Chapter 1

Introduction

1.1 Background

RGCs link the central nervous system with the visual processing hub (Kim et al., 2021). As the RGCs direct their axon to a region in the center of the retina called the optic nerve head (ONH), they converge, creating the optic nerve (ON) to relay visual information as electrical impulses to the brain (Miltner & Torre, 2018). Being the sole output of the retina, injuries or abnormalities to RGCs may result in diseases and eventually irreversible blindness (Fudalej et al., 2021). Examples include inherited optic neuropathies, such as LHON (Leber Hereditary Optic Neuropathy) and ADOA (Autosomal Dominant Optic Atrophy), and acquired optic neuropathies, such as glaucoma, demyelinating optic neuritis, and toxic optic neuropathy (Kim et al., 2021).

Despite being the leading cause of blindness worldwide, the mechanism by which RGCs degenerate and undergo apoptosis is still unknown (Boia et al., 2020; Vernazza et al., 2021). Different RGCs have specific functions, such as optokinetic reflex, gaze control, and circadian rhythm (Dhande et al., 2015). Though understanding RGC on a deeper level would contribute to novel treatment development for slowing down or stopping the disease progression, it was difficult due to their different degradation pathways, unknown involved proteins, and complex molecular mechanisms (Kwong et al., 2021).

To further comprehend RGCs and their pathological mechanisms, researchers have used mouse lines to observe the changes in the reporter gene during development, model diseases, identify pathological changes, and evaluate drug effects (Li, H., et al., 2023). Martersteck et al. (2017) also mentioned that understanding mouse RGCs genetics is aided significantly by driver lines, such as Cre mouse lines. Cre (Cyclization Recombination Enzyme) gene is a 38-kDa protein tyrosine site-specific

recombinase (T-SSRs) that originates from a bacteriophage P1, recognizing the 24 amino acids loxP, which would then delete the DNA sequence located in the middle of two loxP sequences (Jiang et al., 2019). According to Kim et al. (2018) and Shcholok & Eftekharpour (2022), this Cre-loxP system is a potent tool for mouse gene editing, making it the standard for producing genetically engineered mice. RGC-Cre knock-in mouse models such as Brn3/Pou4F or Thy1 mice have been created; however, these mouse lines do not represent RGCs comprehensively and target other cells than RGCs, respectively (Parmhans et al., 2020; Wu et al., 2021). Thus, there is an urgent need to induce Cre mouse lines that are both effective and highly specific to RGCs.

In this study, the Rbpms (RNA binding protein with multiple splicing) locus is being targeted, as it is only expressed profusely in RGCs, making it a specific marker for RGCs, contrasting with other cells (Pereiro et al., 2020). Theoretically, these Rbpms1-Cre knock-in mice would allow researchers to use the Cre-loxP system to edit the mice's RGC genes without altering other retina cells or the body. The research on Cre-Rbpms knock-in mice lines is minimal, urging the need for novel Cre knock-in mice (Li, H., et al., 2023).

Therefore, this research aimed to effectively create Rbpms1-Cre knock-in mice, allowing the Cre enzyme to be expressed only in the RGCs, and consequently, the utilization of the Cre-lox system exclusively for the RGCs. By providing RGC-specific Cre mouse lines, this research hopes to develop a more advanced tool for manipulating genes in RGCs, ultimately contributing to the study of the genetic basis of RGC disease.

1.2 Objective

This research aimed to generate and validate Rbpms1-Cre knock-in mice with Cre expressed explicitly in retinal ganglion cells.

1.3 Hypothesis

From the objective, the following hypotheses were formulated:

H_0: The Cre gene is absent in the KI mouse genome

H_1: The Cre gene is integrated into the KI mouse genome and is expressed exclusively in the retinal ganglion cells of mice

H_2: The Cre gene is integrated into the KI mouse genome and is expressed in the retinal ganglion cells as well as other retinal cells of mice