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Polysaccharides from *Basella alba* protect post-mitotic neurons against cell cycle reentry and apoptosis via suppression of sonic hedgehog expression induced by amyloid-beta peptide

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Abstract

Aims: Amyloid-beta peptide (Aβ), the main neurotoxic component of senile plaques in the Alzheimer's disease (AD) brains, may trigger cell cycle reentry followed by apoptosis in post-mitotic neurons. Polysaccharides from *Basella alba*, a perennial vine that is also a vegetable, possess anticancer and immuno-modulatory properties. However, whether polysaccharides extracted from the perennial vine (PPV-6) may exert beneficial effects against AD and, if so, the underlying mechanisms remain unclear.

Methods: Primary rat cortical neurons exposed to Aβ were used as the *in vitro* AD model. MTT assay and Hoechst staining were used to investigate the neuroprotective effects of PPV-6 against Aβ-induced cytotoxicity. Western blotting and immunocytochemistry were performed to examine the expression levels of proteins. The change of oxygen consumption rate (OCR) was determined by Seahorse analyzer. Flow cytometry and BrdU incorporation were conducted to measure DNA synthesis as an index for cell cycle progression. **Results**: We found that, as compared with Aβ25-35 alone, cell viability and neuronal morphology were recovered by co-treatment with PPV-6. Aβ25-35-mediated induction of cell cycle markers, including cyclin D1, phosphorylated retinoblastoma protein (pRb-Pi), proliferating cell nuclear antigen (PCNA), and histone H3 phosphorylated at Ser-10 (His-H3-Pi), as well as caspase-3 cleavage were all suppressed by PPV-6. Further mechanistic investigation revealed that PPV-6 inhibited Aβ25-35-induced expression of sonic hedgehog (SHH) as well as neuronal cell cycle reentry induced by the biologically active N-terminal fragment of SHH (SHH-N). **Conclusions:** The neuroprotective mechanisms of PPV-6 against Aβs involve, at least in part, downregulation of Aβ-induced SHH expression and suppression of neuronal cell cycle reentry.

Fig. 1

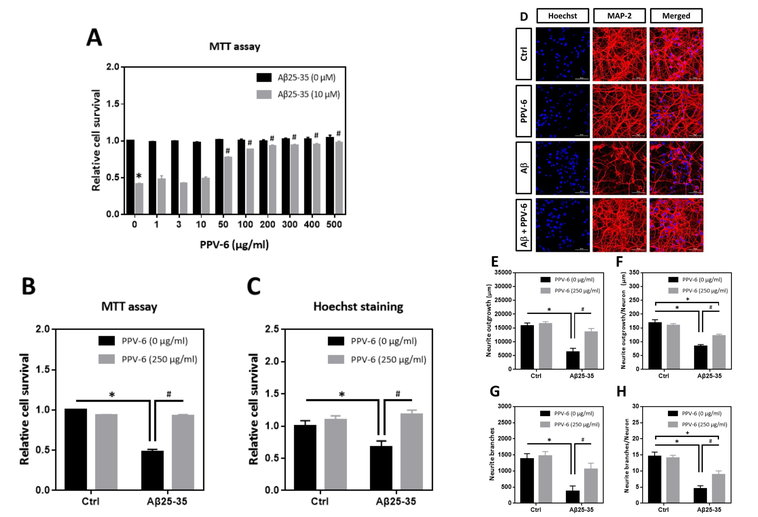

Fig. 1: PPV-6 dose-dependently protects cortical neurons against Aβ25-35 toxicity with restoration of neuronal morphology.

Fig. 2

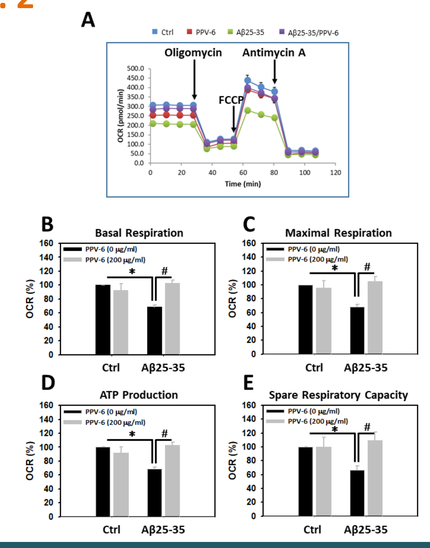

Fig. 2: PPV-6 restores mitochondrial bioenergetics impaired by Aβ25-35.

Fig. 3

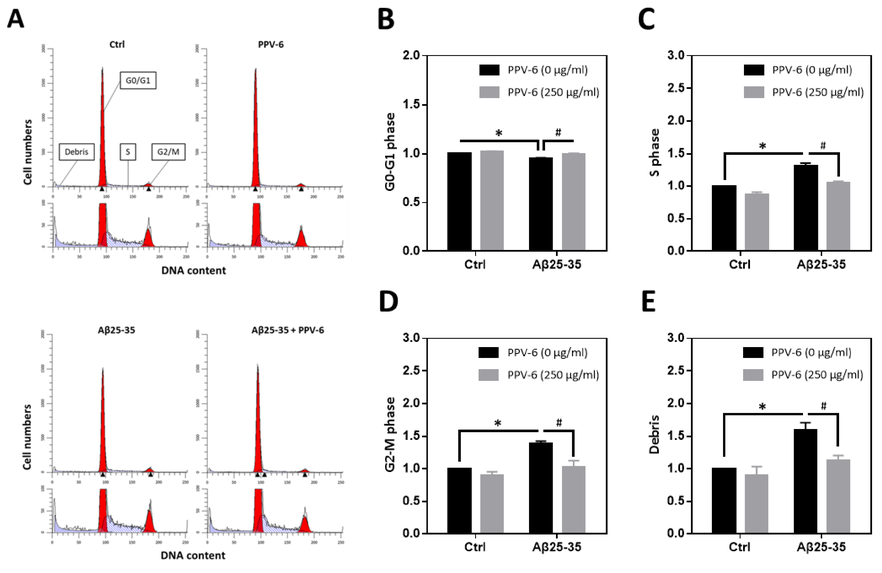

Fig. 3: PPV-6 attenuates the extents of cell cycle progression induced by Aβ25-35 based on flow cytometry.

Fig. 4

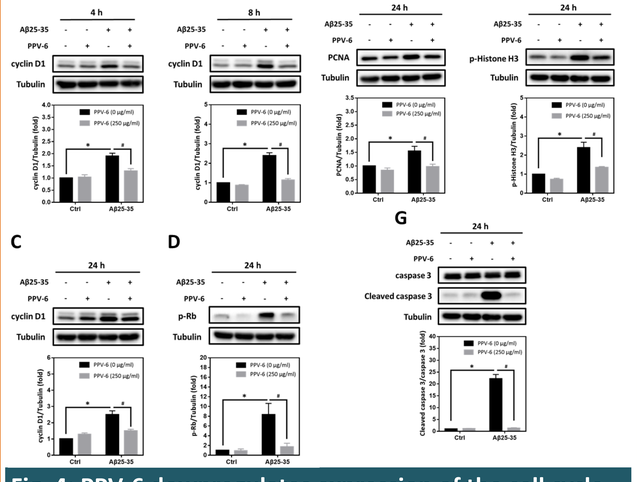

Fig. 4: PPV-6 downregulates expression of the cell cycle markers and caspase-3 cleavage induced by Aβ25-35.

Fig. 5

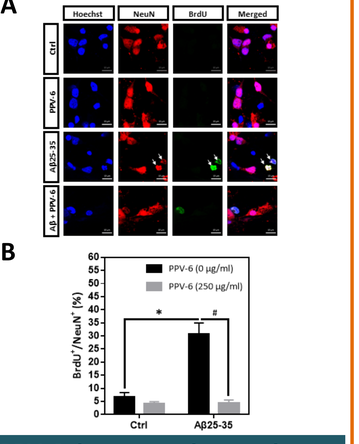

Fig. 5: PPV-6 decreases the numbers of post-mitotic neurons incorporating BrdU upon Aβ25-35 exposure.

Fig. 6

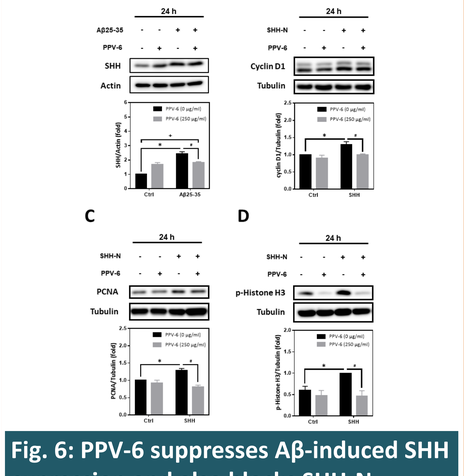

Fig. 6: PPV-6 suppresses Aβ-induced SHH expression and also blocks SHH-N-mediated neuronal cell cycle reentry.

Acknowledgements

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