Identifying the role of autophagy in the protection against protein aggregation

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Chung, R.[1], Sunchu B.[1,2], and Perez, V.I.[1,2]

Department of Biochemistry and Biophysics[1], Oregon State University, Corvallis, Oregon, 97331, USA; Linus Pauling Institute[2], Oregon State University, Corvallis, Oregon, 97331, USA

OSU Oregon State University

Introduction

Aging is a physiological process that most, if not all, living species experiences, but unfortunately this process is accompanied by several age-related diseases. Therefore, there is a great interest to study this process and understand the mechanisms involved with the aim of extending human health span. One of the mechanism that has been involved in the aging process is the decline of protein homeostasis (mechanisms that maintain the quality of proteins). A decline in protein homeostasis leads to an accumulation of protein aggregation, which over time cause cellular disorders and play a role in many chronic diseases, including age-related neurodegenerative diseases such as Alzheimer's, Parkinson's, ALS and Huntington's disease, among others.

Based on our previous data, cells from a long lived species, the naked mole rat, have higher activity of protein homeostasis mechanisms, like autophagy, proteasome, and heat shock chaperone proteins than mouse, the short lived counterpart. We also observed that naked mole rat cells are more resistant to the toxicity induced by polyQ82, a protein that easily aggregates in cells.

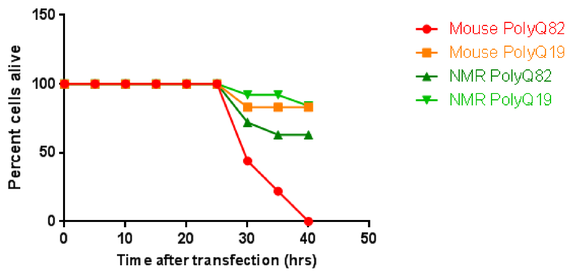


Figure 1. The graph above showed that the percent of cells alive between long lived species and short lived species after transfection with poly Q 82 (prone aggregated protein) and its control poly Q 19 (protein does not aggregate). (1)

The objective of this work is to investigate the role of autophagy in the protection against polyQ82 toxicity. In order to perform the experiment, we isolated and purified a plasmid that expresses LC3B, a marker for autophagy, that contains two fluorescence tags: GFP and mcherry that allow us to follow autophagy activity in naked mole rat cells using fluorescent microscopy. Our preliminary data showed that LC3B plasmid is working in naked mole rat cells and we can use it to identify whether autophagy is activated (because LC3B shows a red color) when cells are transfected with polyQ82 protein.

Materials and Methods

DH5α Bacteria: Growth strain and resistant to ampicillin
Qiagen® Plasmid Maxi Kit: Isolated LC3B DNA from the DH5α bacteria
DNA Digestion and Agarose gel Electrophoresis: digestion enzyme, Nhe I and Pst I to determine the correct bands through DNA agarose gel
Transfection: Using lipofection reagent (X-tremeGene HP Transfection reagent, Roche) to insert target LC3B and Poly Qs into the Naked Mole Rats' cells. Green Fluoresces Protein as control.

Figure 2. The image above showed the double tagged autophagy maker, LC3B-GFP-mcherry used in our experiments. (2)

Results

Figure 3. Identification of the colony obtaining our plasmid of interest. The LC3B GFP mcherry plasmid was isolated by performing miniprep assay. After plasmid purification, all samples were digested enzymatically with Nhe1 and Pst1 restriction enzymes which will result in two bands of 9.53kb and 1.7kb. All digested samples were subjected to an agarose electrophoresis.
As showed in Figure 3, the colony #7 has the right pattern. Therefore sample #7 was growth and the plasmid was isolated and purify using a Maxi perp (Qiagen®).

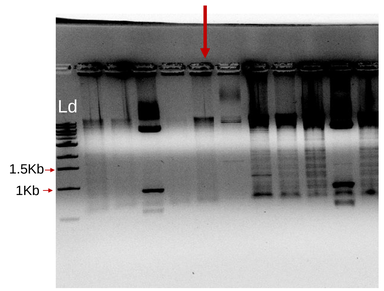


Figure 4. LC3B GFP mcherry plasmid works in naked mole rat cells. LC3B DNA plasmid was transfected into naked mole rat cells by using the Xtreme HP DNA transfection reagent (Lipofectamine reagent). Figure 4 showed that our plasmid is working in naked mole rat cells. Image A showed the bright field of the naked mole rat cells, image B showed the LC3B GFP of the naked mole rat cells. Image C showed the mcherry from the LC3B marker tag.

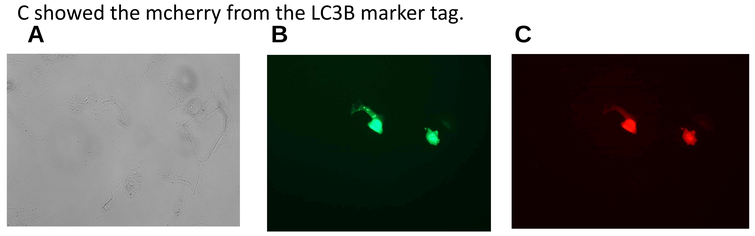
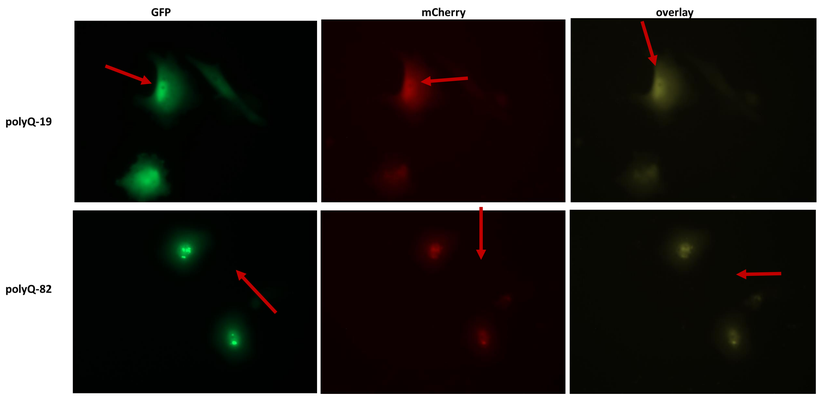


Figure 5. Expression of Poly Q 82 in the naked mole rat cells activates LC3B. Naked mole rat cells were co-transfected with poly Q82 or poly Q19 plus LC3B GFP mcherry. Poly Q19 didn't show the protein aggregation as we were expecting for control (GFP top row). However, poly Q82 showed formation of inclusion bodies (protein aggregation, GFP picture second row. , we also observed the mcherry fluorescence that indicate autophagy activation. The last pictures labeled as "overlay", showed both GFP and mcherry together.



Discussion and Future Direction

The results showed that the plasmid was successful expressed with a high transfection efficiency. Also our data indicates that autophagy get active when poly Q82 is expressed, suggesting that autophagy may have a protective role against poly Q82 aggregation. For future experiments, we need to repeat these experiments to proved our preliminary data. Also, we need to investigate the activity of the proteasome and the heat shock proteins and their role in the protection against polyQ82 toxicity.

References

1. Sunchu, B., Otto, S., Zhen, Y., Caples, K., Perez, V. (**2016**). *Naked mole rat skin fibroblasts are more resistant to proteotoxicity induced by poly Q82 than mouse.* 46th annual meeting of American Association for Aging Study.
2. Hansen, TE., Johansen, T. (**2011**). *Following autophagy step by step.* BMC Biology. **9**:39.

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