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CELLULAR RESTORATION AND LOCOMOTOR RECOVERY UPON ENDOGENOUS DOPAMINERGIC NEUROREGENERATION IN THE 6-OHDA-LESIONED ADULT ZEBRAFISH PARKINSON'S DISEASE MODEL

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ABSTRACT

Parkinson's disease (PD) is the fastest-growing neurological disorder characterised by progressive degeneration of midbrain dopaminergic neurons (DpN) and consequent motor dysfunction. Unfortunately, there remains no cure for PD and current treatment modalities do not modify the disease's course. These limitations raise the need to focus on regeneration of DpN as a potential approach of PD management. The process is, however, inefficient in mammals, making mammalian-based models unsuited for understanding of neuroregeneration. As such, adult zebrafish is greatly favoured for its close brain homology with human and neuronal self-renewal capability. As part of the effort in elucidating cellular events involved in neuroregeneration *in vivo,* we established and characterised a 6-hydroxydopamine (6-OHDA)-induced zebrafish model of PD which resulted in the targeted ablation of over 85% of tyrosine hydroxylase (TH)-positive DpN within the ventral diencephalon (vDn) day-3 post-lesion. Notably, full cellular restoration was observed at the lesioned site by day-30 post-lesion, indicating effective dopaminergic regeneration of adult zebrafish brain. Locomotor assessment using the open field test revealed >65% reduction in both distance travelled and mean speed ($p < 0.0001$) at day-3 post-lesion, followed by motor recovery by day-30 post-lesion, indicating functional recovery concurrent upon cellular regeneration. In order to examine the proliferative and neurogenic dynamics underlying this recovery, a double-pulse chase using 5-Bromo-2'-deoxyuridine (BrdU) and 5-ethynyl-2'-deoxyuridine (EdU) was employed across early (days 5–7) and late (days 11–13) proliferative phases. BrdU-positive cells were predominantly detected in anterior brain regions such as the olfactory bulb (OB) and pallium-subpallium (Pa-SPa), while EdU-positive cells accumulated in the subpallium (SPa) and preoptic area (POA), adjacent to the lesion site. These findings along with whole brain mapping of both sagittal and coronal planes, indicated a potential migration of newly generated cells from OB and telencephalon (Tel) towards vDn. Subsequent co-labelling of EdU and TH at day-30 showed these proliferative precursors differentiate into mature DpN. In order to facilitate 3-dimensional (3D) visualisation of neuroregenerative processes, we optimised a tissue-clearing protocol employing ethyl cinnamate (ECi) immersion, which demonstrated superior optical clarity and long-term fluorescence preservation compared to the conventional benzyl alcohol and benzyl benzoate (BABB) tissue-clearing method. The cellular contributions of proliferative cells from OB and pallial wall of Tel were then validated through targeted ablation of proliferative cells using N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT, γ-secretase inhibitor). The absence of full recovery of TH-positive cells in 6-OHD+DAPT group underscores the critical role of both proliferative populations at OB and Tel in supporting dopaminergic neuroregeneration in the vDn of the adult zebrafish brain. Whole brain imaging of transgenic zebrafish showed a significant expression of astrocytic (GFAP), oligodendrocytic (Olig2) and general glial (Glula) markers in both POA and vDn by day-14 post-lesion ($p < 0.05$), suggesting the possible role of glial cells in guiding newly generated cells towards vDn. At day-22 post-lesion, expression of the differentiation markers (*foxa2*, *nr4a2a*, and *pitx3*) was detected in the Tel-POA, indicating ongoing late-stage DpN differentiation in area adjacent to the lesion site. Taken together, these findings characterise the phase-specific dynamics of DpN regeneration in the vDn of the 6-OHDA-induced adult zebrafish model, offering insights that may guide strategies to restore loss DpN in PD patients.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Parkinson's disease (PD) is the most common movement disorder and the second leading neurodegenerative disorder in the world (Balestrino & Schapira, 2020; Su et al., 2025). It is commonly characterised by the presence of motor-related symptoms such as muscle rigidity, rest tremor, postural instability and bradykinesia as well as non-motor-related symptoms such as loss of smell and slowness of thinking (Radad et al., 2023). It affects 1-2 per 1,000 people of the population in 2016 and is projected to rise substantially, with the number of affected individuals projected to increase by 112% between 2021 and 2050, reaching an estimated 25.2 million cases worldwide (Bhidayasiri et al., 2024). Notably, individuals with PD have a threefold higher mortality rate compared to the general population, a statistic that has remained unchanged despite advances in modern medical treatment (Benito-Rodríguez et al., 2025; Ryu et al., 2023). In Malaysia, PD affects at least 90 per 100,000 of the total population, a figure which had increased by 56% when compared to data collected in 1990. Similar to other countries in the Southeast Asia region, this increasing trend is projected to continue with the rapidly growing aging population in Malaysia (Ascherio & Schwarzschild, 2016; Maserejian et al., 2020).

The aetiology and pathogenesis of PD remains poorly understood. Early-onset of PD are commonly left undiagnosed as detection of the disease depends solely on evidence of clinical signs that manifested towards the later stage. At present, the vulnerability of dopaminergic neurotransmitters and the loss of dopaminergic neurons (DpN) in PD patients are found to be strongly linked to the manifestation of motor symptoms (Rademacher & Nakamura, 2024). Capitalising on this relationship, several treatments have been designed either to act directly as dopamine replacement (i.e. levodopa) or to compensate the loss of DpN (i.e. deep brain simulation). Although these treatments bring about symptomatic benefit, they do not modify the deteriorating course of the disease (Bloem et al., 2021). In view of this significant weakness, cell replacement therapy has been proposed. The efficacy of this approach is, however, inconsistent given the challenges of graft preparation, cell growth control and