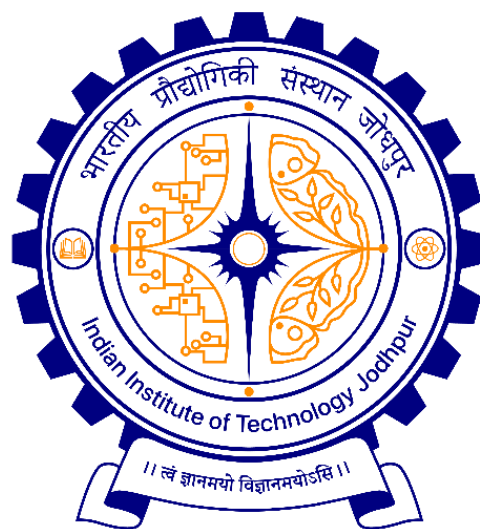


Exploring the Potential of Cell and Tissue Derived Extracellular Vesicle for the Development of Novel Neuro Therapeutics for Adult Neurogenesis

A Thesis submitted by
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Chapter 5

Summary and Future Perspective

5.1 SUMMARY

Aging and neurodegenerative diseases notably impair adult neurogenesis, leading to a reduction in neuroplasticity and hindered neuronal regeneration. This decline, especially in areas like the subventricular zone (SVZ) and hippocampus, is marked by a decrease in the proliferation of neural stem cells (NSCs) and an increase in the number of quiescent NSCs. As aging progresses, neurogenic populations in the brain dwindle, contributing to cognitive decline and the development of neurodegenerative diseases such as Alzheimer's and Parkinson's. While existing treatments like stem cell transplantation and neurodegeneration prevention strategies show potential, their ability to reverse age-related damage or significantly promote regeneration remains limited. Therefore, there is a critical need for innovative approaches to boost NSC proliferation and differentiation to overcome these challenges. Extracellular vesicles (EVs), small lipid bilayer vesicles secreted by various cells, have gained attention as a promising therapeutic platform due to their ability to cross the blood-brain barrier and their non-invasive, non-immunogenic, and non-tumorigenic properties. Specifically, exosomes derived from stem cells and other tissues have shown potential in promoting cellular communication and regeneration. These exosomes can encourage the differentiation, proliferation, and survival of NSCs, making them a promising candidate for developing effective treatments for neurodegenerative diseases. Research has shown that EVs can aid in the differentiation of neural stem or progenitor cells into functional neurons, further supporting their potential in neurotherapeutic applications.

1. The thesis begins with an introductory chapter that outlines the current advancements in the field of extracellular vesicles/exosomes. It discusses how exosomes have been utilized for studying disease mechanisms, discovering biomarkers, and developing neurotherapeutics. The main findings of the research presented in this thesis are summarized as follows:
2. The second chapter discusses the development of an efficient peptide-based ligand specifically targeting the EphA4 receptor using an in-silico approach. This strategy involves the chemical conjugation of the peptide with extracellular vesicles (EVs) derived from adipose tissue, resulting in the creation of Exo-pep-11. Notably, this engineered EV, Exo-pep-11, selectively targets neural stem cells (NSCs) via EphA4 receptors, providing promising therapeutic benefits by enhancing NSC proliferation and differentiation. Our findings demonstrate that NSCs effectively internalized Exo-pep-11 under both *in vitro* culture conditions and *in vivo* aging rats. Furthermore, we observed that the uptake of Exo-pep-11 decreased by approximately 2.3-fold when NSCs were pre-treated with an EphA4 antibody prior to incubation with Exo-pep-11, confirming the receptor-specific uptake of this engineered EV. Treatment with Exo-pep-11 resulted in a 1.9-fold increase in NSC proliferation and a 1.4-fold and 2.4-fold increase in the expression levels of Nestin and ID1, respectively. Additionally, Exo-pep-11 demonstrated potential for enhancing neurogenesis in aging rats, as evidenced by a 1.6-fold and 1.5-fold increase in the expression of tyrosine hydroxylase (TH) and Tuj1, respectively, in the olfactory bulb of these animals. Overall, our results

underscore the potential of Exo-pep-11 for therapeutic applications aimed at countering age-related declines in NSC activity and neurogenesis.

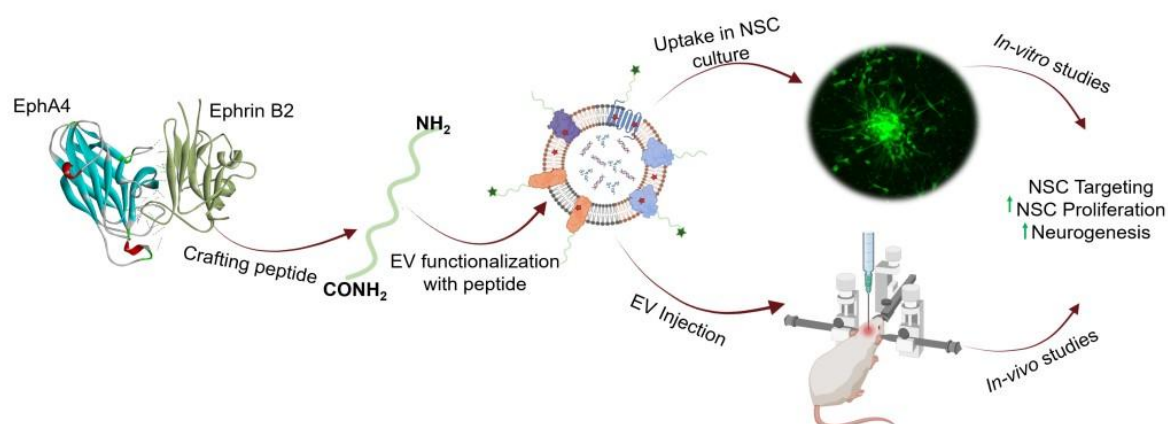


Figure 5.1 A representative figure summarizing Chapter 2.

3. In The third chapter, we explored the potential of Extracellular Vesicles (EVs) derived from the substantia nigra (SN) of rats from different age groups to address age-related declines in neural stem cell (NSC) activity, as well as their neurogenic and neuroprotective effects, using an aging rat model treated with 6-hydroxydopamine (6-OHDA). This model shows diminished NSC activity due to aging and dopaminergic neurodegeneration caused by 6-OHDA treatment. Our findings revealed that 6-OHDA induced neurodegeneration and microglial activation at the injection site in the rat olfactory bulb, further aggravated by reduced adult neurogenesis in the aging rats. *In vitro* studies showed that EVs derived from 1-month-old rat SN (SN-EV-1) were most effective in promoting NSC differentiation into functional dopaminergic neurons. *In vivo* experiments demonstrated that SN-EV-1 increased the expression of dopaminergic markers, enhanced NSC differentiation, and facilitated their migration to the olfactory bulb while reducing 6-OHDA-induced damage. Behavioural assessments confirmed the neuroprotective benefits of SN-EV-1. This study underscores the therapeutic potential of SN-EV-1 in enhancing dopaminergic neurogenesis and providing protection against neurodegenerative damage in aging rats, offering promising new approaches for treating neurodegenerative disorders such as Parkinson's disease.

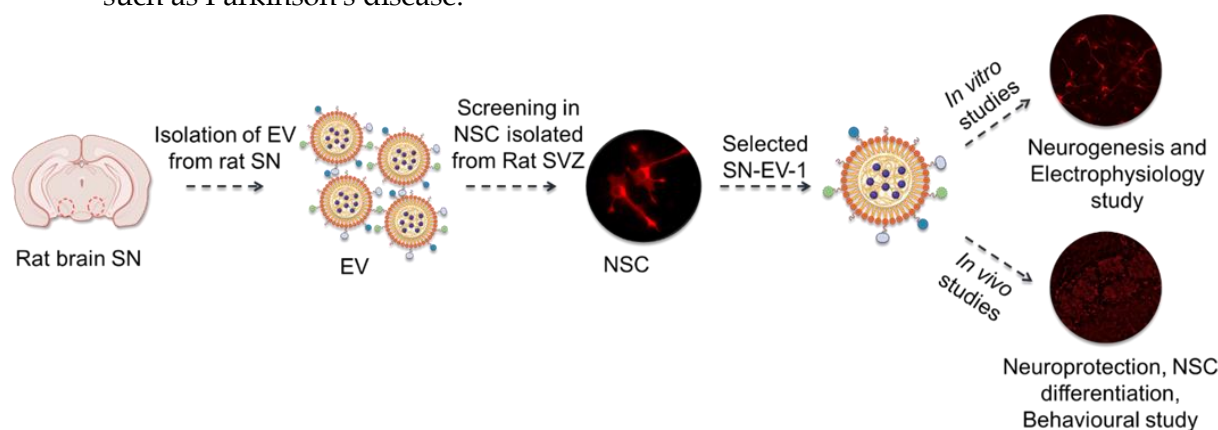


Figure 5.2 A representative figure summarizing Chapter 3.

4. The fourth chapter of the thesis outlines a protocol for isolating extracellular vesicles (EVs) derived from the substantia nigra (SN) and conducting subsequent proteomic analyses. Given that neurodegeneration in the substantia nigra is linked to Parkinson's disease, comparing EVs from healthy and diseased SNs, along with their proteomic profiles, will enhance our understanding of disease mechanisms and aid in identifying exosome-based biomarkers. This study presents a novel protocol that combines differential centrifugation and immunocapture techniques to achieve a purer and higher yield of exosomes compared to traditional ultracentrifugation methods. Our proteomic analysis identified 51, 48, and 70 proteins from three distinct exosome samples (SN-EV-1, SN-EV-2, and SN-EV-4), with Gene Ontology annotations revealing their involvement in various biological functions. This research establishes a reliable method for isolating brain-derived exosomes and lays the groundwork for comparative studies between healthy and neurodegenerative conditions. Ultimately, our findings aim to deepen the understanding of exosomal roles in disease mechanisms and contribute to the identification of potential biomarkers and therapeutic targets for neurodegenerative disorders.

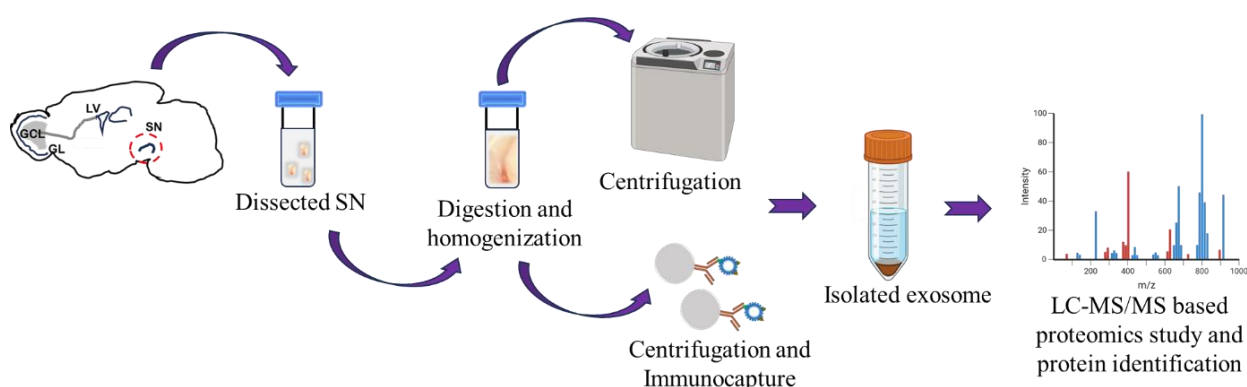


Figure 5.3 A representative figure summarizing Chapter 4.

5.2 POTENTIAL OF EVs FROM DIVERSE TISSUE SOURCES

While this chapter primarily focuses on EVs derived from specific tissues—such as adipose-derived stem cells and neural tissues—there is a growing body of evidence suggesting that EVs from a range of tissue and cell types can exert beneficial effects on neurogenesis and neuroprotection. For example, EVs isolated from mesenchymal stem cells (MSCs) of bone marrow, umbilical cord, and dental pulp have been shown to cross the blood-brain barrier, modulate inflammatory responses, and promote neuronal survival and differentiation in various models of neurodegeneration. Similarly, EVs from immune cells and endothelial cells have demonstrated the capacity to influence neural cell fate and repair processes. The tissue origin of EVs is a critical determinant of their molecular cargo—proteins, lipids, RNAs, and metabolites—which in turn shapes their functional properties. Thus, while EVs from different tissues may share some common neuroprotective mechanisms like delivery of regulatory molecules (miRNA, protein, DNA etc.), modulation of signalling pathways, transfer of

metabolic enzymes, they may also possess unique attributes that could be harnessed for tailored therapeutic strategies.

5.3 FUTURE PERSPECTIVE

The presented thesis encompasses three distinct studies, each contributing valuable insights into the role of engineered extracellular vesicles (EVs) in addressing neurodegenerative challenges. The first study investigates peptide-functionalized EVs designed to target neural stem cells (NSCs) and mitigate aging-related neuro-complications. The second study focuses on substantia nigra-derived EVs, examining their effects on dopaminergic neurogenesis and neuroprotection following 6-hydroxydopamine (6-OHDA) treatment. The third study introduces a protocol for isolating EVs from the substantia nigra and conducting subsequent proteomic analyses. Given that substantia nigra neurodegeneration is linked to Parkinson's disease, comparing EVs derived from healthy and diseased substantia nigra and analyzing their proteomic profiles could enhance our understanding of disease mechanisms and facilitate the identification of exosome-based biomarkers. The promising findings from this research highlight the potential of engineered EVs to target NSCs and promote neurogenesis, providing significant insights into therapeutic strategies for age-related neurodegenerative diseases and neural injuries. This work also represents the first exploration of substantia nigra-derived EVs, documenting their role in dopaminergic neurogenesis and neuroprotection. Furthermore, the thesis outlines a protocol for isolating EVs from rat substantia nigra and performing proteomic analyses.

Building on these findings, several key directions for future research and development emerge. The use of adipose tissue-derived EVs (Exo-pep-11) establishes a foundation for investigating other sources of EVs that may possess enhanced targeting capabilities or regenerative properties. Exploring EVs derived from various tissues, particularly in the context of dopaminergic neurodegeneration, presents a promising avenue for addressing specific neurodegenerative diseases such as Parkinson's disease. Additionally, investigating induced pluripotent stem cell (iPSC)-derived or mesenchymal stem cell (MSC)-derived EVs could yield versatile therapeutic candidates with tissue-specific targeting abilities.

Transitioning from preclinical studies to clinical applications will require addressing challenges related to manufacturing, quality control, and scalability. As engineered EVs progress to clinical trials, it will be essential to evaluate their safety, immunogenicity, and efficacy in human subjects. Employing personalized medicine approaches could further tailor EV-based therapies to individual patients based on their unique genetic profiles and disease characteristics. Identifying biomarkers that predict individual responses to EV treatments will be crucial in advancing this personalized approach and ensuring its success in treating neurodegenerative diseases.

A deeper understanding of the mechanisms underlying age-related declines in NSC function and neurodegeneration is vital for refining therapeutic strategies. Investigating how aging and neurodegeneration specifically affect NSC niches, signalling pathways, and cellular interactions in the brain will provide insights into optimizing therapies. Research into the cellular and molecular changes occurring in aged NSCs and their microenvironments will help identify additional targets for intervention.

Moreover, beyond their therapeutic potential, EVs can serve as powerful diagnostic tools. Future studies should explore the role of substantia nigra-derived EVs in developing brain disorders and identify potential biomarkers for assessing neurodegenerative diseases. Analyzing the contents of SN-EVs described in this thesis may lead to a better understanding of the mechanisms underlying neurodegenerative diseases and treatment responses.

In conclusion, this study lays a solid foundation for utilizing EVs to promote NSC rejuvenation and neurogenesis, particularly concerning aging and neurodegenerative diseases. It also provides methods for isolating SN-EVs and conducting proteomic studies. However, extensive research is necessary to optimize EV production, enhance blood-brain barrier penetration, evaluate long-term efficacy, and explore combination therapies to maximize the therapeutic potential of EV-based strategies while advancing biomarker discovery for neurodegenerative diseases. Addressing these challenges may pave the way for the clinical application of EVs in treating neurodegenerative disorders, offering a powerful tool for restoring brain function, understanding disease mechanisms, and combating the effects of aging and neurodegeneration.