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Role of transcriptional factor EB (TFEB) in the pathogenesis of *Acinetobacter baumannii*

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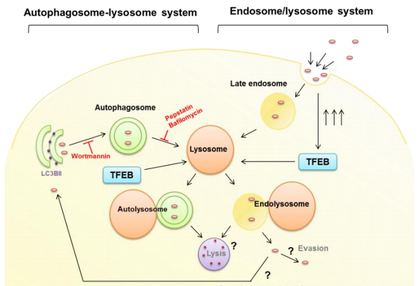
Introduction and purpose

Acinetobacter baumannii is a gram-negative coccobacillus with high clinical relevance due to the different nosocomial infections that it causes.

Given the lack of treatment options against *A. baumannii* infections, it is prioritary the development of novel and effective treatments. To this end, we propose to study an intracellular host cell factor as a potential target to develop inhibitors to block the entry and persistence of *A. baumannii* in the host.

Endosome/lysosome and autophagosome/lysosome systems play an important role in the bacterial intracellular trafficking.



These systems are regulated, among others, by TFEB. The role of TFEB in the entry of *A. baumannii* in the host is unknown.

The aim of this study is to determine the involvement of TFEB in the entry and persistence of *A. baumannii* in the host.

Objectives

1. To study the expression of TFEB in infected A549 cells by *A. baumannii*.
2. To study the bacterial adherence and invasion in A549 cells deficients in TFEB expression.
3. To study the bacterial adherence and invasion in A549 cells overexpressing TFEB.
4. To demonstrate the implication of autophagosome-lysosome system in *A. baumannii* intracellular trafficking.
5. To determine the lysosome lysis by *A. baumannii* infection in A549 cells.
6. To determine the role of TFEB in cell death caused by *A. baumannii* infection.

Conclusions

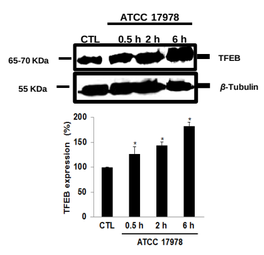
The results of this study help to clarify the role of endosome/lysosome and autophagosome/lysosome systems TFEB-dependent in the pathogenesis of *A. baumannii*. Exploitation of this network "node" could potentially represent a novel therapeutic approach to treat *A. baumannii* infections by modulating autophagy/lysosome function, and may boost the design of a novel class of antimicrobial therapeutics targeting host factors.

Methods and Results

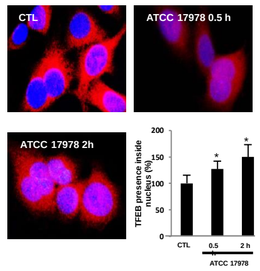
1. Expression of TFEB in A549 cells by *A. baumannii*

Human lung epithelial cells (A549) were infected with ATCC17978 strain (10^8 cfu/ml) during 0.5, 2 and 6 h to analyze the TFEB expression by Western Blot and immunofluorescence assay.

Western Blot



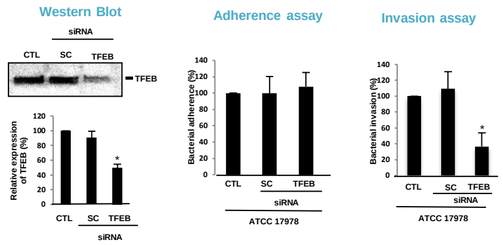
Immunofluorescence



The infection with ATCC 17978 strain increases progressively and significantly the expression of TFEB.

2. Bacterial adherence and invasion in A549 cells deficients in TFEB expression

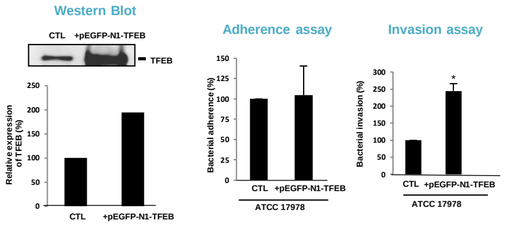
A549 cells were TFEB down-expressed by small interference RNA (siRNA) transfection, and were subsequently infected with ATCC17978 strain (10^8 cfu/ml) during 2 h to study bacterial adherence and invasion.



Bacterial invasion in A549 cells was reduced in TFEB deficient cells by 64% compared to the control non-infected cells, while bacterial adhesion didn´t show any difference.

3. Bacterial adherence and invasion in A549 cells overexpressing TFEB

A549 cells were TFEB overexpressed by plasmid pEGFP-N1-TFEB transfection, and then were infected with ATCC17978 strain (10^8 cfu/ml) during 2 h to study bacterial adherence and invasion.

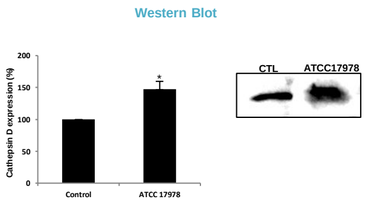


Bacterial invasion in A549 cells was increased in TFEB overexpressing cells by 150% compared to the control non-infected cells, while bacterial adhesion didn´t show any difference.

4. *A. baumannii* effect on lysosomes lysis in A549 cells

Lysosome lysis in infected cells was studied by determining Cathepsin D expression after A549 cells incubation with ATCC 17978 for 2 h.

Western Blot

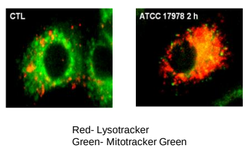


Lysosome lysis is higher in infected cells.

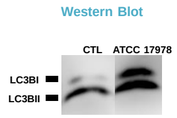
5. Implication of autophagosome-lysosome system

Lysosome biogenesis and autophagy activation were studied using lysotracker, a marker of lysosome, and determining LC3BII expression respectively.

Immunofluorescence



Western Blot

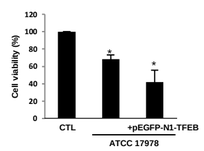


Both lysosome biogenesis and autophagic activity are higher in infected cells.

6. To determine the role of TFEB in cell death caused by *A. baumannii* infection

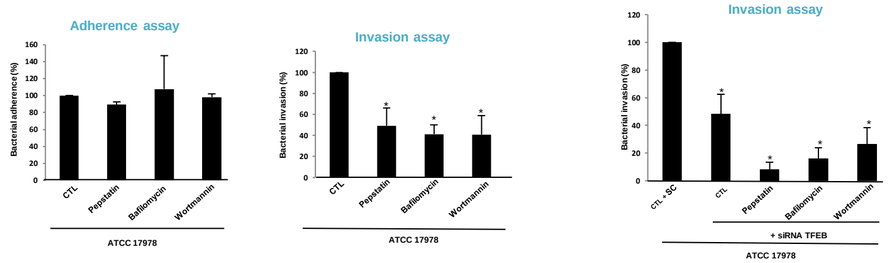
The role of TFEB in cell death caused by ATCC17978 strain using a cell viability assay was analyzed.

Cell viability assay



The viability of A549 cells infected with *A. baumannii* decreases significantly in TFEB overexpressing cells compared to the control A549 cells.

A549 cells were pretreated with autophagosome inhibitors (Bafilomycin 0.8 µM, Pepstatin 20 µg/ml and Wortmannin 1 µM) for 30 min, and infected with ATCC17978 strain (10^8 cfu/ml) during 2 h to study the bacterial adherence and invasion to host cells.



Bacterial invasion was reduced in A549 cells pretreated with autophagosome inhibitors. Interestingly, the treatment of A549 cells with TFEB siRNA and autophagosome inhibitors reduced more significantly the bacterial invasion into these cells in comparison with A549 cells without treatment.

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