microRNA regulates antimicrobial responses expression during the infection of *Cryptococcus neoformans*

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OBJECTIVES:

New treatment strategies for the treatment of cryptocococcosis infection are studied for greater efficiency in combating yeast spread and disease control. This study proposes the possibility to development of new therapies through of study microRNAs (miRNAs) involved in the interaction between yeast and human glioblastoma cells (U87-MG), in order to use them as regulators of infection, since this is a recently disclosed strategy in a variety of diseases.

METHODS:

Interaction Host X Fungal

The interactions between glioblastome cells (U87-MG) (1x10⁴ cels) and *C. neoformans* ATCC 90112 (1x10⁴ cels) were quantified by flow cytometry.

miRNA Expression in Host Cells

The RNA of host cells were extracted, purified and quantified and miRNA expression was evaluated by quantitative PCR using plate miRCURY LNATM Universal RT miRNA Ready-to-Use PCR for human cells.

Protein Analysis

The search for genes that interference by miRNA expression was performed by the miRSystem and GSEA-Broad databases. The analysis of the interfering proteins by miRNAs was carried out by the selection of proteins in the cell membrane and cytoskeleton (GeneCards) and the your functions were analysed by STRING. The proteins expression were performed by immunoflourescence assay. The reading was performed on IN Cell Analyzer 2000 GE and analysis on the IN Cell Investigator 1000 Workstation

RESULTS:

Figure 1: Interaction *C. neoformans* and U87-MG cells by flow cytometry. Statistics were analyzed by GraphPad Prism, one-way ANOVA (Mean $\pm$ SD).

Figure 2: Expression of COP-1 and 3-GTPase proteins in U87-MG cells infected with *C. neoformans*. The expression of proteins is represented by the intensity of cell fluorescence. Data analyzed in GraphPad Prism v.5 software for analysis in two-away ANOVA comparisons with control vs. times by Bonferroni post-test. * P < 0.05.

Table 1: miRNA overexpression of interaction *C. neoformans* and U87-MG cells; signaling pathways, genes and encoding proteins influenced by overexpression and proteins

miRNA	RQ*/IP value	Signaling Pathway	Genes and encoding proteins
hsa-miR-632	9.4300 / 0.0449	TGF- β , MAP kinase (MAPK), Matrix extracellular components (MEC) interaction.	Gene: IQGAP3 – Protein: 3-GTPase Gene: GPS1 – Protein: COP-1
hsa-miR-552	9.87324 / 0.0339		
hsa-miR-621	7.9434 / 0.0307		
hsa-miR-1913	5.7982 / 0.0455		
hsa-miR-582	5.1319 / 0.0489		
hsa-miR-326	4.9529 / 0.0419		
hsa-miR-943	3.2819 / 0.0278		
hsa-miR-1254	2.7979 / 0.0201		
hsa-miR-1249	2.5650 / 0.0478		
hsa-miR-605	2.1723 / 0.0410		

*RQ – Relative Quantification

3-GTPase protein
COP-1 protein

Pathway Rho-GTPase

Essentials for the yeast adhesion

CONCLUSION:

The inhibition of expression of miRNAs during fungus-cell interaction may be interesting to control fungal infection, because we believe that once inhibited, the yeast can reduce invasion of host cells, being the miRNAs interesting new therapeutic targets.