CHAPTER: I

1. GENERAL INTRODUCTION

Fundamental characteristic features shared by almost all neurodegenerative diseases is dysregulated cell death predominantly through activation of cell death cascades by various genetic and environmental triggers. Glutamate excitotoxicity is one such trigger which is implicated in the pathogenesis of neurodegenerative disorders like Alzheimer’s disease (AD) and temporal lobe epilepsy (TLE). In such disease condition, progressive hippocampal neurodegeneration due to excitotoxicity leads to deterioration in both acquiring and recalling spatial information and other co-morbidities such as depression (1). Pharmacological therapeutics targeting excitotoxicity have failed due to their non-specificity along with the undesirable side effects associated with them. The possibilities of stem cell therapy as an alternative approach for treating neurodegenerative diseases holds considerable promise and hope. Stem cell based approaches were designed on the premise that grafted stem cells would differentiate into neurons at the lesion site, thus compensating for the neuronal cell losses induced by degenerative insults. The concept of adult neurogenesis is now largely accredited and is known to occur in two brain regions, i.e. the sub ventricular zone (SVZ) and sub granular zone (SGZ) of the dentate gyrus (DG) (2–6). Even though hippocampal neurogenesis is a continuous process, the rate at which it generates new neurons in SGZ is not sufficient enough to compensate for the extensive neuronal loss observed in many hippocampal neurodegenerative conditions. Stem cell therapy has been investigated extensively in the past and the functional recovery following such therapies were encouraging despite of various debates about the possible mechanisms by which functional recovery would have occurred. Two prospective ways through which functional recovery could be attained includes, stimulation followed by mobilization of endogenous stem cells towards the degenerating sites (7–9) or by transplanting exogenous neural stem/progenitor cells which can proliferate and substitute for the lost neurons (10–12). Stem cells, which are undifferentiated cells have the ability to self-renew and differentiate into other cell types, based on such properties, they are classified as totipotent, pluripotent or multipotent cells. Mesenchymal stem cells (MSCs) being multipotent in nature are explored extensively in recent years as they have less ethical constraints, least possibilities for teratoma formation and high immunomodulatory potential (13). Adult MSCs can be derived from umbilical cord, placenta, wharton’s jelly, adipose tissue,
bone marrow and dental pulp (14–17). Given the plethora and easy isolation procedures, it is not surprising to know that till date ~986 MSCs based clinical trials are exploring their therapeutic potential against a gamut of diseases (Source: Clinicaltrial.gov). Bone marrow mesenchymal stem cells (BM-MSCs) is one of the best characterized MSCs that were primarily considered as “to go” MSCs for treating various diseases (13). Nevertheless, a recent study demonstrated that the migration potential of BM-MSCs is far less compared to other MSCs due to the weak cell surface expression of homing factors (18,19). Additionally, due to the highly invasive bone marrow isolation procedure (20) coupled with lower yields of MSCs (21,22), there is a necessity to comprehensively study the biological properties of alternate source of MSCs that possess clinical benefits alike to BM-MSCs. For instance, identification of dental pulp stem cells (DPSCs), which are ontogenetically related to ectodermal neural crest cells with inherent tendency to differentiate along the neural lineage (23) have provided an opportunity to explore a tailor made MSCs for treating neurodegenerative diseases. However, as compared to BM-MSCs, till date there is no study that investigated the neuroprotective ability of DPSCs against excitotoxicity mediated hippocampal neurodegeneration. Therefore, several comparative studies are warranted to estimate whether highly migratory neural crest originated adult stem cells like DPSCs can engraft and protect degenerating neurons equal to BM-MSCs, if so, what are the molecular differences (e.g. growth factor expressions) between these MSCs such information would assist the scientists as well as the clinicians to choose appropriate MSCs for treating neurodegenerative diseases. Of note, based on the previous studies it has been reached to an accord that the functional recovery following MSCs transplantation does not exclusively depend on the transdifferentiation of transplanted MSCs but also via their paracrine effects (24). In recent years, secretome mediated tissue regeneration has fascinated more attention. This is primarily due to convergence of observations in several studies that in spite of poor engraftment of transplanted cells, there was a significant behavioural recovery in several animal models of neurodegenerative diseases studied thus support the view that grafted cells exhibit a bystander effect by releasing various growth factors and cytokines which might have activated various hosts’ endogenous reparative mechanisms for functional recovery. Nevertheless, there is no comparative study to evaluate the neuroprotective potential of hDPSCs, hBM-MSCs and their respective conditioned medium (CM) in an in vitro as well as in vivo model of hippocampal neurodegeneration. Consequently, in Objective 1 (Chapter III) of the present study, we compared the neuroprotective potential and mechanism of neuroprotection mediated by hDPSCs, hBM-MSCs and their CM in an in vitro model of hippocampal neurodegeneration. In Objective 2 and 3 (Chapter IV), we validated the in vitro
observation of neuroprotection mediated by hDPSCs, hBM-MSCs and their respective CM in an animal model of hippocampal neurodegeneration. Furthermore, the anti-inflammatory, neurogenic potential and hippocampal functional recovery were compared between hDPSCs, hBM-MSCs and their respective CM in an animal model of hippocampal neurodegeneration. As our results revealed that CM could recapitulate the neuroprotective effects of stem cell transplantation, in Chapter V, we systematically characterized the CM by subjecting them to molecular weight-based fractionations and evaluated their neuroprotective potentials in an in vitro model of hippocampal neurodegeneration. We observed that the low molecular weight biomolecules present in the CM demonstrated neuroprotection against excitotoxicity as opposed to high molecular weight biomolecules in the CM. Further exploration of CM revealed that the exosomes isolated from CM particularly from the early passages of DPSCs/BM-MSCs were neuroprotective as compared to later passages of DPSCs/BM-MSCs. Of note, the neuroprotective effect of DPSCs/BM-MSCs derived exosomes are dose dependent. In Objective 4 (Chapter IV), we explored the molecular mechanisms of neuroprotection mediated by hDPSCs, hBM-MSCs and their CM in an in vivo model of hippocampal neurodegeneration. Our results revealed that hDPSCs, hBM-MSCs and their CM treatments, protects degenerating neurons by increasing the expressions of hosts’ endogenous neurotropic factors such as brain derived neurotropic factor (BDNF) and through anti-apoptotic mechanism. Besides, hDPSCs, hBM-MSCs and their CM treatment could activate hosts’ endogenous cell survival mechanisms such as Phosphoinositide 3-kinase (PI3K)-B-cell Lymphoma-2 (Bcl-2) to protect neurons.
OBJECTIVES OF THE STUDY

**Objective 1:** To study the neuroprotective efficacy and to understand the mechanisms of neuroprotection mediated by hDPSCs/hBM-MSCs and hDPSCs/hBM-MSCs derived CM against kainic acid induced hippocampal neurodegeneration in an *in vitro* condition.

**Objective 2:** To study the neuroprotective efficacy of transplanted hDPSCs/hBM-MSCs and hDPSCs/hBM-MSCs derived CM treatment against kainic acid induced hippocampal neurodegeneration in an *in vivo* condition.

**Objective 3:** To study the hippocampal functional recovery following hDPSCs/hBM-MSCs transplantation and hDPSCs/hBM-MSCs CM treatment.

**Objective 4:** To decipher the molecular mechanisms of neuroprotection mediated by hDPSCs/hBM-MSCs and hDPSCs/hBM-MSCs derived CM against kainic acid induced hippocampal neurodegeneration in an *in vivo* condition.