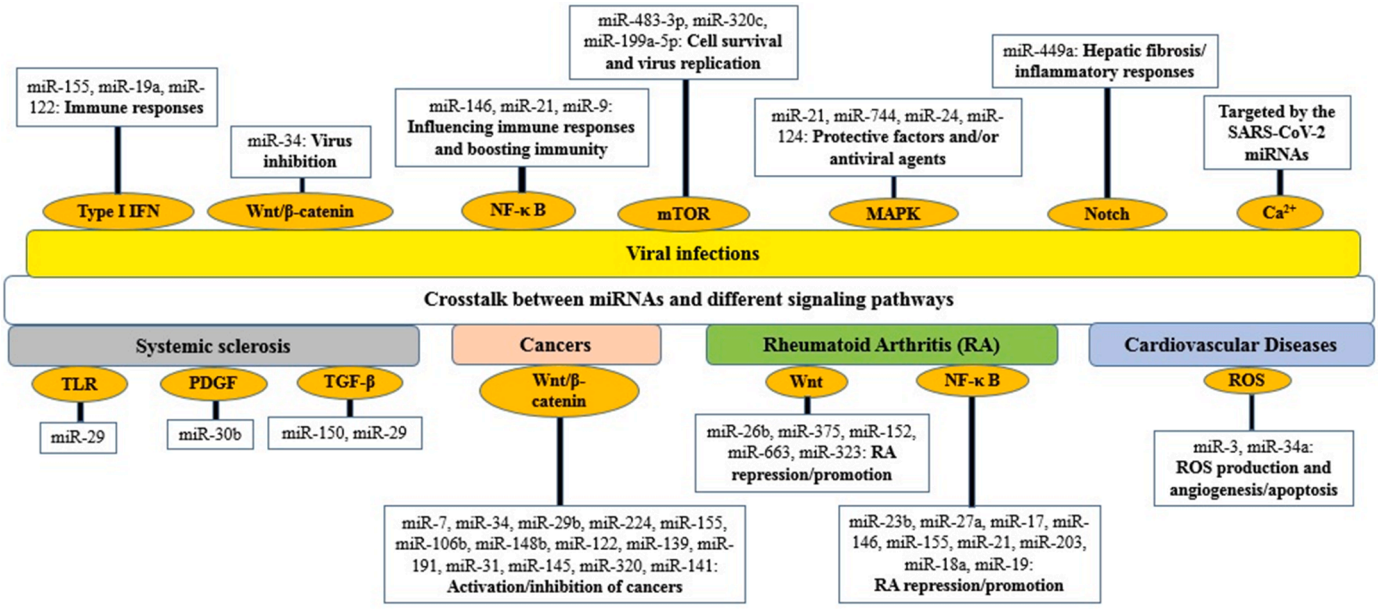

Fig. 1. Crosstalk between miRNAs and different signaling pathways in diseases (Toll-like receptors (TLRs), Platelet-derived growth factor (PDGF), Reactive oxygen species (ROS), Hepatitis C virus (HCV), Coxsackievirus B3 (CVB3), Calcium (Ca^{2+}), Interferon (IFN), Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)) (Barbu et al., 2020; Carbonell and Gomes, 2020; Hong et al., 2018; Khan et al., 2020; Li et al., 2015; Onyido et al., 2016; Rizkita and Astuti, 2021).

Table 1
Crosstalk between miRNA expression and the PI3K/AKT/mTOR signaling pathway in diseases.

miRNAs	Diseases/disorders	Cell signaling/pathway	Cell responses/actions	References
miR-100 and miR-146	Cancers (inducing metastasis)	Suppressing the expression of autophagy inhibitory effectors (mTOR and Bcl2)	Activation of pro-survival autophagy	Eng et al. (2019)
miR-21 and miR-93	Cancers (inducing metastasis)	Activating the PI3K-Akt-mTOR signaling cascade	Targeting PTEN and promoting the blockade of autophagy	Eng et al. (2019)
miR-144 and miR-451	Acute anemia	Represses the LKB1/AMPK/mTOR pathway	Promotes red cell precursor survival and increases apoptosis during recovery from acute anemia	Fang et al. (2018)
miR-138-5p (increasing the levels of EGFR by TRPM2-AS through sponging miR-138-3p)	NSCLC	Activates EGFR and PI3K/AKT signaling	Promotes cell proliferation, migration, and invasion of tumor cells (inhibits apoptosis)	Cui et al. (2020)
miR-199a-5p	Ankylosing spondylitis	Modulating mTOR through directly targeting Rheb	Induces autophagy and inhibits pathogenesis	Wang et al. (2017)
miR-375	Gastric cancer	By mediating AKT/mTOR	Inhibits proliferation and migration of tumor cells and autophagy	Yuan et al. (2018)
miR-146b	Prostate cancer	By targeting (activating) PTEN/AKT/mTOR	Inhibits autophagy	Gao et al. (2018)
miR-34a	Brain aging	- Activation of autophagy by upregulating SIRT1 and downregulating mTOR after treatment with Urolithin A	Induces apoptosis and inhibits autophagy	Chen et al. (2019)
miR-142-3p	Idiopathic pulmonary fibrosis	Protecting bleomycin- induced injury in lung epithelial cells through downregulation of Cox-2 and PI3K/AKT/mTOR activation	Decreases bleomycin-induced apoptosis	Guo et al. (2017)
miR-494	Ischemia/Reperfusion-induced cardiomyocyte	Through PI3K/AKT/mTOR and by targeting SIRT1(target gene of miR-494)	Suppresses hypoxia/reoxygenation-induced cardiomyocyte apoptosis and autophagy	Ning et al. (2020)
miR-15a and miR-16	Human cervical carcinoma	Through mTOR	Promotes drug chemosensitivity through inducing autophagy	Huang et al. (2015)
miR-376b, miR-129	Glioma	Promoting the mTOR signaling pathway	Develops the process of Glioma by autophagy induction	Chen et al. (2016b)
miR-155	Hypoxia	Downregulating mTOR	Attenuates cell proliferation and induces cell cycle arrest and autophagy	Wan et al. (2014)
miR-21	Bladder cancer	Inhibits PTEN (mTOR)	Inhibits beclin-1, caspase-3, and E-cadherin causing cell proliferation, invasion and migration, and inhibition of apoptosis	Zhang et al. (2020)
miR-1911-3p	Lung cancer	Decreases protein levels of both mEAK-7 and mTORC1 downstream effectors p-S6 and p-4E-BP1	Decreases cell proliferation and migration	Mendonça et al. (2020)
miR-27a	Osteoarthritis	Downregulation of PI3K-AKT-mTOR	Increased interleukin-1b (IL-1b)-induced apoptosis	Cai et al. (2019)
miR-1224-5p	Osteosarcoma	Targeting PLK1 Through PI3K/AKT/mTOR	Activates autophagy, cell invasion, inhibits the epithelial-to-mesenchymal transition	Jin et al. (2020)
miR-221	Hypoxia-reoxygenation injury	Disinhibiting the mTORC1/p-4EBP1 pathway)	Inhibition of autophagy (alterations in autophagic cell injury)	Chen et al. (2016a)
miR-155	HBV	Regulating SOCS1/Akt/mTOR axis (inhibits phosphorylated AKT and mTOR protein concentrations)	Reinforcing autophagy and increasing HBV replication	Chen et al. (2020)
miR-497 and miR-99a	Hepatocellular carcinoma	Suppressing IGF1R/mTOR signaling	Tumor suppressing	Cheng et al. (2017)
miR-660-5p	Breast cancer	Modulating TET2 and PI3K/AKT/mTOR	Tumor progression	Peng et al. (2020)
miR-199a	Cardiovascular diseases	Targeting GSK3β involving mTOR	Inhibits autophagy and induces cardiac hypertrophy	Samidurai et al. (2018)
miR-182		Targeting AKT/mTORC1	Regulates angiogenesis	

pathway in different diseases (Chong et al., 2021; Gozuacik et al., 2017; Nahand et al., 2021; Zhao et al., 2019). Table 1 illustrates some of these crucial cell responses. In addition, several pieces of evidence highlight the direct and indirect interactions between regulatory miRNAs and mTOR signaling and its downstream component cascades in the different types of immune cells described in innate and adaptive immunity responses (Gagnon and Ansel, 2019; Nazari et al., 2021). It has been shown that the miR-126-mTOR-vascular endothelial growth factor receptor 2 (VEGFR2) axis plays an important role in plasmacytoid dendritic cell (pDCs) homeostasis and function. miR-21 is also a key positive regulator for the potential production and secretion IFN-α and IFN-λ by pDCs and the promotion of cell defense against viral infections. miRNAs also affect the interpretation of T cell antigen receptor (TCR) signaling by regulating the expression patterns of mTOR signaling in T cells. Furthermore, the downregulation of miR-633 activated the mTOR pathway and increased IL-4, IL-17, and IFN-γ levels in $CD4^+$ T cells in systemic lupus erythematosus. Additionally, miRNAs regulate the development and functions of other $CD4^+$ cell subtypes such as regulatory T cells (Tregs) and T helper17 (Th17) cells, as well as the Treg/Th17 balance by controlling mTOR expression as a negative regulator of Treg and also a positive regulator of Th17 differentiation (Nazari et al., 2021).

Since immune cell differentiation and activation are affected by mTOR, the aforementioned crosstalk crucially induces immune response regulation. Therefore, the identification of miRNAs targeting mTOR signaling is essential to determine different cell responses, especially immune responses and inflammation, in different infections and disorders. Consequently, such miRNAs could be used as potential therapeutic

targets, for instance in immunotherapy-based approaches, for the treatment or management of different diseases.

5. miRNA expression and mTOR signaling in *Leishmania*-infected cell interactions

miRNAs exert crucial functions in innate and acquired immunity, regulating macrophages and DCs, including differentiation, activation, polarization, maturation, response to infection, tolerance, inflammation, immune memory, and wound repair and regeneration. They can also directly modulate lineage-determining transcription factors (LDTFs) and signal-dependent transcription factor (SDTFs) expression or indirectly control transcription factor (TFs) activities, both of which are exploited by some intracellular pathogens such as *Leishmania* parasites (Lecoeur et al., 2021; Rashidi et al., 2021a). On the other hand, the survival, propagation, and the pathogenic progress of intracellular pathogens including protozoan parasites are crucially dependent on the alteration of host cell signaling and the relevant pathways such as mTOR and other vital cell responses. Furthermore, mTOR exerts critical functions in regulating the development and differentiation of cell subsets of both innate and adaptive immune responses. Therefore, intercellular pathogens and parasites use complicated strategies to target the metabolism of immune cells to alter potential deleterious immune responses acting on the metabolic pathways controlled by mTOR during pathogen-host interactions (Rashidi et al., 2021c). Overall, intercellular pathogens employ different strategies to exploit the infected-host cell conditions during infection and to change/manage host cell responses (such as mTOR) and biomolecules (such as miRNAs) to favor the persistence of pathogenesis (Madhry et al., 2021; Rashidi et al., 2021c).

Leishmania parasites are vector-borne protozoa targeting mainly host macrophages thus leading to the disease called Leishmaniasis with different clinical manifestations including cutaneous, mucocutanous and visceral forms (Rashidi et al., 2021a, 2018). Recently, the modulation of miRNA expression has been comprehensively investigated in *Leishmania* infection (Rashidi et al., 2021b; Soares et al., 2021). It was revealed that the up-regulation of several miRNAs including miR-15a, miR-21, miR-24-3p, miR-30e-5p, miR-148a, miR-194, miR-294-3p, miR-302d-3p, miR-615, miR-210, miR-155, let-7a, miR-346, miR-721, miR-371 and miR-466i, and the down-regulation of miR-763, miR-1264, miR-494, miR-3473f and miR-122, correlated with the increase of parasite survival, replication and infectivity (Rashidi et al., 2021b). This information highlights the importance of further studies on the influence of host miRNAs in the pathogenicity of *Leishmania* parasites in infected cells. Moreover, this research needs to be performed at different molecular levels and with different networks to find essential miRNA-associated points.

The host-mTOR pathway and its correlation with parasitic disease pathogenesis especially intracellular parasites including *Leishmania, Toxoplasma* and *Plasmodium* has been recently described (Rashidi et al., 2021c). It has been shown that, the activation of mTOR signaling in *Leishmania*-infected cells led to the control of IL-12/IL-10 expression levels and also up-regulated the chemokine (C-X-C motif) ligand 16 (CXCL16) (a factor related to parasite propagation). In addition, the mTOR activation seems to alter (decrease or increase) the expression of M1 and M2 macrophage markers, respectively. Furthermore, it has been revealed that infection induced mTOR to differentially activate or inhibit autophagy (as a crucial cell response associated with mTOR) through the successful progression of the *Leishmania*-infection of target cells. mTOR inhibition could also occur through the parasite glycoprotein 63 (GP63) virulence factor by inducing the suppression of host specific mRNA translation thus increasing parasite proliferation.

The abovementioned data described host cell-specific miRNAs, modulated in parasite-infected host cells, as fine pathogen infectivity modulators (He et al., 2020; Judice et al., 2016; Paul et al., 2020; Rashidi et al., 2021b). In addition, it seems that host mTOR, as a crucial master regulator of cell signaling, exerts tangible effects on the modulation of pathogenicity during parasitic infections. The relevance of the differential expression of regulatory miRNAs for several signaling pathways including Hippo, TGF-β, FoxO, fatty acid biosynthesis, ErbB, apoptosis, adenosine 3′,5′-cyclic monophosphate (cAMP), MAPK, Ras, AMPK, as well as PI3K-Akt, has been described in *Leishmania* infections. However, the information on miRNAs and the relevant mechanisms employed by such biomolecules to target signaling remains unclear (Nimsarkar et al., 2020). The discovery of specific miRNAs with highly specialized roles linked to cellular signaling/responses (such as mTOR) might lead to novel insights into the molecular biology of parasite-hosts interactions and consequently the better management of parasitic diseases. Accordingly, some of these associations and existing gaps are discussed below,with a specific focus on *Leishmania* parasites.

6. The interplay of miRNAs and mTOR signaling in *Leishmania* infection

The modulation of miR-548d-3p in *Leishmania* infection leads to interference with chemokine production and inflammatory processes towards increasing parasite pathogenesis (Souza et al., 2021). Several regulatory miRNAs including miR-7a-1-3p, miR-690, miR-6994-5p, miR-574-5p and miR-7235-5p have been also identified as biomolecules suppressing transcription factors involved in the differentiation of naive $CD4^+$ T cells into the Th1 phenotype in *Leishmania* infection. The pathway enrichment analysis of modulatory miRNAs in cell cultures infected with *L. donovani* revealed the involvement of some important pathways such as mTOR in $CD4^+$ T cell biology (Kumar et al., 2020). Given the crucial roles of mTOR signaling in regulating immune B and T cells and its downstream signals and cytokines, the function of miRNAs in orchestrating mTOR signaling in *Leishmania* infection should be also considered (Iwata et al., 2017; Nazari et al., 2021).

It has been revealed that the management and control of the monocytopoiesis and the differentiation of macrophages significantly influence the outcomes of leishmaniasis (Raybarman and Bhattacharjee, 2021). The epigenetic alterations such as the histone modifications of genes related to pro-inflammatory cytokines and genes of the mTOR pathway could be involved in the regulation of such phenomena in the pathogenesis of *Leishmania* parasites and host immune responses. Moreover, the process of monocyte processing is also affected by miRNAs due to the role of these non-coding RNAs in the regulation and control of gene expression, differentiation and monocyte maturation. Therefore, further investigation into crosstalk between miRNAs and mTOR might reveal more details regarding monocytopoiesis and other important cell responses during leishmaniasis and other parasitic infections.

The autophagic digestion of *Leishmania* parasites in infected-host cells could be dependent or independent of the mTOR pathway throughout the different stages of infection. Several mice miRNAs including mmu-miR-101c, mmu-miR-210-5p, mmu-miR-129-5p have been recognized as regulators of mTOR-regulated autophagy during *Leishmania* infections (Frank et al., 2015; Thomas et al., 2018). Although, the underlying mechanisms were unclear, the investigation of more interactions at the molecular level between miRNAs and mTOR signaling in the regulation of autophagy or other possible cell responses could provide further useful information on parasitic infections (López-Rosas et al., 2019).

Furthermore, Leishmania parasites could induce a hypoxic environment inside the infected macrophages which activates hypoxia-inducible factor-1 alpha (HIF-1α). Consequently, HIF-1α upregulates miR-210 that provides favorable conditions for parasite survival inside infected cells by downregulating NF-κB-mediated pro-inflammatory immune responses (Kumar et al., 2018b). On the other hand, HIF-1α has been described as a host-protective factor against *Leishmania* infection. The absence of this factor leads to greater susceptibility to infection through the modulation/activation of the BNIP3 (BCL2 interacting protein 3)/mTOR/SREBP-1c (sterol regulatory element binding protein)

axis. Moreover, *HIF1α* polymorphisms are associated with susceptibility to infection. In line with this, it has been observed that single-nucleotide polymorphism (SNPs) are localized in the *HIF1α* gene and might be under the control of some miRNAs such as miR-199a thus leading to the suppression of *HIF-1α* transcription. Therefore, the genetic variations and *HIF1α* polymorphisms may be implicated in *Leishmania* growth in macrophages during infection and such a process might be indirectly or directly linked to the miRNA expression and mTOR signaling. However, the exact nature of the crosstalk between such signaling and biomolecules remains unknown (Mesquita et al., 2020).

The dynamic interchanges between M1 and M2 macrophages can significantly modulate leishmaniasis outcomes (Tomiotto-Pellissier et al., 2018). *Leishmania* infection triggers mTOR, as well as other signaling and pathways, facilitating the M2 polarization of macrophages (Das et al., 2021; Kumar et al., 2018a). Interestingly, the enrichment of miR146a-5p has been reported during leishmaniasis both "*in vivo*" and "*in vitro*". It seems that the expression of miR146a is regulated by the BET bromodomain 4 (BRD4)/p300-dependent super-enhancer during *Leishmania* infection and this miRNA down regulates tumor necrosis factor (TNF) receptor associated factor 6 (TRAF6), IL-1 receptor--associated kinase 1 (IRAK1) and inducible nitric oxide synthase (iNOS) which facilitates M2 polarization (Das et al., 2021). Consequently, due to the involvement of mTOR in the M2 polarization of macrophages, the investigation of the expression of other miRNAs in this process and their possible crosstalk with mTOR could be attractive and reveal more details regarding parasite pathogenicity and host immune responses in *Leishmania* infection.

Arginine competition between *Leishmania* parasites and their infected host cells is a metabolically relevant process during infection and thereby arginine sensing responses and the relevant biomolecules and pathways are crucial in the management of parasite pathogenesis (Goldman-Pinkovich et al., 2020). Recently, a functional interaction between SLC38A9 (the uncharacterized human member 9 of the solute carrier family 38), as a host-arginine-activated amino acid transporter, and amino acid permease 3 (AAP3), as a parasite arginine transporter, has been identified in *Leishmania*-infected cells. The upregulation of SLC38A9 is essential to increase and maintain high levels of AAP3 from the parasite during the infection. Therefore, the *Leishmania* parasite activates SLC38A9 arginine sensing in infected host cells through mTOR activation in conditions of arginine deprivation (Goldman-Pinkovich et al., 2016; Madan et al., 2021; Pawar et al., 2019). In addition, it has been shown that miRNA regulation in *Leishmania*-infected cells is crucially controlled by parasite arginase (an enzyme that uses L-arginine to produce ornithine and urea) (Muxel et al., 2017). L-arginine availability is required for the activation of the microbicidal response in *Leishmania*-infected host cells. Thus, the host and parasite consumption of this amino acid by arginase enzymes could change its accessibility for the activation of host responses during the infection. For instance, L-arginine deprivation leads to the suppression of T cell responses. miR-122 repression of the cationic amino acid transporter 1 (CAT1) can also regulate the availability of intracellular L-arginine. Furthermore, miR-155 suppresses arginase 2 expression in DC, inhibiting L-arginine depletion and leading to the activation of T cells (Acuña et al., 2020). The role of mTOR signaling in the arginine sensing process in infected host cells and the implication of regulatory miRNAs in L-arginine availability and in the expression of arginase enzyme in immune cells might suggest a possible correlation between host immune responses and arginine pathways, modulated by the interplay of mTOR and miRNAs during *Leishmania* infection. More investigations are required to clearly identify such networks and downstream/related components.

It has been shown that *Leishmania*-infected macrophages activate the PI3K/AKT pathway (Ruhland et al., 2007) which is probably partially regulated by miR-155 in TLR-stimulated macrophages (O'Connell et al., 2008). Additionally, *Leishmania*-infected macrophages increased the activation of the AKT-dependent pro-survival signaling pathway, and this activation process was amplified and prolonged in the presence of Leishmania RNA virus 1 (LRV1). Thus, miR-155 is involved in the TLR-3 mediated activation of the pro-survival PI3K/AKT signaling in LRV1^{+} *Leishmania*-infected cells. Accordingly, it could be concluded that LRV1^{+} *Leishmania*-induced macrophage survival via the TLR-3/AKT axis is partially dependent on miR-155 (Eren et al., 2016). This information further highlights the importance of crosstalk between mTOR signaling and miRNAs especially in LRV1^{+} *Leishmania*-infected cells.

7. The interplay of miRNAs and mTOR in other parasites

During the early stage of murine alveolar echinococcosis, the hepatic miRNA transcriptome is altered. Both mmu-miR-15a-5p and mmu-miR-101a/b-3p, are negative regulators of mTOR signaling which are downregulated in the AE-infected liver thus increasing mRNA expression of *mTOR*. mTOR was reported as a regulator linking inflammation to angiogenesis via activation of TNFα/IκB kinase β (TNFα/IKKβ) signaling, which in turn led to the production of the extracellular matrix remodeling enzyme (Boubaker et al., 2020). In another study, miR-71-5p (expressed in germinative cells and in other cells of the germinal layer in *Echinococcus multilocularis* metacestodes) has been detected as the regulator of mTOR in *E. multilocularis* infection (Macchiaroli et al., 2021). In addition, monocytic-type myeloid-derived suppressor cells (M-MDSCs) have an angiogenic role during *E. granulosus* infection. In this sense, differentially/modulatory miRNAs expressed in the infected M-MDSCs might affect the immune response and angiogenesis via the regulation of several signaling pathways such as PI3K/AKT/mTOR and/or the MAPK. These pathways can increase the secretion of vascular endothelial growth factor (VEGF), which is the most crucial pro-angiogenic factor (Yin et al., 2018).

The identification of the miRNA profile of *Toxoplasma gondii*-infected porcine alveolar macrophages indicated that miR-127 and miR-143-3p regulate nitric oxide synthase 1 (NOS1) and NOS3, respectively, both of which exert important functions in synthesizing nitric oxide (NO) by oxidizing L-arginine. These genes are targeted by the aforementioned miRNAs being involved in several signaling (all of which were crucial for the normal activity of macrophages) including mTOR (Macchiaroli et al., 2021).

Although the focus of this review was to highlight the crosstalk between miRNAs expressed in the infected host cells and host-mTOR signaling, several miRNAs have also been identified in parasites to target host cell signaling such as mTOR. For instance, Let-7 (as a regulator for developmental timing in *Caenorhabditis elegans*) was found to be a main regulator of mammalian glucose metabolism through targeting different genes of insulin-PI3K-mTOR signaling such as the insulin receptor (Gutierrez-Loli et al., 2017). Furthermore, mTOR was detected as providing enriched signaling for target genes of miR-235. This miRNA suppresses the metabolic and transcription factor activity essential for Haemonchus contortus development from the L3 to the L4 stage (Marks et al., 2019).

8. Extracellular/secretory (circulating) miRNAs and mTOR in parasitic infections

miRNAs can be also secreted into extracellular fluids and transported to their target cells through vesicles (exosomes) or by binding proteins (Argonautes). miRNA expression in vesicles or their combination with proteins increased the protection and stability of extracellular miRNAs in the extracellular milieu. Although the mechanisms of uptake/action of such miRNAs remain unknown, some, including vesicle-associated extracellular miRNAs, probably enter cells via phagocytosis, endocytosis, or direct fusion with the cell membranes. Meanwhile vesicle-free secreted miRNAs could be taken up through specific receptors on the cells. Overall, extracellular/secretory miRNAs have been identified as potential biomarkers, as well as intracellular/cytosolic acting miRNAs, for different disorders such as parasitic diseases. Extracellular/secretory miRNAs have also been suggested as master signaling molecules to

mediate various cell-cell communications (both intercellular and extracellular communications) (O'Brien et al., 2018).

The EVs secreted from helminths contain miRNAs able to regulate the host immune system through different strategies and pathways (Ancarola et al., 2017; Hansen et al., 2019; Mu et al., 2021; Nicolao et al., 2019). The microfilarial stage of *B. malayi* inhibits mTOR in human DC and this process was correlated with the DC dysfunction observed in filarial infections. Further investigations clarified that several miRNAs (miR-7, miR-9, miR-34, let-7, miR-71, miR-99, miR-100, miR-92, miR-31, and miR-4299) were expressed in the microfilarial-EVs of *B. malayi* target genes associated with the mTOR signaling cascade. miR-100 in microfilarial-EVs targeted mTOR (and also its downstream regulatory protein 4E-BP1) and downregulates mTOR phosphorylation. These results suggest that microfilarial-derived EVs (containing regulatory miRNAs) are important effectors in the immune modulation and reprograming induced by filarial infections (Narasimhan et al., 2016; Ricciardi et al., 2021). These data highlight that excretory/secretory products including helminth EVs could be one of the most important strategies used by the parasites to manipulate the different barriers of the immune system (Drurey and Maizels, 2021). Thus, future research should focus on the miRNAs expressed in EVs as potential regulators of host immune responses in parasitic infections.

9. Conclusions

miRNAs have been described as effective regulators of metabolic reprogramming and inflammatory responses in several immune cells, exerting crucial functions in the regulation of different cellular processes and signaling in different disorders (Virga et al., 2021). The next-generation sequencing platforms for miRNA profiling have the potential to uncover novel miRNAs and to corroborate known miRNAs involved in diseases (Onyido et al., 2016). In addition, the identification of functional miRNAs (especially relevant to cell signaling) could also lead to the characterization of several significant target genes simultaneously modulated by miRNAs. Therefore, miRNA expression profiling and further identification of noteworthy targets in cell signaling could be potentially useful for the evaluation of disease phenotype, progression, or treatment responses. Accordingly, miRNAs have been suggested as potential therapeutic targets to control various disorders such as parasitic infections (Hong et al., 2018).

Currently, there is growing evidence showing that *Leishmania*-infected host cells activate key signaling pathways regulated by miR-155, miR-21 and other miRs. On the other hand, such high levels of miR-155 or miR-21 correlate with the increase of parasite survival, replication and infectivity (Rashidi et al., 2021b). However, the (direct or indirect) molecular mechanisms of miRNAs-mTOR interactions are unclear in *Leishmania* parasites and this remains an interesting field of study. In fact, further investigation into the crosstalk between miRNAs and mTOR pathways might shed some light on monocytopoiesis, on the regulation of autophagy as well as cell responses during leishmaniasis and other parasitic infections. In addition, based on the implication of mTOR pathway in the M2 polarization of macrophages, in the regulation of immune (B and T) cells, in the modulation of downstream signals and cytokines during *Leishmania* infection, miRNAs might play a role in the activation/inhibition of mTOR signaling. Therefore, since the exact nature of miRNAs and the mechanisms involved in targeting relevant signaling is still unknown, several issues including those dealt with above need to be clarified to fully understand certain biological processes.

Although the crosstalk between miRNAs and the mTOR pathway appears to be context dependent, it could be also employed as a potential therapeutic target for the management of different diseases. Targeting miRNA-modulated cell responses and signaling pathways (such as mTOR) might lead to novel therapies. A number of novel pharmacological platforms including small molecules, locked nucleic acid technology, and RNA-based therapeutics (antisense, aptamers, small interfering RNA (siRNAs), miRNA mimics/anti-miRs and synthetic mRNA) seem suitable to this end (Bajan and Hutvagner, 2020; Eng et al., 2019; Qu et al., 2019; Sevgin and Sevgin, 2021). However, the complexity of overlapping signaling networks highlights the need for detailed molecular investigations to confirm and suggest key regulatory miRNAs as therapeutic targets. Additionally, mTOR-mediated translational control and miRNA-mediated posttranscriptional regulation/modification of gene expression could affect the expression of various biomolecules involved in different cell responses and be targeted for the reduction or enhancement of the pathogenesis of diseases.

We here offer some insights into the crosstalk between miRNAs and the mTOR signaling pathway in leishmaniasis and other parasitic infections. Further studies on the miRNA/mTOR signaling network in different parasites and the role of miRNAs in other key signaling networks implicated in parasitic infections are required to fully understand the pathobiology of parasites especially in infected host cells, all of which may lead to the better management and elimination of the parasitic infections.

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CRediT authorship contribution statement

Sajad Rashidi: Conceptualization, Writing – original draft, Writing – review & editing. **Reza Mansouri:** Writing – review & editing. **Mohammad Ali-Hassanzadeh:** Writing – review & editing. **Esmaeel Ghani:** Writing – review & editing. **Mohammadreza Karimazar:** Writing – review & editing. **Antonio Muro:** Writing – review & editing. **Paul Nguewa:** Supervision, Writing – review & editing. **Raúl Manzano-Román:** Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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