

Introduction

- Aging leads to a decline in cognitive function and a greater risk of neurodegenerative, cardiovascular, psychological, and immunological disorders. (ThyagaRajan et al. 2011, Sipe et al. 2017, Cole et al. 2019).
- As the brain ages, senescent cells accrue and lead to neurodegenerative disease. Senescent cells secrete pro-inflammatory cytokines, growth-factors, and proteases due to the acquisition of senescence associated secretory phenotype (SASP) (Tan et al. 2014).
- Preliminary data obtained from past studies conducted in our laboratory suggested that cellular senescence occurs in mice brainstem. Our main goal is to establish a model for replicating the results obtained from the previous studies on mice.

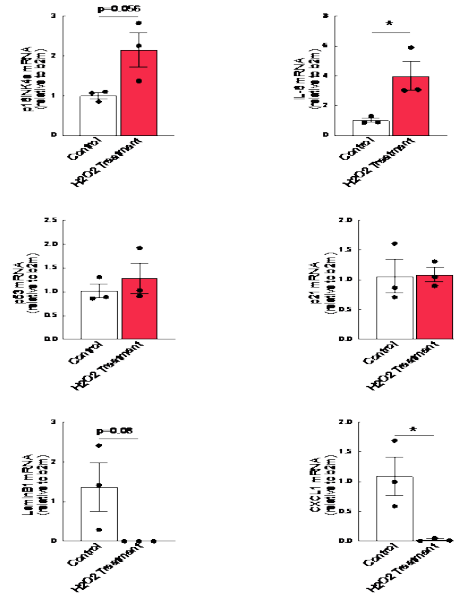
Hypothesis

- Increased oxidative stress will lead to an increased accumulation of senescent cells in the cultured human brain stem astrocytes.

Methods

- We cultured human brainstem astrocytes and treated them with hydrogen peroxide in concentrations of 200 μmol and 300 μmol for 7 days.
- The cells were then collected in trizol, then RNA was extracted using Directzol RNA MiniPrep (Zymo Research) according to the manufacturer's protocol.
- Reverse transcription was done to convert the RNA to cDNA using the Applied Biosystems High Capacity cDNA synthesis kit.
- The cDNA samples were analyzed using the real time quantitative PCR.

Changes in the mRNA expression of astrocytes in cell culture after H2O2 treatment



Results

- p16, p21 and p53 are the major cyclin dependent kinase inhibitors which are well documented senescent cell markers. IL6 is a pro-inflammatory cytokine and is a major SASP factor that is upregulated in senescence. Lamin B1 is a gene that encodes Lamin proteins which are a part of the nuclear envelope. CXCL1 is a chemokine that is responsible for recruitment of neutrophils and oligodendrocytes.
- In my study, we observed a non-significant increase in the levels of p16, p21 and p53. The levels of IL6 were found to be significantly higher in the H2O2 treated astrocytes as compared to the control (untreated) astrocytes.
- A non significant decrease was observed in the levels of Lamin B1 in the H2O2-treated astrocytes.
- A significant downregulation was seen in the levels of CXCL1 in the treated cells

Summary and Conclusions

- All these results from my study indicate that the oxidative stress induces senescence in cultured human brain stem astrocytes as indicated by higher levels of IL6 and non significant increase in the p16, p21 and p53.
- The non significant decrease in the levels of LaminB1 implies damage to the nuclear lamina indicating senescence
- But there was an increase in the level of CXCL1, which is a very interesting finding and contrary to what we expected to find.
- Thus we were able to establish a model replicating the cellular senescence in the mice and our future goal would involve use of the senolytic drugs in aged (C57BL/6J) mice for clearing of senescent cells and to see its effect on the animal brainstem.

References

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