Identifying factors that contribute to oligodendrocyte precursor cell senescence in aging

Introduction

Oligodendrocyte precursor cells (OPCs) are the progenitor cells responsible for forming mature, myelinating oligodendrocytes (OLs) in the central nervous system during development. While the majority of OPCs differentiate early in life, there is a small pool that generate OLs over the life span and can differentiate into OLs following white matter injury (WMI). Previous work has shown that this differentiation is impaired in aging, reducing the ability to recover from WMI.[1] Significant upregulation of senescence markers in old vs young OPCs suggests senescence, a stress induced state in which cells no longer proliferate, contributes to the impaired ability of OPCs to differentiate[2]. Studies of senescence in other cell types has shown that it leads to reduced functional capacity and mediates the physiological consequences of aging. Thus, better characterizing OPC senescence and the mechanisms involved would greatly improve our understanding of the role of OPCs in brain aging.

Although studies have identified some canonical senescence genes in OPCs, OPC senescence genes have not been completely characterized, making accurate identification of senescent OPCs more difficult. Furthermore, due to our lack of understanding of OPC senescence mechanisms, there is a need to identify novel regulators of senescence in OPCs. Understanding these mechanisms would greatly enhance both our understanding of OPC development, and our ability to promote CNS myelination in injury. Given this, I propose to identify an OPC-specific senescence signature, and to identify novel regulators of senescence via a genome-wide CRISPR-Cas9 knockout screen. I intend to carry out this project with two faculty experts in glial cell biology and genome-wide CRISPR screens.

Research Plan:

Aim 1: Define canonical and OPC-specific senescence markers in vivo by single-cell RNA-seq

Rationale: A comprehensive OPC-specific senescence signature has yet to be established. Such a signature would allow for a more accurate identification of senescent phenotypes in different OPC populations and may provide insight into the mechanisms underlying senescence in OPCs.

Experimental Design: OPCs will be taken from 20-24 month old mice and senescent cell clusters identified by flow cytometry using fluorescent antibodies to established senescence markers such as NOTCH3, B2MG and DEP1, which have been shown to have high levels of expression in senescent cells[3]. Following identification of senescent OPCs, RNA from both proliferating (non-senescent) and senescent OPCs will be extracted and single-cell RNA-seq performed, with non-senescent OPCs serving as a control to identify genes implicated in aging, but not senescence. Data will then be analyzed to identify differentially expressed transcripts in senescent OPCs, which will comprise our OPC senescence signature. This experiment will yield genes both up- and downstream of senescence and will allow for more accurate identification of senescent OPCs. In the future, candidates may be validated through in vivo knockout studies.

Possible Outcomes: It is possible that with multiple senescence markers, we may miss OPCs which display lower levels of markers or do not display some at all. We can account for this in adjusting the sensitivity of our gating and analysis, or utilizing fewer antibodies for selection.

Aim 2: Identify regulators of OPC senescence via a CRISPR-Cas9 knockout screen

Rationale: Discovery of OPC-specific regulators of senescence will establish mechanisms underlying OPC senescence and can be used to inform the development of mechanistic in vivo studies.

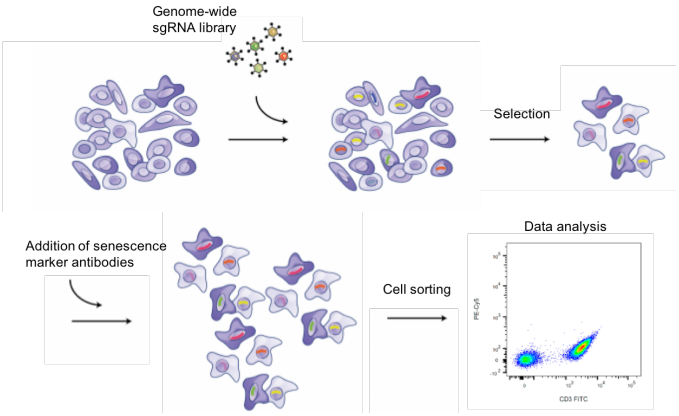

Fig 1. Experimental outline for a senescence marker-based pooled CRISPR screen.

Experimental Design: To identify regulators of OPC senescence, I will conduct a genome-scale CRISPR-Cas9 knockout screen with a pooled lentiCRISPR library. Complex pooled DNA libraries will be combined, and delivered in lentiviral constructs in 4-8 week old OPCs, which should not display high levels of senescence. OPCs can be cultured and transduced at a large scale which allows for genome-wide screens. After transduction and selection, I will select senescent cells using the flow-cytometry approach described in Aim 1 with multiple antibodies against senescence markers. Sorted cells will then be analyzed to identify genes whose knockout increases or decreases senescence, yielding insight into mechanisms of OPC senescence.

Possible Outcomes: If Aim 1 yields OPC-specific markers for which there are robust reporters or antibodies, these can be used in a parallel screen to support our initial findings. Future studies may also use the signature from Aim 1 to validate hits from Aim 2 in vivo.

Intellectual Merit

While senescence has been characterized in microglia and astrocytes, significantly less work has been done in understanding OPC senescence and its relevance in aging. Given that the efficiency of myelination has been shown to decline over time, this proposal, which aims to better characterize OPC senescence, will greatly contribute to our understanding of the effects of aging on oligodendrocyte development and myelination. Furthermore, characterization of OPC senescence opens up the possibility of mechanistic and in vivo studies, which will broadly increase our knowledge of the roles of OPCs, and how they change over time.

Broader Impact

This work has implications for the understanding and treatment of neurological diseases, as aging is linked to an increase in disorders such as dementia and Alzheimer's. Dementia is linked to loss of white matter and decreased myelination, and myelin breakdown is implicated in Alzheimer's, pointing to a critical role for OPCs. This work could eventually allow for the reversal of senescent phenotypes in OPCs, potentially leading to curative treatments. Additionally, understanding OPC senescence has important implications for demyelinating diseases such as MS, for which there are no remyelinating therapies. Recovery from such diseases is impaired by lack of understanding of OPC differentiation; thus, understanding blockades to differentiation such as senescence may inform future therapeutic development.

Feasibility

My previous work with oligodendrocyte development will significantly contribute to the success of the project. Furthermore, I am rotating in the lab of Dr. Ophir Shalem, whose lab has a strong and successful history of genome-wide CRISPR-Cas9 knockout screens, and who will provide the resources and mentorship need. I am also rotating with Dr. Chris Bennett, who has expertise in the isolation, culture, and sequencing of glia, and can offer the resources and mentorship needed for this project. Having access to a wide breadth of experts in my fields, as well as Penn's world-class facilities and resources, also ensures the feasibility of this project.

References: (1) Swenson et al. *Translational Medicine of Aging* 2019; (2) Neumann et al. *Cell Stem Cell* 2019; 3) Althubiti and Macip, *Methods in Molecular Biology* 2019.