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The Role and Relationship of S1P and Reelin in the Horizontal-to-Radial Transitioning of Newly Generated DGCs in the Hippocampus

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Abstract: The radial-to-horizontal positioning of newborn dentate granule cells (DGCs) is a crucial step in adult hippocampal neurogenesis, yet the molecular machinery governing this process remains largely obscure. Two extracellular proteins, sphingosine-1-phosphate (S1P) and Reelin, are implicated independently from each other in cell migration and differentiation. However, whether these proteins act cooperatively to accomplish DGC repositioning has not been established. This study aims to examine the interaction between S1P and Reelin during horizontal-to-radial transitioning and whether Reelin modulates S1P signaling. To address this question, RNA sequencing, antibody staining and retroviral knockdowns were used to identify the sites of S1P production and assess the effects of S1P and Reelin deficiency on DGC migration. Our results show that both proteins are required for proper transitioning, as knockdown of either S1P or Reelin leads to loss of radial orientation, reduced dendrite growth, and impaired synaptic activity. Furthermore, our findings suggest that Reelin functions upstream of S1P signaling, uncovering a cooperative action between them in DGC repositioning. By elucidating the roles of S1P and Reelin in neurogenesis through these findings not only gives us a better understanding of hippocampal circuit integration but also open insights into possible therapeutic targets in diseases with impaired neurogenesis, such as cognitive decline and neurodegenerative diseases.

Keywords: Neurogenesis, Horizontal-to-Radial Transition, Sphingosine-1-Phosphate (S1P), Reelin, Neural Circuit Integration

1. Introduction

Neurogenesis in the adult mammalian brain is important for the facilitation of learning, memory retention, and cognitive adaptability and has been associated with diverse neurological conditions such as depression and age-related cognitive deterioration (Teixeira et al., 2012). Neuronal formation occurs in the subventricular zone and the subgranular zone of the dentate gyrus in the hippocampus; the latter is the region in the brain responsible for memory processing and spatial orientation (Abbott & Nigussie, 2020). Here, immature neurons undergo multiple maturation steps, where it has to go through a process known as horizontal-to-radial transitioning before it becomes integrated into the circuit in DG (Yang et al., 2020). One such protein present in the extracellular environment, sphingosine-1-phosphate (S1P), has been found to act on various G protein-coupled receptors S1PR1-5 in the DG.

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S1P serves to modulate cell–cell and cell-matric adhesion, which in turn impacts cell migration and differentiation (Mendelson et al., 2014). Studies have indicated that mice lacking SphK isoenzymes that convert sphingosine to S1P display disrupted neurogenesis, a developmental process suggestive of the role of S1P in neurogenesis (Mizugishi et al., 2005). Other research has shown that S1PR1 is both necessary and sufficient for cell repositioning in the dentate gyrus (Yang et al., 2020), thus highlighting the importance of sphingosine-1-phosphate (S1P) in the horizontal-to-radial transition of neurons. Another extracellular matrix protein, Reelin, regulates neuron migration and dendritic development in the DG. Reelin overexpression accelerates dendritic maturation, while inactivation of the Reelin pathway reduces dendritic growth and disrupts neuron migration, leading to abnormal synaptic circuits (Teixeira et al., 2012). It remains to be determined by contemporary research whether S1P and Reelin exert similar functions in the repositioning of newly generated DGCs or if an interaction exists during the process of repositioning. Given the roles for both S1P and Reelin in cell migration, together with the findings that a deficiency in either protein leads to defects in neurogenesis, we hypothesized that S1P and Reelin cooperate to promote the reorientation of DGCs from a horizontal to a radial position in the DG.

To investigate their relationship, we first aim to identify which cell type or layer within the DG produces S1P by using RNA sequencing and antibody staining of DG tissue for sphingosine kinase. Through pinpointing exactly which cell layer S1P impacts, we can further explore whether Reelin and S1P work cooperatively or independently to contribute to the horizontal-to-radial transitioning of newly generated DGCs. Lastly, we intend to examine if Reelin regulates S1P signalling during horizontal-to-radial transitioning of new DGCs and if Reelin functions upstream or downstream of S1P.

2. Experiment

2.1. Sphingosine kinase sequencing

S1P is synthesized from sphingosine through sphingosine kinase and is naturally expressed in adult mice brains. By extracting the hippocampal stem cells one day after differentiation in cell culture, we can identify cells with high levels of S1P by using high-throughput RNA sequencing to determine the levels of sphingosine kinase in each type of hippocampal cell (Figure 1).

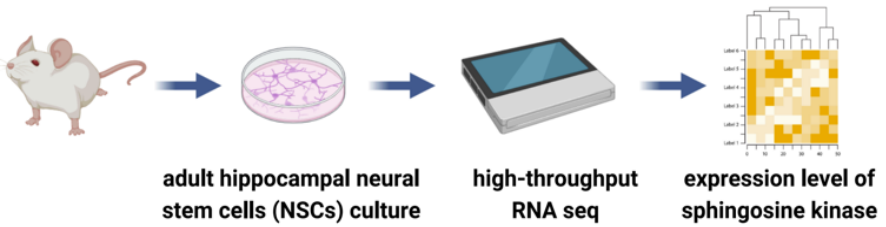


Figure 1: Experiment 1 method: Extracting adult mice hippocampal neural stem cells (NSC) one day after differentiation and sequence S1P kinase using high-throughput RNA sequence. The graph shows a density of sphingosine kinase in different types of hippocampal cells

2.2. Reelin, S1PR1 and Reelin/S1PR1 gene knockdown

To determine if S1P and Reelin are in the same pathway Reelin-only knockdown, S1PR1-only knockdown, as well as Reelin/S1PR1 knockdown, will be completed to explore the phenotype of horizontal to radial transitioning of new DGCs. Retrovirus containing shReelin, shS1PR1 and shReelin/shS1PR1 knockdown gene vectors, as well as GFP, will be injected into the dentate gyrus subgranular zone of adult mice. On day 3, doxycycline water will be fed to the mice and induce the

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expression of the shRNA/GFP genes. Then, on days 5-, 7- and 21-days post-infection, we will look at the neuron phenotypes using fluorescence microscopy and neuron function using electrophysiology recording (Figure 2).

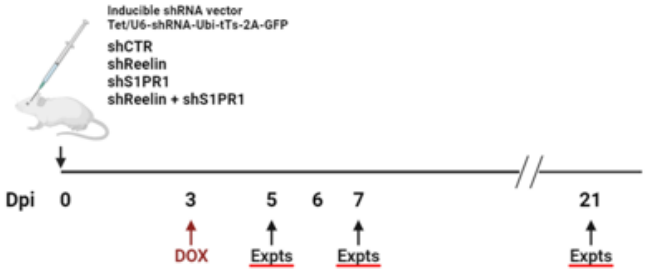


Figure 2: Experiment 2 method: Inject shRNA/GFP into adult mouse dentate gyrus subgranular zone. Feed doxycycline water on day 3. Measure fluorescence image and electrophysiology on days 5, 7 and 21

2.3. Retroviral knockdown and reelin sequencing

To explore whether Reelin regulates S1P signalling, we will use a retrovirus containing shRNA targeting Reelin mRNA to inhibit Reelin production. After 5 days, we will examine levels of S1P and capture fluorescence image DGCs. Additionally, RNA sequencing will be completed to further examine the downstream effects of Reelin inhibition on other genes.

3. Discussion

For experiment 1, within the sub granular zone, we expect to find high levels of sphingosine kinase expression in the middle granular cell layer and inner polymorphic layer (Figure 3). High levels of sphingosine kinase strongly indicate high levels of S1P production, which suggests high levels of cell migration and repositioning in these regions.

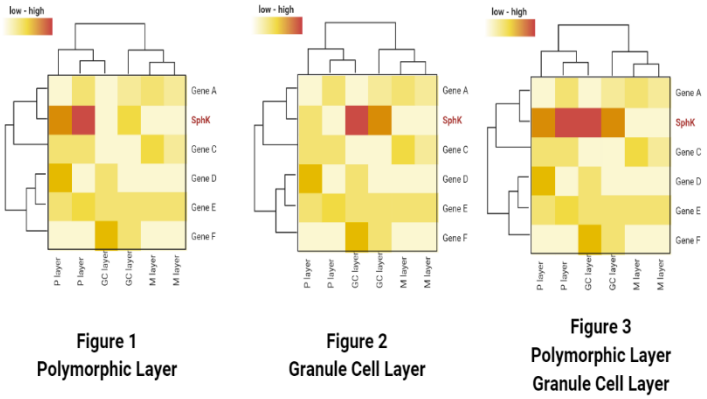


Figure 3: Levels of sphingosine kinase in 1) polymorphic layer, 2) granule cell layer and 3) polymorphic and granule cell layer combined

For experiment 2, imaging is expected to show a significant decrease in fluorescence in shReelin and shS1PR1 compared to the control (shLuc), indicating a decrease in Reelin and S1PR1 expression in the DG, therefore successfully knocking down the gene in the mice.

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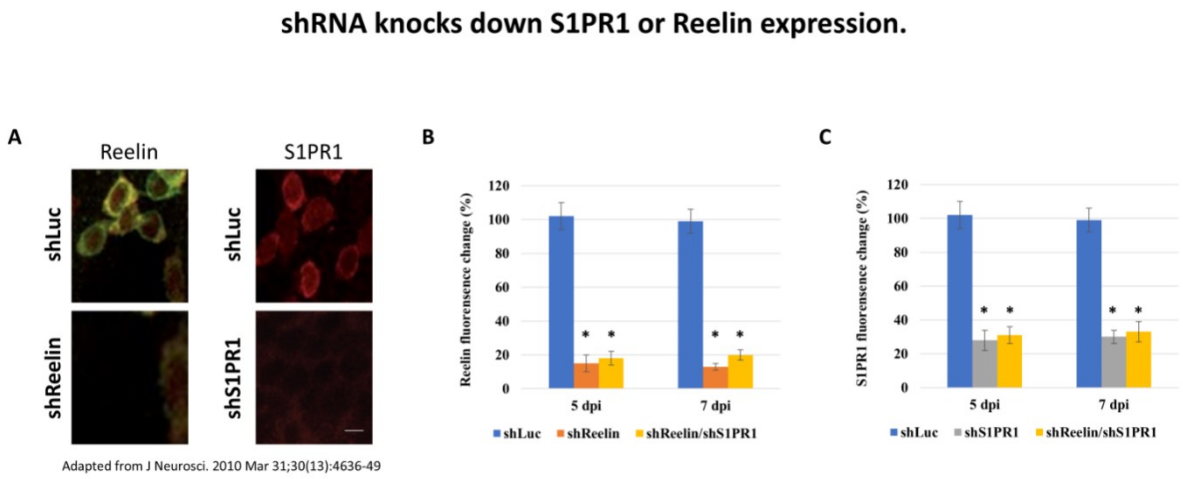


Figure 4: A) Fluorescence image comparison of shLuc and shReelin for Reelin, and fluorescence image comparison of shLuc and shS1PR1 for S1PR1. B) Summary chart of Reelin fluorescence change in percentage for shLuc, shReelin and shReelin/shS1PR1. C) Summary chart of S1PR1 fluorescence change in percentage percentage for shLuc, shReelin and shReelin/shS1PR1

After seven days, the shLuc mice will have a greater number of cells in the radial direction, whereas the shReelin, shS1PR1 and shReelin/shS1PR1 groups will have significantly fewer cells in the radial direction, further supporting the role of S1P and Reelin in the horizontal-to-radial transition. In terms of neurite growth, we expect to see a significant decrease in shReelin, shS1PR1and shReelin/shS1PR1 groups compared to shLuc. Similarly, anticipate a significant reduction in Excitatory postsynaptic potential (ESPC) after 21 days in shReelin, shS1PR1and shReelin/shS1PR1 mouse groups in comparison to shLuc. However, we expect to see similar results across all these phenotypes, or at least non-significant differences between shReelin, shS1PR1 and shReelin/shS1PR1. (Fig. 4). Such non-significant differences would indicate a cooperative interaction between S1P and Reelin in facilitating repositioning.

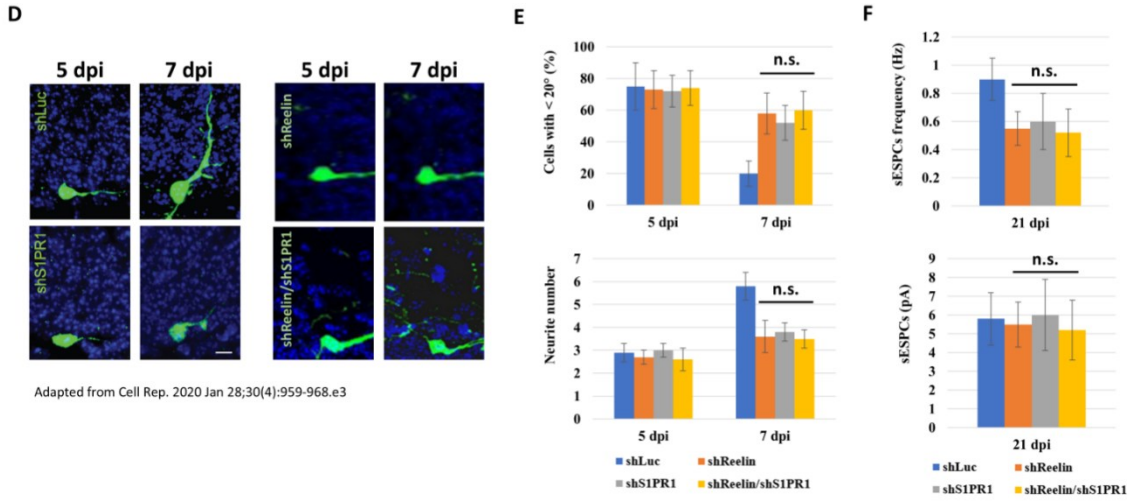


Figure 5: D) Fluorescence imaging of shLuc, shReelin, shS1PR1and shReelin/shS1PR1 at 5 dpi and 7 dpi. E) Cells with <20% fluorescence (top) and neutrite number (buttom) at 5 dpi and 7 dpi. F) sESPCs frequncy (top) and sESPCs (PA) (buttom) at 21 dpi

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In terms of experiment 3, we anticipate a decrease in S1P signaling, suggesting that Reelin functions upstream of S1P. Moreover, we also expect an increased number of horizontal cells in DGCs due to defective transitioning, underscoring the importance of Reelin and S1P in horizontal to radial transition. Additionally, sequencing Reelin could reveal whether S1P signalling is the only process involved in horizontal and radical transition, or if additional mechanisms contribute.

These findings may deepen our understanding of the molecular mechanisms guiding the horizontal-to-radial transitioning of new DGCs and how genetic mutations causing S1P and Reelin loss-of-function may impact the dentate. Additionally, understanding how neurons integrate into existing circuits could shed light on understanding the broader processes of learning and memory.

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