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Leishmania Regulated MTDH Expression to Suppress Dendritic Cells

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This study aimed to investigate the correlation between *Leishmania* infection and dendritic cell infiltration and explore the underlying molecular mechanism how *Leishmania* infection regulates dendritic cell infiltration. Three datasets, GSE63931, GSE80008 and GSE77528 were combined and their batch effects were removed by Combat function in sva R package. Immune cell infiltrations were estimated using the Microenvironment Cell Populations-counter (MCP-counter) R package. Statistical results were verified by Student's *t* test. The differential expression of metadherin (MTDH) was identified by Limma R package. The correlation between MTDH expression and dendritic cell infiltration was estimated by Pearson's product moment correlation coefficient. GDS5086 was used to explore MTDH expression pattern in dendritic cells infected with *Leishmania*. Compared with normal samples, 5 types of immune cells showed differential infiltration in leishmaniasis samples, including T cells, $CD8^+$ T cells, dendritic cells, cytotoxic lymphocytes and B lineage cells. Among these, only DCs were significantly suppressed in leishmaniasis samples. Notably, MTDH expression was differential between leishmaniasis and normal samples. There was a significant correlation between MTDH expression and dendritic cell infiltration. In conclusion, these results demonstrate that *Leishmania* infection leads to the downregulation of MTDH expression and the suppression of dendritic cell infiltration.

Keywords: dendritic cell; gene expression; infiltration; *Leishmania*; MTDH
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Introduction

Leishmania species are protozoan parasites transmitted to human by phlebotomine sandflies and are the causative agents of leishmaniasis, a complex of vector-borne diseases (Murray et al. 2005; Chappuis et al. 2007). Leishmaniasis affects approximately 20 million people in 98 countries in two main forms, visceral leishmaniasis and cutaneous leishmaniasis (Gutierrez-Rebolledo et al. 2017). Visceral leishmaniasis can be lethal if untreated, while cutaneous leishmaniasis is characterized by chronic evolution, which affects the skin and cartilaginous structures (Goto and Lindoso 2010). Genetic diversity of *Leishmania* species results in the difficulty of controlling the disease and the increasing number of cases that are resistant to conventional treatment (Guerin et al. 2002). Therefore, it is of great sig-

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nificance to explore the pathogenesis of *Leishmania*.

In human immune system, dendritic cells (DCs) play an essential role in killing pathogenic microorganisms. DCs are a family of professional antigen presenting cells (APCs) and present antigens to naive T cells and modulate their responses (Reis e Sousa 2004). Previous reports showed the role of DCs in orchestrating immune responses in leishmaniasis. DCs are one of the principal host cells of *Leishmania* (Rodriguez-Gonzalez et al. 2016). During *Leishmania* infection, monocytes are recruited, migrate into the draining lymph nodes (LN), and finally differentiate into "lymph node monocyte-derived DCs", which control the induction of protective T helper 1 response against *Leishmania* (Leon et al. 2007). In response to pathogens which fail to trigger IL-12 production by macrophages, DC-T cells provide microenvironment for initial NK cell activation (Gorak et al. 1998). Skin DCs are also induced to express increased amounts of the major histocompatibility complex (MHC) antigens and costimulatory molecules and release cytokines to initiate protective T helper cell type 1 immunity (von Stebut et al. 1998). All these findings suggest that DCs are essential in the immunity against pathogens.

Metadherin (MTDH), also called Astrocyte elevated gene-1 (AEG-1), is a key player in tumor progression, including transformation, evasion of apoptosis, invasion, metastasis, and chemoresistance (Sarkar et al. 2009; Hu et al. 2009; Liu et al. 2009). However, the role of MTDH in other pathological processes remains poorly understood. Recently, bioinformatics methods have been employed to explore the data of gene expression profiles and elucidate molecular mechanism of physiological and pathological processes. Therefore, in this study we aimed to perform bioinformatics analysis to reveal the mechanism how MTDH plays a role in the link between *Leishmania* infection and DCs.

Materials and Methods

Immune cell analysis

GSE63931 (16 samples containing 8 patients infected with *Leishmania* and 8 healthy donors), GSE80008 (30 samples containing 18 patients infected with *Leishmania* and 12 healthy donors) and GSE77528 (23 samples containing 8 infected with *Leishmania* and 15 healthy donors) were obtained from GEO database (https://www.ncbi.nlm.nih.gov/gds/) (Oliveira et al. 2015; Gardinassi et al. 2016; Vivarini et al. 2017). The three datasets were combined and their batch effects were removed by Combat function in sva R package (Wang et al. 2021). Immune cell infiltrations were estimated using the Microenvironment Cell Populations-counter (MCP-counter) R package (Becht et al. 2016) and subjected to statistical analysis using Student's *t* test.

WGCNA analysis

GDS5086 (3 repeated experiments in dendritic cells for up to 24 hours following infection with *Leishmania*) (Favila et al. 2014) was obtained from GEO database to explore MTDH expression patterns in DCs infected with *Leishmania*. To analyze the gene expression landscape associated with *Leishmania* infection, co-expressed genes were identified by weighted gene co-expression network analysis (WGCNA) (Langfelder and Horvath 2008). All correlation analyses in this study were performed by using Pearson's product moment correlation coefficient. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed by clusterProfiler R (p-adj < 0.01 and Q-value < 0.01) and visualized by pathview R for the co-expression module (Yu et al. 2012; Shen et al. 2020).

Results

Immune cell infiltration

GSE63931, GSE80008 and GSE77528 were combined and their batch effects were removed by sva R. The result showed a comparable difference between leishmaniasis (Lei) and normal (Nor) samples (Fig. 1).

Immune cell infiltrations were estimated by MCP-counter between normal (Nor) and leishmaniasis (Lei) samples (Fig. 2). There were 5 types of immune cells presenting differentially infiltration between the two sample groups, including T cell, DC, cytotoxic lymphocyte, $CD8^+$ T cell and B lineage cell. We analyzed whether each immune cell type was upregulated (activated) or downregulated (suppressed) in Lei samples compared with Nor samples, and only DCs were significantly suppressed in leishmaniasis samples compared with normal group.

Correlation between MTDH and DCs infected with Leishmania

GDS5086, in which gene expression was detected in DCs infected with *Leishmania* for up to 24 hours, was used to analyze the specific gene expression pattern. MTDH expression was downregulated after *Leishmania* infection (Fig. 3A). The Topological Overlap Matrix (TOM) for all genes was analyzed to investigate *Leishmania* specific gene modules (Fig. 3B). WGCNA was applied to select the consensus gene modules which showed negative correlation with *Leishmania* infection time (Fig. 4). Blue ($r = -0.52$, $p = 0.05$), white ($r = -0.45$, $p = 0.09$) and green ($r = -0.46$, $p = 0.08$) gene modules (with black arrows) were chosen due to significant negative correlation. KEGG pathway analysis showed a *Leishmania* pathway with 6 downregulated genes (Fig. 5). Correlational analyses were performed to detect the correlations between MTDH and the module genes in this pathway, including *NFKBIA*, *TNF*, *JUN*, *IL1B*, *PTGS2* and *IL1A*. We found significant correlation between MTDH and all these genes, especially for *NFKBIA* ($p = 0.011$, $r = -0.63$) and *IL1B* ($p = 0.044$, $r = -0.53$) (Fig. 6).

Discussion

Despite high prevalence of leishmaniasis, there are only several treatments available for *Leishmania*, and drug

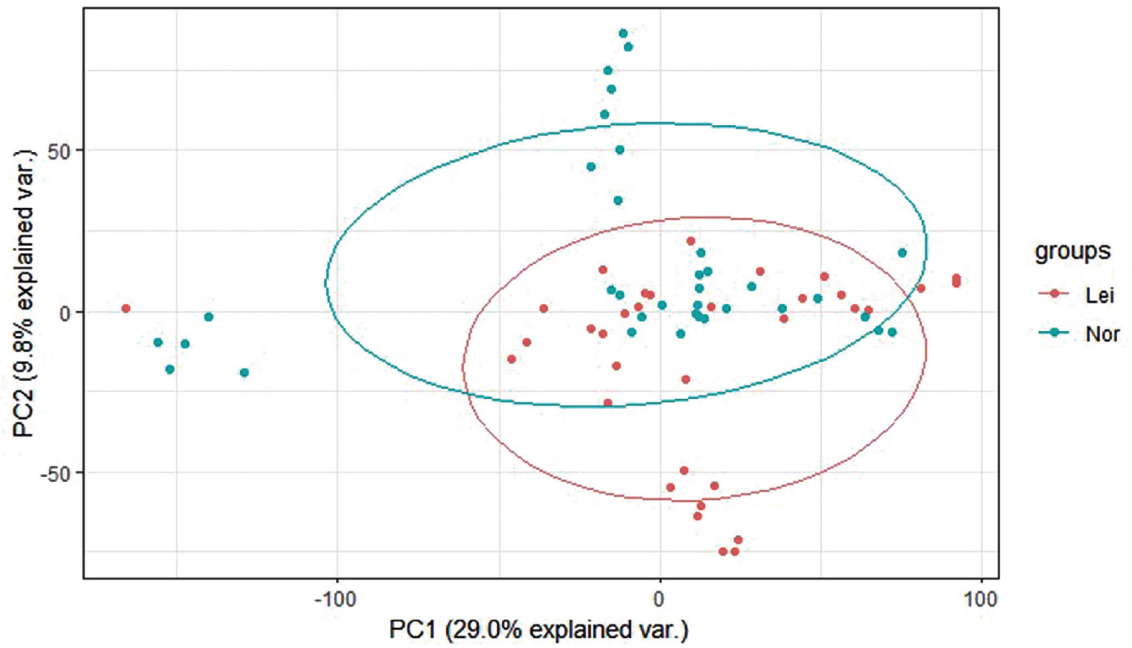

Fig. 1. Principal component analysis (PCA) for leishmaniasis (Lei) and normal (Nor) samples. The results showed a comparable difference between Lei and Nor.

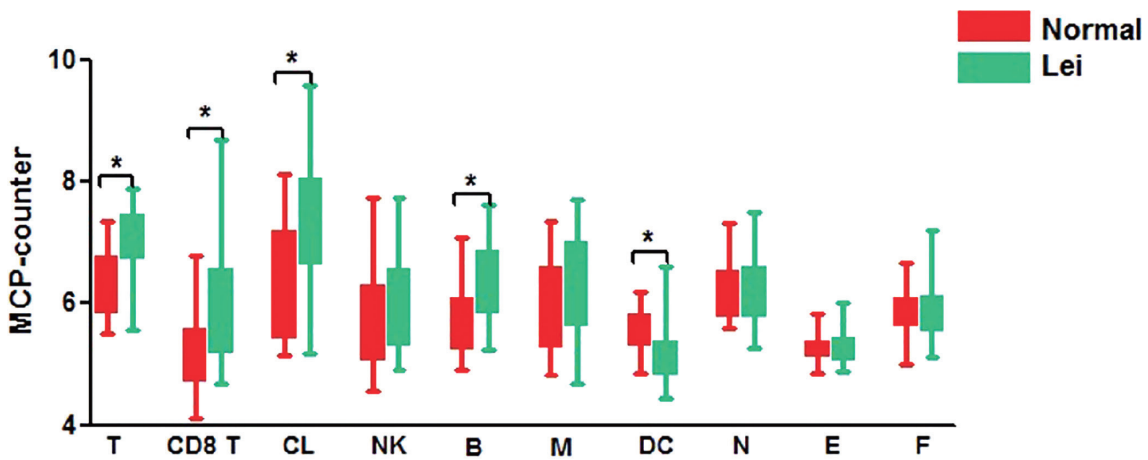

Fig. 2. Estimation of immune cells infiltrations in Lei and Nor samples.
There were 5 types of immune cells differentially infiltrated between leishmaniasis and normal samples, including T cells (T) (p = 2.11E-08), $CD8^+$ T cells (CD8 T) (p = 1.58E-04), myeloid dendritic cells (DCs) (p = 5.83E-06), cytotoxic lymphocytes (CL) (p = 1.33E-05) and B lineage cells (B) (p = 3.27E-04). $*p < 0.05$
NK, NK cells; M, monocytic lineage cells; N, neutrophil granulocytes; E, endothelial cells; F, fibroblasts.

resistance is a fundamental determinant of treatment failure (Ponte-Sucre et al. 2017; Varikuti et al. 2018). Immunotherapies have a major impact on oncology and human health (Chaudhuri et al. 2021; Dai et al. 2021). MTDH is involved in cancer and becomes an attractive target for cancer immunotherapy (Dhiman et al. 2016). We reported that MTDH promoted the invasion and proliferation of glioma cells (Tong et al. 2017). Moreover, MTDH overexpression was associated with macrophage activation in hepatocellular carcinoma (Robertson et al. 2018). In this study, we investigated the correlation of MTDH expression with *Leishmania* infection, and our results suggest that *Leishmania* may inhibit DC infiltration through the down-regulation of MTDH expression (Fig. 7). However, our speculation is based on bioinformatics analysis and further experimental studies are needed to validate the model proposed in Fig. 7.

In order to avoid destruction by the immune system, *Leishmania* hides inside the host cells. DCs are one of the principal host cells of *Leishmania* (Rodriguez-Gonzalez et al. 2016). *Leishmania* uses lipophosphoglycan to promote its survival in DCs, preventing natural killer T cells from recognizing the infected DCs. Therefore, *Leishmania* multiplies rapidly in DCs, leading to the death of host cells eventually. After DCs are destroyed, free *Leishmania* amastigotes move into new host cells.

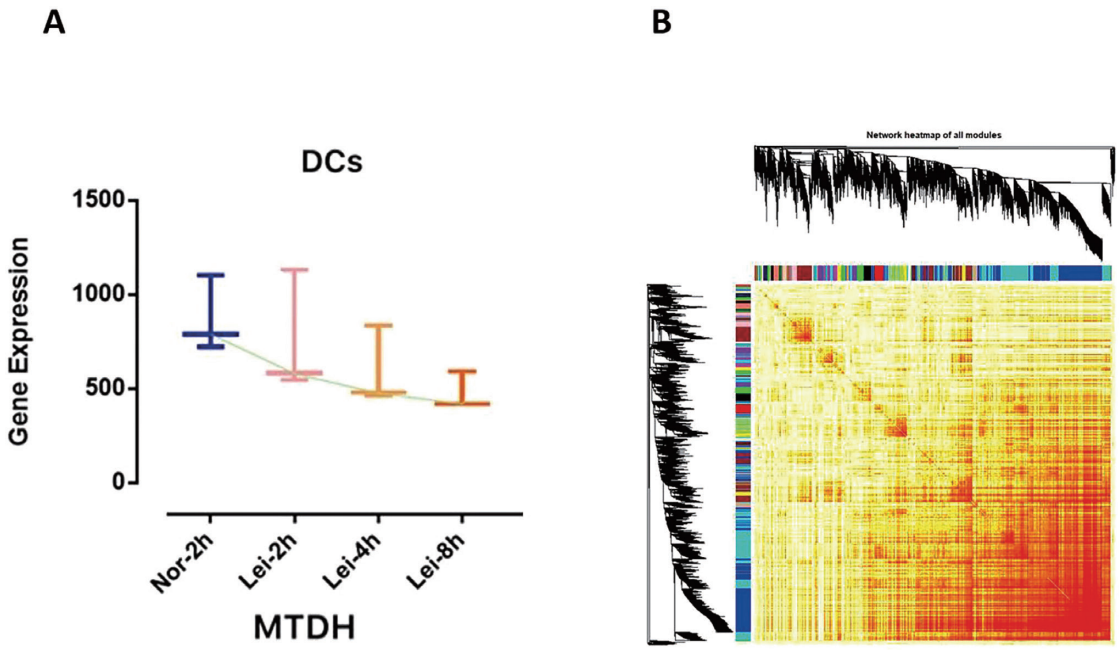

Fig. 3. Gene expression network in dendritic cells (DCs).
A. MTDH expression pattern in DCs infection. B. Heat map depicted the Topological Overlap Matrix (TOM) for all genes analyzed. Light to progressively darker red color indicated increasing overlap. Blocks of darker color along the diagonal were modules. Gene dendrogram and module assignment were shown at left and above, respectively.

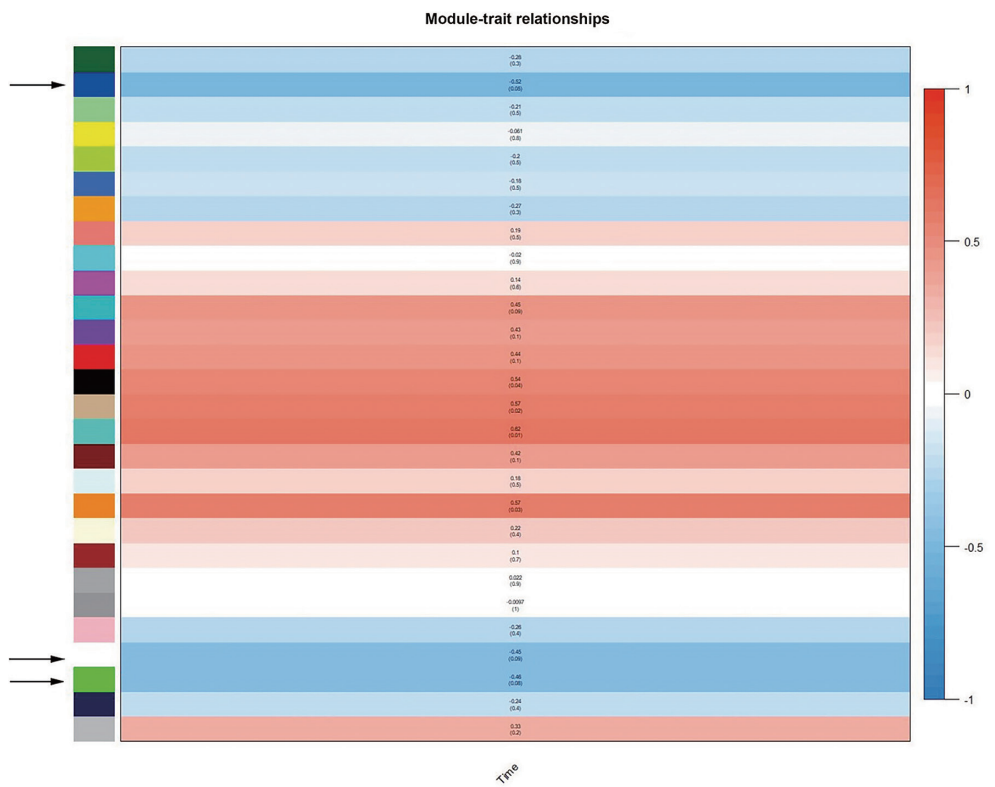

Fig. 4. Consensus module and clinical trait relationships.
Each row in the table corresponded to a consensus module. Column indicated infection times. Each cell contained corresponding correlation and p-value. Table was color-coded to indicate correlation (according to color legend). Blue ($r = -0.52$, $p = 0.05$), white ($r = -0.45$, $p = 0.09$) and green ($r = -0.46$, $p = 0.08$) modules with arrows showed most significantly negative correlations.

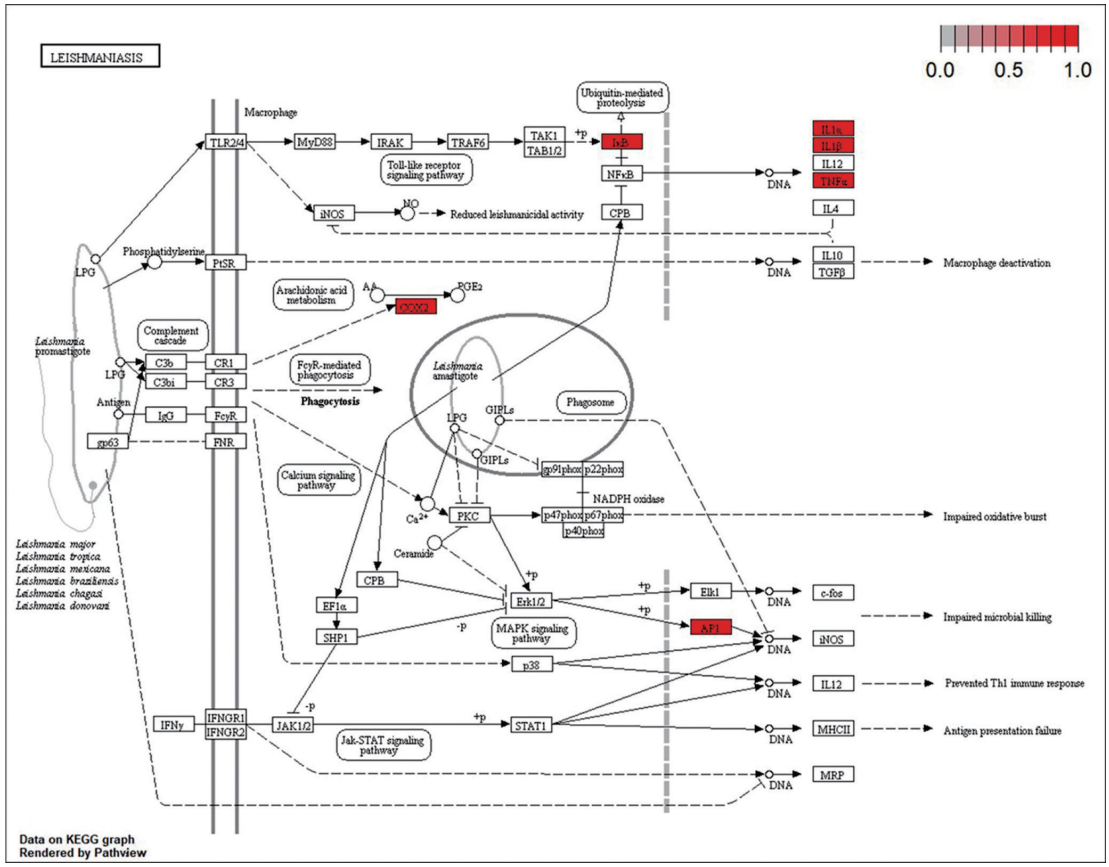

Fig. 5. Functional analysis based on differentially expressed genes.
Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichments were applied to predict the functional pathways. A *Leishmania* pathway with 6 downregulated genes was found (red).

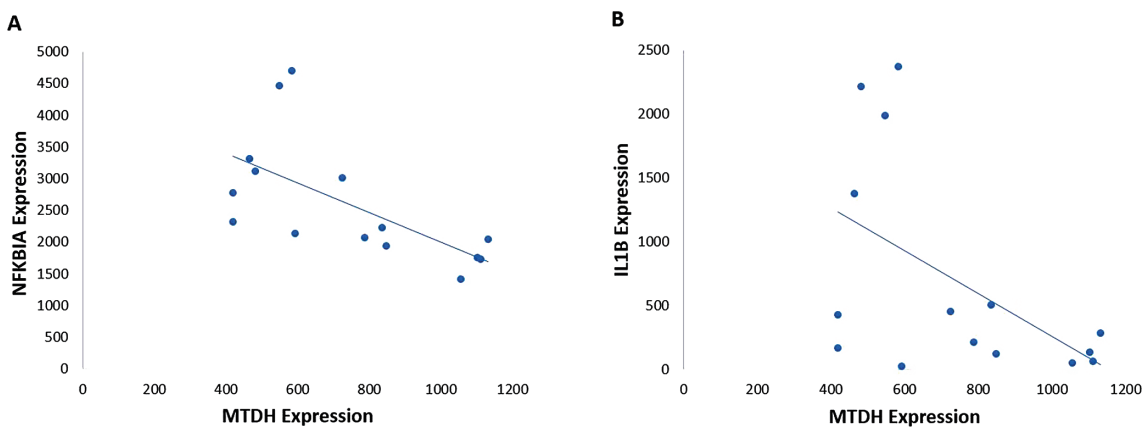

Fig. 6. The correlation of expression between MTDH and major module genes.
A. NFKBIA ($r = -0.63$, $p = 0.011$). B. IL1B ($r = -0.53$, $p = 0.044$).

Leishmania can keep its persistence in host cells by inhibiting the apoptosis of DCs. A previous report showed that *Leishmania* could delay apoptosis induction in the infected DCs by diminishing mitogen-activated protein kinase (MAPK) activation (Rodriguez-Gonzalez et al. 2016). It is known that MTDH plays a key role in the activation of MAPK pathway. It is possible that infected DCs diminish MAPK activation by downregulating the expression of MTDH to avoid apoptosis, resulting in the persistence of *Leishmania*. Indeed, we found significant negative correlation between MTDH expression and the expression of *NFKBIA* and *IL1B*. *NFKBIA* and *IL1B* are known to be strongly induced during inflammatory response. These results suggest that *Leishmania* infection could downregu-

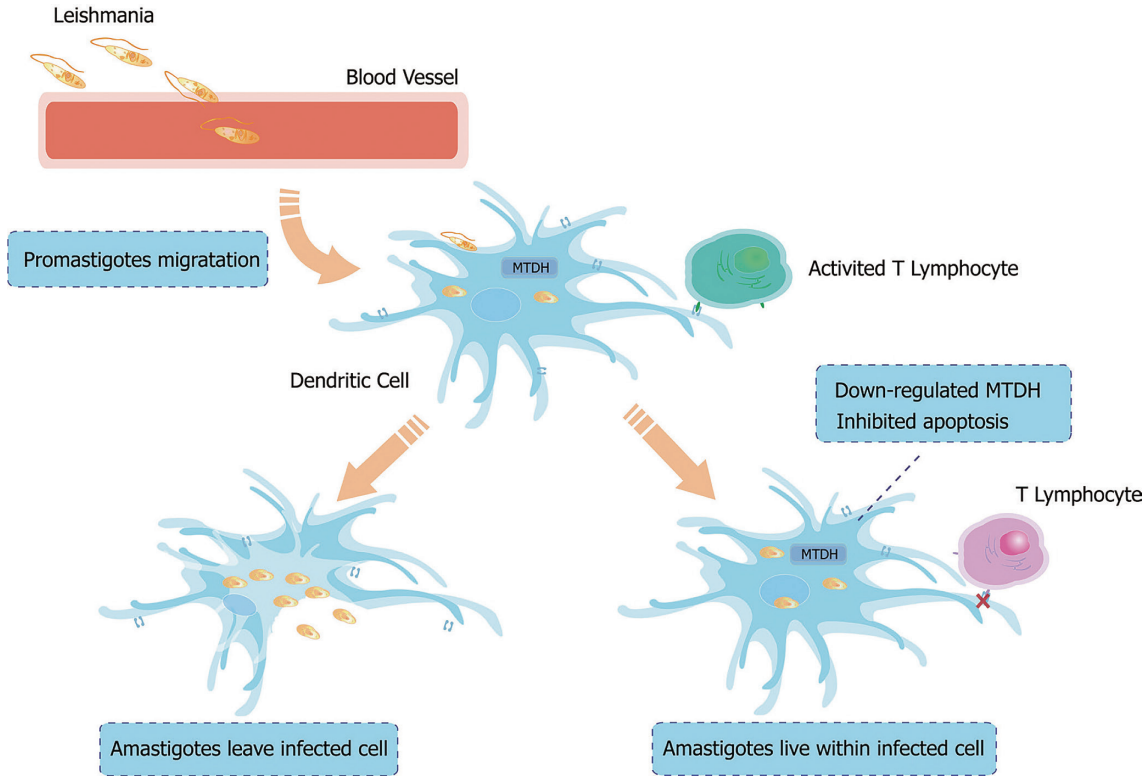

Fig. 7. A hypothetical model of *Leishmania* infection and dendritic cells (DCs). *Leishmania* hides and multiplies in DCs, eventually leading to the death of DCs (left). *Leishmania* can inhibit the apoptosis of DCs by downregulating MTDH expression (right).

late MTDH expression to promote inflammatory response.

In summary, our results based on bioinformatics analysis suggest that *Leishmania* infection leads to the downregulation of MTDH expression and the suppression of DC infiltration.

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Author Contributions

L.T., S.Y., B.D., S.Z., and W.X. collected and analyzed the data. L.W. and M.C. designed and supervised the study. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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