1. INTRODUCTION

Introduction:

Parkinson's disease (PD) is the second most common neurodegenerative movement disorder after Alzheimer’s disease, affecting millions of elderly individuals worldwide (Savitt *et al.*, 2006; Calabrese, 2007). This is clinically characterized by resting tremor, rigidity, bradykinesia and postural instability due to the preferential loss of dopamine-producing neurons in the *substantia nigra pars compacta* region of the midbrain (Beitz, 2014). At the time of clinical presentation, approximately 50–70% of DAergic neurons in the nigrostriatal system have been lost (Orth and Schapira, 2002). The other pathological hallmark of PD is the presence of round eosinophilic intracytoplasmic proteinaceous deposits termed Lewy bodies (LBs) and dystrophic neurites (Lewy neurites) in surviving neurons. These aggregates are mainly composed of fibrillar α-synuclein, molecular chaperones, ubiquitin and neurofilaments (Spillantini *et al.*, 1997). However, the LB is not an obligate marker of the disease as some familial PD cases. (Takahashi *et al.*, 1994; Mori *et al.*, 1998; Hayashi *et al.*, 2000). Clinically well-defined markers and designed therapy are still lacking for neurodegenerative diseases. The mechanisms leading to progression and selective loss of dopaminergic neurons in PD had been a focus of research for the last several years; however, it is not well understood. There is no effective therapy and dopamine (DA) supplementation only provides symptomatic relief, however, no effect on disease progression. It is important to further understand the neuronal cues leading to selective neurodegeneration in PD to find the next generation of therapeutic strategies.

Neuroinflammation and its association with neurodegenerative diseases are emerging. The first evidence of the association of neuroinflammation and PD was concluded from the presence of HLA-DR-positive reactive microglia within the substantia nigra of patients with PD and in the experimental animal studies. (McGeer *et al.*, 1988). Recently, neuroinflammation has been suspected as the major cause of the gradual loss of dopaminergic neurons promoted by microglial activation and release of pro-inflammatory cytokines and chemokines (Block *et al.*, 2007). It had been observed that particularly tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6 and interferon- γ (IFN-γ), are upregulated in the DAergic nigrostriatal system and other brain regions outside the SN and as well as in CSF and serum in PD patients (Mogi *et al.*, 1994a; Mogi *et al.*, 1994b; Blum-Degen *et al.*, 1995; Stypula *et al.*, 1996; Dobbs *et al.*, 1999; Reale *et al.*, 2009). The increased levels of TNF-α and its cognate receptor in serum and also in the substantia nigra have been observed in PD patients sample (Mogi *et al.*, 2000a; Scalzo *et al.*, 2009). The mechanism of inflammation induced pathogenesis of PD specifically selective neuronal loss is not well understood.

Now there are established evidence suggesting mitochondrial dysfunction is one of the major hallmarks of PD (Schapira, 1993; Lang and Lozano, 1998; Bender *et al.*, 2006). Several lines of direct evidence shows the dysfunction of mitochondria is observed in tissue derived from PD patient samples as well as the high level of mtDNA depletion was seen in SN neurons from PD patients (Schapira, 1993; Lang and Lozano, 1998; Bender *et al.*, 2006). The other direct evidence of mitochondrial dysfunction in PD came from the toxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), a toxin induced neurodegeneration in animal similar to PD where is leads to decrease the complex-1 activity, leading to ATP depletion and reactive oxygen species (ROS) production is observed. Emerging evidence strongly shows defective mitochondria is associated with inflammation. Mitochondrial dysfunction activates NLRP3 inflammasome activation as well as induces redox-sensitive factor of the NF-κB pathway. This leads to increased level of pro-inflammatory cytokines like TNF-α and IL1-b (Su *et al.*, 2008; Chesselet *et al.*, 2012; Watson *et al.*, 2012). These cytokines further induce mitochondrial dysfunction. The implication of mitochondrial mediated inflammation and its association with the pathogenesis of PD is not well understood. These evidence strongly suggest that maintenance of healthy mitochondrial network is critical in PD pathogenesis. The equilibrium of network of the healthy mitochondria is maintained through the process of dynamic fusion and fission (Chen and Chan, 2009). The stressed or damaged mitochondria is labelled through ubiquitination and selectively degraded through a process known as mitophagy (Chen and Chan, 2009; Gomes and Scorrano, 2013). Mitochondrial homeostasis is critical for maintaining normal neuronal cellular function hence it is important to maintain a healthy pool of mitochondria. The process of autophagy plays a vital role in neuronal survival through clearing defective mitochondria, miss-folded or un-folded protein aggregates as well as regulates the level of key regulatory proteins. Autophagy is regulated by the network of protein complexes in different stress and pathological condition. The defect in autophagy flux leads to accumulation of degradable cargo. It had been shown that two familial gene PINK1/PARKIN frequently observed to be mutated in PD patients, which have an important role in selective autophagy, mainly mitophagy by regulating the identification of defective cargo (Deas *et al.*, 2011; Pickrell and Youle, 2015). In the last step of the autophagy process selective defective cargo containing autophagosome fuses with lysosomal for degradation. As lysosome plays the vital role in clearing process and its role in the pathogenesis of PD is emerging. The mutations in genes glucocerebrosidase (GBA) and lysosomal type 5 P-type ATPase (ATP13A2) of lysosomal genes leads to impaired lysosomal membrane instability, impaired lysosomal acidification, decreased processing of lysosomal enzymes, and is known to be associated with PD. Mitochondria and lysosomal cross talk in PD pathogenesis are emerging. Chemical induced mitochondrial dysfunction leads to impair in autophagic flux and lysosomal function in an animal as well as a cell based model of Parkinson's disease (Wu *et al.*, 2015). The regulation of autophagic flux and its cross-talk with mitochondrial function/dynamic in PD is not understood. The alteration in homeostasis of autophagy pathway may lead to aggregation of defective mitochondria and leads to neurodegeneration hence the regulators of autophagy may be regulated in narrow physiological range. The miRNAs, emerging class of small non-coding RNAs, play important role in the regulation of mRNA copy number and protein level in the narrow physiological range (Tran and Hutvagner, 2013). Our group already demonstrated the association of non-coding RNA mainly miRNAs with mitochondria (Sripada *et al.*, 2012). This association of miRNAs with mitochondria shows the regulation of nuclear-encoded mitochondrial transcript as well as regulation of the mitochondrial homeostasis related transcript at different stress condition. The experimental evidence suggests the miRNAs has special significance to PD. It has been observed that genetic and pharmacological intervention of miRNA biogenesis in mid brain leads to loss of dopamine neurons and produces symptoms like PD (Kim *et al.*, 2007)**.** The role miRNAs in the regulation of autophagy and mitochondrial homeostasis in PD stress condition is not well understood.

In the current study, we systematically investigated the differentially expressed miRNAs in PD stress conditions using human SH-SY5Y cell line as a model system. Further, their role in the regulation of autophagy and mitochondrial functions was explored. We developed an inflammatory model of PD and characterized novel set of miRNAs playing an important role in mitochondrial function and organellar cross talk in a neuronal cell.