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Editorial Comment

Use of Zebrafish in Senescence-Related Neuro-Muscular Degeneration Research

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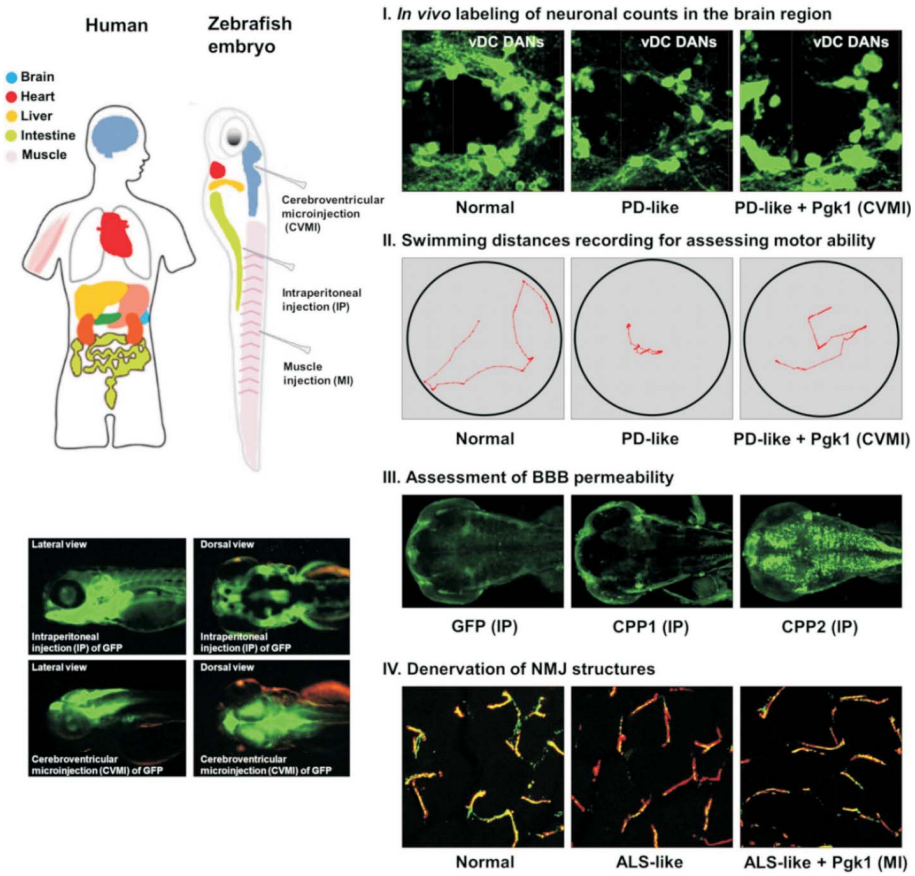

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Central Illustration. Zebrafish as an animal model for studying age-related neuro-muscular degenerative diseases: Left panels: Compared to humans, five-day-old zebrafish embryos, measuring 4-5 mm in length, already possess primary functional organs. Exogenous proteins or drugs can be directly delivered into zebrafish embryos through CVMI, IP, or MI. I. *In vivo* labeling of neuronal counts in the brain region of zebrafish embryos. The zebrafish strain *Tg(dat:EGFP)* embryo immersion in MPTP caused damage to DANs, leading to PD-like symptoms. Addition of exogenous Pgk1 to the brain ventricles of PD-like embryos reduced DANs death. II. Recording swimming distances to assess motor ability. When embryos were immersed in MPTP, which resulted in PD-like symptoms, they exhibited decreased swimming distance. However, addition of exogenous Pgk1 to the brain ventricles of these PD-like embryos helped maintain better swimming distances. III. Assessment of blood-brain barrier (BBB) permeability. Zebrafish embryos were intraperitoneally injected with GFP or fluorescently labeled cell-penetrating peptide (CPP) 1 or CPP2. Only CPP2 was detected in the brain of zebrafish embryos, indicating the efficacy of CPP2 in penetrating the BBB. VI. Denervation of neuromuscular junction (NMJ) structures. Synaptic vesicles at the motor neuron terminals were labeled with SV2 (green fluorescent), and the postsynaptic motor endplates were labeled with acetylcholine receptor by α-BTX (red fluorescent). In a normal NMJ structure, co-localization of the nerve terminals and motor endplates appeared as a yellow signal. ALS-like symptoms were induced in adult zebrafish using the *Tg(Zα: TetON-Rtn4al)* transgenic line, resulting in denervation of the NMJ structures. Exogenous addition of Pgk1 alleviated the denervation of motor neurons in ALS-like adult zebrafish.

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide and remains incurable. The pathology of PD is characterized by the presence of intracellular protein inclusions called Lewy bodies (LBs) and Lewy neurites, formed by the misfolding and aggregation of α-synuclein within dopaminergic neurons (DANs). Other pathological changes in patients with PD include the formation of neurofibrillary tangles caused by hyperphosphorylated tau protein and the deposition of amyloid-β. The accumulation of LBs leads to the degeneration and death of DANs in the substantia nigra pars compacta (SNpc). A reduction in the number of DANs in the SNpc results in insufficient dopamine secretion at synaptic terminals in the dorsal striatum, which affects the control of voluntary movements and cognitive functions in the basal ganglia, thereby causing PD symptoms. Clinically, PD can be identified by se-

veral motor symptoms, such as bradykinesia, rigidity, tremor, and postural instability. Non-motor symptoms, such as sleep disturbances, psychiatric disorders, autonomic dysfunction, anosmia, depression, and cognitive impairment, can also occur.[1]

Statistically, the annual incidence rate of PD is 0.015%, with a prevalence of nearly 2–3% in people over 65 years of age. Approximately 20% of afflicted patients have a family history of PD, whereas more than 80% have sporadic PD. Although several genes associated with PD have been identified, the etiology of most cases remains unknown. Age is considered the most significant risk factor for PD, with men being 1.5–2 times more likely to develop the disease than women. Other contributing factors include environmental and genetic factors.[1] The average age at the onset of PD is approximately 60 years. Owing to the diverse pathogenic mechanisms of PD, its treatment efficacy is limited. Therefore, there is a need to develop neuroprotective drugs that can prevent the loss of DANs while alleviating autonomic and cognitive dysfunction in patients.[2]

Zebrafish are small, easy to breed, maintain, and exhibit high genomic homology with humans, making them important model organisms in pharmacology, developmental genetics, and human disease studies. Symptoms of PD in zebrafish can be induced by exposing embryos to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or by intraperitoneal injection of adult zebrafish, resulting in the damage and death of DANs. A transgenic zebrafish line, *Tg(dat:EGFP)*, with the green fluorescence-labeled dopamine active transporters, which allows us for non-invasive imaging of DANs due to the transparency of zebrafish embryos.[3] Furthermore, by recording and analyzing the swimming distance of embryonic and adult zebrafish using imaging software, zebrafish motor dysfunction can be quantitatively assessed. Our previous study demonstrated that extracellular Pgk1 promotes neurite outgrowth in motor neuron cell lines.[4] When further administering exogenous Pgk1 into the zebrafish brain ventricle via cerebroventricular microinjection, it was found that Pgk1 has a novel function in reducing MPTP-induced DAN death and maintaining better motor performance in zebrafish.[5] To enhance the application of Pgk1, it is necessary to develop methods for delivering Pgk1 through the bloodstream across the blood-brain barrier (BBB) into the central nervous system. Interestingly, zebrafish have similar BBB structures and functions as mammals at 3 days post-fertilization.[6] This characteristic allows for the rapid assessment of whether exogenous Pgk1 combined with cell-penetrating peptides[7] can be delivered to the zebrafish brain.

In addition, adult human skeletal muscles shrink with age, leading to loss of muscle mass (sarcopenia) and frailty, which increases the risk of falls, injuries, and mortality. However, the functions of nerves and muscle contractions are closely interconnected. The synaptic contact area between motor neurons and their target muscle fibers is the neuromuscular junction (NMJ). The synaptic site on the muscle membrane is the motor endplate. The NMJ is a highly specialized synapse that ensures efficient transmission of electrical impulses from presynaptic motor neurons to postsynaptic muscle fibers to stimulate contraction. Aged NMJs have been shown to have a reduced number of neurotransmitter-containing vesicles at presynaptic nerve terminals. In addition, aged NMJs exhibit fragmented endplates with shallower folding, leading to a decreased surface area and a reduced number of postsynaptic acetylcholine receptors.[8] Functional muscle denervation is currently hypothesized to be a key factor in the pathogenesis of sarcopenia, with some researchers even describing sarcopenia as an NMJ disease. Therefore, enhancing NMJ stability may improve neuromuscular diseases and delay sarcopenia. Using a zebrafish transgenic line *Tg(Zα:TetON-Rtn4al)* to mimic the disease progression of amyotrophic lateral sclerosis,[9] we injected Pgk1 into the back muscles of adult zebrafish. Pgk1 maintains the NMJ structure and reduces motor neuron denervation at the injection site.[4] This study demonstrated that Pgk1 has the potential to protect neurons and slow the progression of neurodegenerative diseases. Similar to mammals, zebrafish exhibit typical vertebrate aging characteristics, leading to a functional decline in phenotypes that are highly similar to human pathology.[10] Hence, zebrafish can significantly contribute to the biology of aging and the development of therapeutic strategies to mitigate age-related degeneration.

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Footnotes

All procedures described herein were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC; A111005) of MacKay Medical College and were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals.

Conflicts of interest

All authors declare no conflicts of interest.

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