ESAT-6 abrogates IL-18 mediated phagosome lysosome fusion via microRNA-30a upon Calcimycin treatment in mycobacteria infected macrophages

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Abstract

The major immunodominant antigens of Mycobacterium tuberculosis (M. tb) confiscate the host immune system to give a survival advantage to this intracellular pathogen. But, the mechanism of action behind this control is not entirely known. Since we have previously reported the mechanism of autophagy inhibition by early secreted antigenic target 6 kDa (ESAT-6) through microRNA (miR)-30a-3p in Calcimycin-treated differentiated THP-1 (dTHP-1) cells, the present study was undertaken to evaluate the effect of miR-30a on the immunomodulatory profile of ESAT-6 treated cells, and the mechanism involved thereof, if any. The effect of recombinant ESAT-6 (rESAT-6) on the immunomodulatory profile in Calcimycin-treated phorbol 12-myristate 13- acetate (PMA) dTHP-1 cells was checked. Later, transfection studies using miR-30a-3p inhibitor or -5p mimic highlighted the contrary roles of different arms of the same miRNA in regulating IL-18 response by ESAT-6 in dTHP-1 cells upon Calcimycin treatment. By using either IL-18 neutralizing antibody or inhibitors of phosphoinositide 3-kinase (PI3K)/NF-κB/phagosome-lysosome fusion in the miRNA-30a transfected background, IL-18 mediated signaling and intracellular killing of mycobacteria was reversed in the presence of ESAT-6. Overall, the results of this study conclusively prove the contrary roles of miR-30a-3p and miR-30a-5p in modulating IL-18 signaling by ESAT-6 in dTHP-1 cells upon Calcimycin treatment that affected phagosome-lysosome fusion and intracellular viability of mycobacteria.

Introduction

TB, caused by M. tb, is one of the leading causes of death worldwide. The challenges in eradicating TB demands the development of novel TB therapeutics based on host-pathogen interaction. Upon entry inside the respiratory tract, the alveolar macrophages act as the first line of defense against M. tb. The host employs multiple strategies to eliminate intracellular bacteria. IL-18, a member of the IL-1 family of cytokines, is generally present in all nucleated cells of the body. Recently, it has been reported that IL-18 and IL-12 activate autophagy to kill the intracellular M. tb by inducing IFN-γ. Despite the cooperative role of many cytokines to suppress intracellular mycobacterial growth, the M. tb genome encodes several effector molecules, such as early secreted antigenic target 6kDa (ESAT-6) secretion system 1 (ESX-1) that suppress the host immune response. Recently, we have found that miR-30a-5p favors the host autophagic response in killing the intracellular mycobacteria, contrary to miR-30a-3p in the presence of ESAT-6 nullifies this pathway. Next, we attempted to understand that ESAT-6 specifically downregulated IL-18-mediated phagosome lysosome fusion by modulating miR-30a-3p and miR-30a-5p levels that affected the fate of mycobacteria inside the dTHP-1 cells.

Methodology

For determining intracellular bacterial viability by CFU assay, miRNA or plasmid transfected dTHP-1 cells were infected at an MOI of 1:1/1:10 for 3 h/4 h with M. smegmatis/M. bovis BCG. rESAT-6 was overexpressed in the BL21 (λDE3, pLysS) strain of E. coli by adding 1mM isopropyl ß-D-1-thiogalactopyranoside (IPTG) then overexpressed protein was purified from bacterial lysates by Ni-NTA chromatography. dTHP-1 cells were pre-treated with various concentrations of rESAT-6 followed by incubation with Calcimycin for different time points. In some experiments, dTHP-1 cells were initially transfected with either pcDNA3.1(-)/pcDNA3.1(-)-IL-18 or miR-30a-5p/3p mimic/inhibitor along with negative control before pre-treatment with rESAT-6 and Calcimycin addition. Experiments involving p65 translocation and IκBα phosphorylation, cytoplasmic and nuclear extracts were isolated separately. The percentage of cells expressing IL-18R1 on the cell surface was measured by flow cytometer. In experiments involving phagosome lysosome fusion, miR-30a-5p/3p mimic/inhibitor or negative controls transfected dTHP-1 cells were first infected with GFP-M. bovis BCG followed by pre-treatment with either rESAT-6 or other inhibitors before adding Calcimycin. For overexpression of IL-18 in vitro, dTHP-1 cells were transfected with pcDNA3.1(-)-IL-18 or pcDNA3.1(-) empty vector.

Results

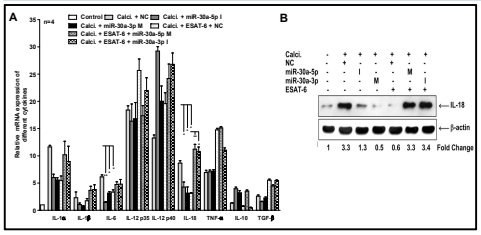

Figure 1. Effect of rESAT-6 on the immunomodulatory profile of Calcimycin (Calci.) treated dTHP-1 cells through miR-30a.

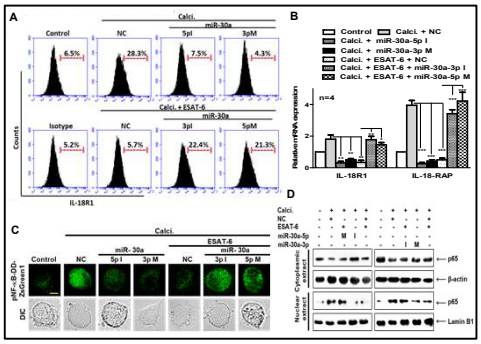

Figure 2. Effect of miR-30a-3p/5p on IL-18 receptor expression, and NF-κB activation in Calci. treated dTHP-1 cells.

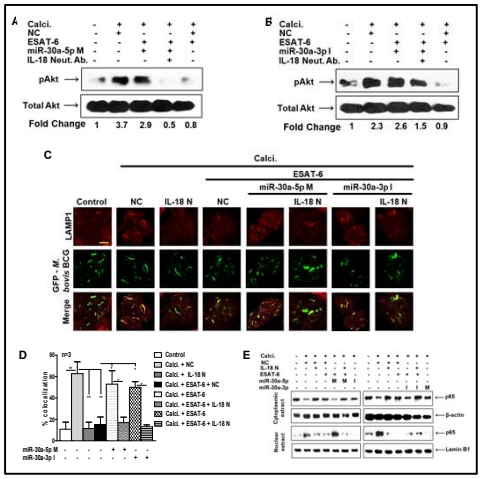

Figure 3. Effect of miR-30a-3p/5p on phosphorylation of Akt and phagosome lysosome fusion in dTHP-1 cells.

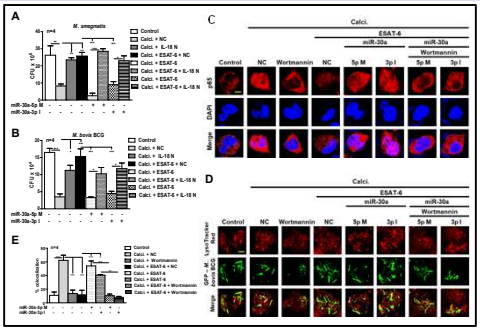

Figure 4. Effect of Wortmannin (100 nM) on IL-18R downstream signaling and phagosome lysosome fusion in dTHP-1 cells.

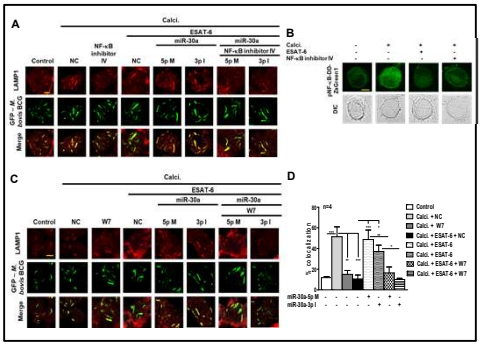

Figure 5. Effect of NF-κB inhibitor and W7 (7.5 µM) on intracellular mycobacterial viability and role of W7 on phagosome lysosome fusion in dTHP-1 cells.

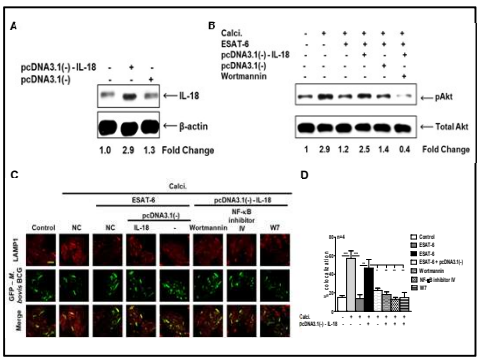

Figure 6. Effect of IL-18 overexpression on Akt phosphorylation, and phagosome lysosome fusion in dTHP-1 cells.

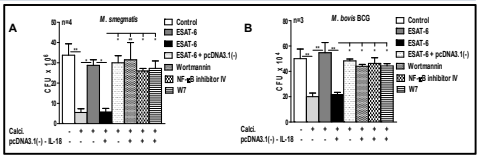

Figure 7. Effect of IL-18 overexpression on intracellular survival of M. smegmatis or M. bovis BCG in dTHP-1 cells.

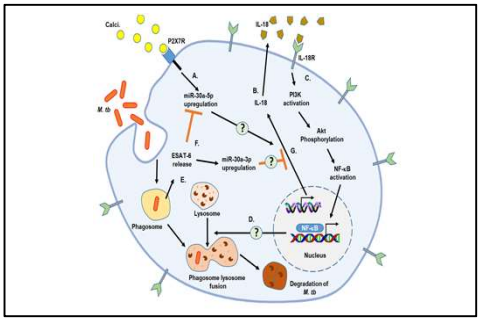

Figure 8. rESAT-6 inhibits IL-18 regulated phagosome lysosome fusion through contrary regulation of 3p and 5p arms of miR-30a in Calci. treated dTHP-1 cells.

Conclusion

- Calcimycin upregulates the expression of miR-30a-5p to enhance autophagy and IL-18 expression
- rESAT-6 upregulates the expression of miR-30a-3p and downregulates miR-30a-5p to inhibit autophagy and IL-18 expression
- Binding of IL-18 to its receptor activates PI3K-Akt-NF-κB signaling cascade
- Activation of IL-18R downstream signaling enhances the fusion of phagosome and lysosome leading to killing of mycobacteria

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References

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