Cellular Senescence in Cultured Human Brainstem Astrocytes: Effect of Oxidative Stress

Authors: Zane Leibhart and Dr. Madhan Subramanian*

Abstract: Cardiovascular diseases are one of the leading causes of mortality in the elderly. A central role in the contraction of cardiovascular diseases by means of aging could be played by cellular senescence. Senescent cells accumulate with age in various tissues of the body including the brain. Recent studies from our lab have shown that there is increased senescent cell accumulation in the brainstem in aged mice. Neurons in the brainstem is responsible for the control of sympathetic nerve activity to several vascular beds and overactivity of the sympathetic neurons would lead to increased risk for cardiovascular diseases. It is well documented that neurons are post mitotic and do not undergo senescence, however glial cells could undergo senescence and have implications in neuronal function. In this study we developed a cell culture model to study senescence in vitro. We have used cultured human astrocytes and subjected them to oxidative stress by means of hydrogen peroxide treatment. We believe that increasing levels of hydrogen peroxide will lead to an increase in the number of senescent cells present within the cultured human brain stem astrocytes. Our results showed that there are increased levels of senescence marker p16 and senescence associated secretory phenotype IL6 upregulated after hydrogen peroxide treatment along with decreased levels of laminB1. These results suggest that we were able to induce senescence in human brainstem astrocytes and it could be used as a suitable model to study senescence in vitro. We were able to establish a model using human cells, replicating the cellular senescence in the mice and our future goal would involve understanding the mechanism by which senescence cells in the brainstem would affect the sympathetic regulation.

Keywords: Cellular Senescence, Human, Brainstem, Astrocytes

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). Neurons were shown to not undergo senescence. However, they are surrounded by several other types of cells that do undergo this process. Specifically, it has been shown that astrocytes within the brain undergo senescence, however this study has not taken place at the level of the brain stem (Salminen et al. 2011). Cellular senescence has been observed within the brainstem, but it is unknown what cell is undergoing this process. Astrocytes have been shown to senescence within the brain yet have not yet been observed specifically within the brainstem, which is a central region for autonomic cardiovascular control. Understanding this process at the level of the brainstem could mediate the sympathetic nervous system dysregulation shown with aging. Previous research in this lab has shown that brainstem astrocytes will senescence; however, we are attempting to create a model using cultured human brainstem astrocytes.

Methods

We used cell culturing to grow human brainstem astrocytes and introduced them to hydrogen peroxide treatments in concentrations of 200 and 300 µmol for 7 days replicating oxidative stress to induce cellular senescence. Senescent cells were then removed from the culture and placed in trizol, RNA was extracted using Directzol RNA MiniPrep (Zymo

* Faculty Mentor, Department of Physiological Sciences

Research) according to the manufacturer's protocol. Reverse transcription was utilized 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.

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 (Figure 1). A non-significant decrease was observed in the levels of Lamin B1 in the H2O2-treated astrocytes (Figure 1). A significant downregulation was seen in the levels of CXCL1 in the treated cells (Figure 1).

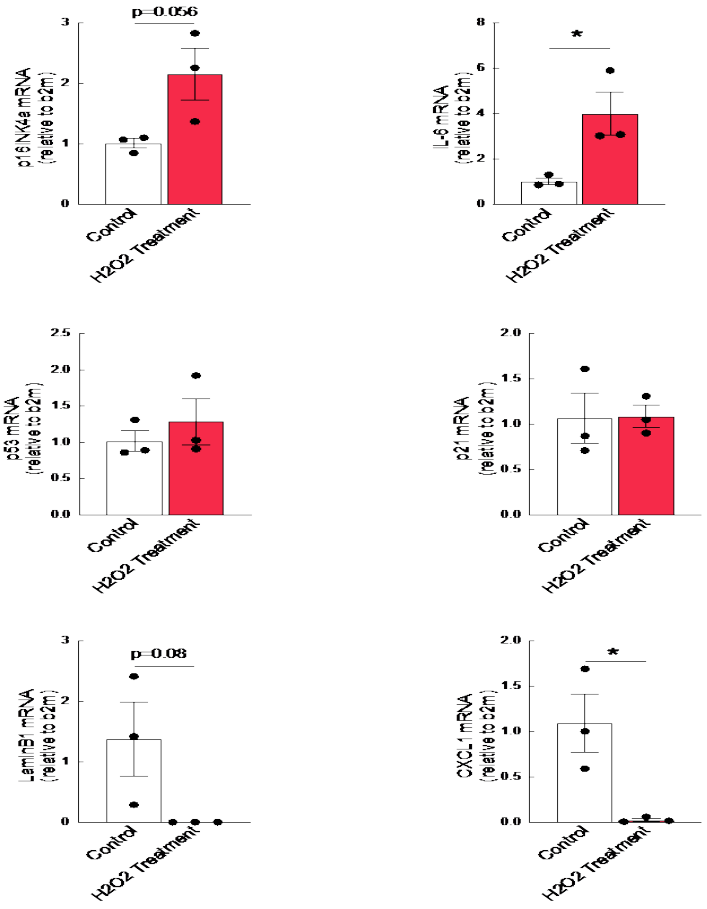

Figure 1: There are increased levels of p16 and IL6 after hydrogen peroxide treatment in the cultured astrocytes accompanied with decreased levels of LaminB1. $p<0.05$ is considered significant. $n=3$.

Discussion

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 p16 and increase in the IL-6. The significant decrease in the levels of LaminB1 implies damage to the nuclear lamina indicating senescence. Based on these preliminary findings, we were able to establish a model to study cellular senescence in vitro and our future goal would be understanding the mechanism by which senescence cells in the brainstem would affect the sympathetic regulation.

Literature Cited

Cole, J. H., R. E. Marioni, S. E. Harris, and I. J. Deary. 2018. Brain age and other bodily 'ages': Implications for neuropsychiatry. Molecular Psychiatry 24:266-281.

Salminen, A., J. Ojala, K. Kaarniranta, A. Haapasalo, M. Hiltunen, and H. Soininen. 2011. Astrocytes in the aging brain express characteristics of senescence-associated secretory phenotype. European Journal of Neuroscience 34:3-11.

Sipe, L. M., C. Yang, J. Ephrem, E. Garren, J. Hirsh, and C. D. Deppmann. 2017. Differential sympathetic outflow to adipose depots is required for visceral fat loss in response to calorie restriction. Nutrition and Diabetes 7.

Tan, F. C. C., E. R. Hutchison, E. Eitan, and M. P. Mattson. 2014. Are there roles for brain cell senescence in aging and neurodegenerative disorders? Biogerontology 15:643-660.

ThyagaRajan, S., K. S. Madden, B. Teruya, S. Y. Stevens, D. L. Felten, and D. L. Bellinger. 2011. Age-associated alterations in sympathetic noradrenergic innervation of primary and secondary lymphoid organs in female Fischer 344 rats. Journal of Neuroimmunology 233:54-64.