XVII INTERNATIONAL SYMPOSIUM ON
SCHISTOSOMIASIS
PERSPECTIVES ON SCHISTOSOMIASIS ELIMINATION
NOVEMBER 10-13TH 2024 | SALVADOR - BAHIA

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TITLE

DIFFERENTIAL EXPRESSION OF MIRNAS IN SUSCEPTIBLE AND RESISTANT STRAINS OF BIOMPHALARIA SPP. TO SCHISTOSOMA MANSONI AND ESTABLISHMENT OF BIOMPHALARIA GLABRATA EMBRYONIC CELLS (BGE) CELL CRYOPRESERVATION

AUTHORS

Vieira, J.G.R.*[1]; Dutra, I.T.X.[1]; Lima, M.G.[1]; Gava, S.G.[1]; Queiroz, F.R.[2]; Jeremias, W.J.[4]; Montresor, L.C.[5]; Caldeira, R.L.[1], Gomes, M.S.[3]; Mourão, M.M.[1]

AFFILIATIONS

[1] Laboratório de Helmintologia e Malacologia Médica, Instituto René Rachou (IRR)/Fiocruz Minas, Belo Horizonte, Brazil
[2] Instituto Mário Penna, Belo Horizonte, Brazil
[3] Universidade Federal de Uberlândia - Campus Patos de Minas (UFU), Minas Gerais, Brazil
[4] Universidade Federal de Ouro Preto (UFOP), Ouro Preto, Minas Gerais, Brazil
[5] Moluscário Lobato Paraense, Instituto René Rachou (IRR) / Fiocruz Minas, Belo Horizonte, Brazil

ABSTRACT

Schistosomiasis is prevalent in impoverished populations. In Brazil, the disease is caused by Schistosoma mansoni and three species of the genus Biomphalaria are the intermediate hosts: Biomphalaria glabrata, Biomphalaria tenagophila, Biompalaria straminea. The susceptibility of these molluscs to S. mansoni is influenced by environmental, physiological, genetic, and behavioral factors. Differential expression of microRNAs also may play a role in parasites development, and influence hosts susceptibility. MicroRNAs expression may be studied in vivo or in vitro. However, studies with cells are less complex and cost and labor-effective; and the use of cell cultures is a viable approach for developing techniques and practices that can be initially established and later transferred to whole organisms. Thus, we are working with both approaches. First we focused on quantifying and comparing the expression of two microRNAs (miR-750 and miR-1984) in Biomphalaria spp. strains exposed or not exposed to S. mansoni. To further explore molecular aspects, this study also aims to establish an effective protocol for cryopreservation of B. glabrata embryonic cells (Bge) and evaluate miRNAs expression in this model. Snails: Hemolymph and head/foot region RNA extraction from infected and non-infected molluscs was followed by Reverse Transcription-quantitative PCR (RT-qPCR) assessments, revealing the microRNAs expression profiles. Both miRNAs were expressed in all evaluated molluscs. In other invertebrates, miR-750 is related to development and immune system regulation. Expressions in the susceptible B. straminea population do not significantly change between the two miRNAs. In B. glabrata and B. tenagophila species the negative regulation of miR-750 and miR-1984 in strains resistant to S. mansoni infection suggests a possible relationship with the host's immune response, conferring resistance to the parasite. BgE: Selected and nonspecific miRNA inhibitors (anti-miR) were designed and used in the in vitro transfection of Bge cells. The cells were co-cultured for 24 h with S. mansoni sporocysts transformed in vitro. Cell morphology will be evaluated, and the cell adhesion index will be calculated. A decrease in miR-750 and miR-1984 expression was observed in Bge cells after exposure to S. mansoni sporocysts. Also, no high mortality rate was observed in Bge cells exposed to anti-miRs. In parallel, for developing an effective cryopreservation protocol, Bge cells were frozen and thawed using different media and procedures. We standardized a protocol with the addition of 5% DMSO during freezing, followed by a specific thawing process using a water bath, centrifugation, and subsequent cell maintenance for one week, ensuring better viability of Bge cells. Elucidating the function of miRNAs, in Bge model and molluscs, is an important point in understanding the susceptibility and resistance to infection of the intermediate host.

KEYWORDS

Biomphalaria spp.; Cell Culture; miRNA; Schistosoma mansoni; Susceptibility

FINANCIAL SUPPORT

FAPEMIG