10. SUMMARY AND CONCLUSION

10.1. Summary:

Several lines of the evidence supports the view that defect in mitochondrial function, inflammation, cellular quality control of mechanisms like the ubiquitin-proteasome system (UPS) and autophagy play a major role in the further pathogenesis of PD.

The active quality control mechanisms are required for the neuronal survival. The defect in these mechanisms leads to accumulation of defective mitochondria leading to inflammation, ROS and neuronal cell death. In the current study it was planned to systematically study the miRNAs role as an endogenous modulator of autophagy and mitochondrial pathway in the cell based model of Parkinson's disease.

The role of miRNAs in inflammatory mediated PD stress condition:

Emerging evidence suggest that chronic neuroinflammation is one of the major causes of progression of neurodegeneration in PD. It has been finely demonstrated that mitochondria is a central player of inflammation. However, the systematic study has not been yet done in a case of expression of miRNAs and its role in mitochondrial function in the inflammatory stress condition. In the current study, we first time demonstrated inflammatory mediated cell based model of PD and type of cell death. We investigated the role of miRNAs in the regulation of TNF-α induced mitochondrial functions using, DAergic cell line, SH-SY5Y (model of dopaminergic neuron degeneration akin to PD). The major findings can be summarized as below:

- Prolonged exposure of TNF-α induces apoptotic cell death in SH-SY5Y.
- TNF-α sensitizes SH-SY5Y cells to PD stress (6-OHDA, Rotenone and α-synuclein) induced cell death.
- TNF-α induces mitochondrial oxidative stress and decreases mitochondrial membrane potential in SH-SY5Y cells.
- TNF-α decreases mitochondrial complex-I and ATP in dopaminergic cells.
- TNF-α modulates mitophagy in SH-SY5Y cells.
- The cells treated with TNF-α showed decreased level of nuclear encoded transcript of mitochondrial complexes.

- TNF-α differentially regulates miRNAs expression involved in the pathogenesis of PD.
- TNF-α regulates miRNAs expression targeting mitochondrial functions in SH-SY5Y cells.
- The levels of hsa-miR-27a, hsa-miR-17, hsa-miR-103 and hsa-miR-155 significantly increased in the presence of TNF-α.
- Inhibition of hsa-miR-155 rescues TNF-α induces cell death in SH-SY5Y cells.

The evidence in the current study demonstrated that TNF-α is a potential regulator of miRNAs which may regulate mitochondrial functions and neuronal cell death, having important implication in pathogenesis of PD

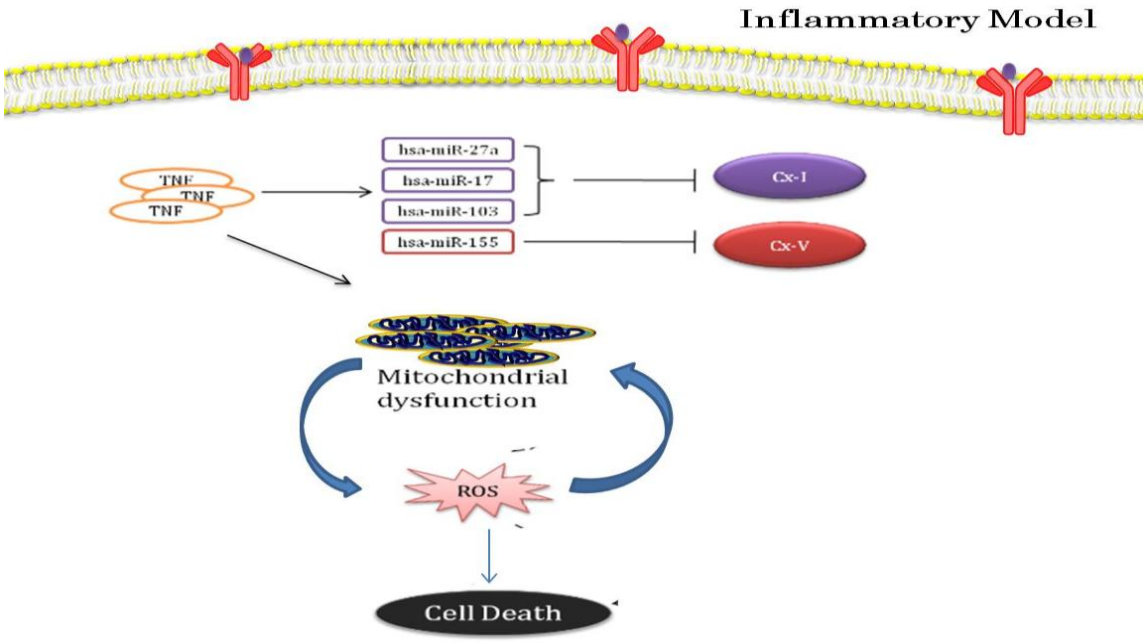

Figure 10.1: Summary of role of miRNAs in inflammatory mediated PD stress condition

Identification of expression pattern of miRNAs and its implication in toxin mediated PD stress condition:

6-hydroxydopamine (6-OHDA) is a neurotoxicant which induces PD like conditions in the experimental animal model and induces degeneration of DAergic neurons. It had been observed that 6-OHDA inhibits the mitochondrial respiratory chain complexes I and IV activity and inducing oxidative stress. In the current study, we first time identified the signature miRNAs expression pattern in 6-OHDA induced PD stress condition.

- miRNAs expression are altered in 6-OHDA induced PD stress model of SH-SY5Y
- Identified top five up and down regulated miRNAs target gene which involved in Neurotrophin Signaling pathway, mTOR pathway and cell death pathway.
- The level of hsa-miR-5701 was significantly downregulated in the presence of 6-OHDA in SH-SY5Y.
- The putative targets of miR-5701 target the genes involved in lysosomal biogenesis and mitochondrial quality control.
- The transcript level of VCP, LAPTM4A and ATP6V0D1 decreased significantly in the presence of miR-5701 mimic.
- The expression of miR-5701 mimic showed mitochondrial dysfunction and defect in autophagy flux.
- The expression of miR-5701 sensitizes SH-SY5Y cells to 6-OHDA induced cell death.

The evidences in the current study demonstrated that signature pattern of miRNAs expression in PD stress conditions and characterized a novel miR-5701 as a potential regulator of mitochondrial and lysosomal function leading to neuronal cell death and may have important implication in the pathogenesis of PD.

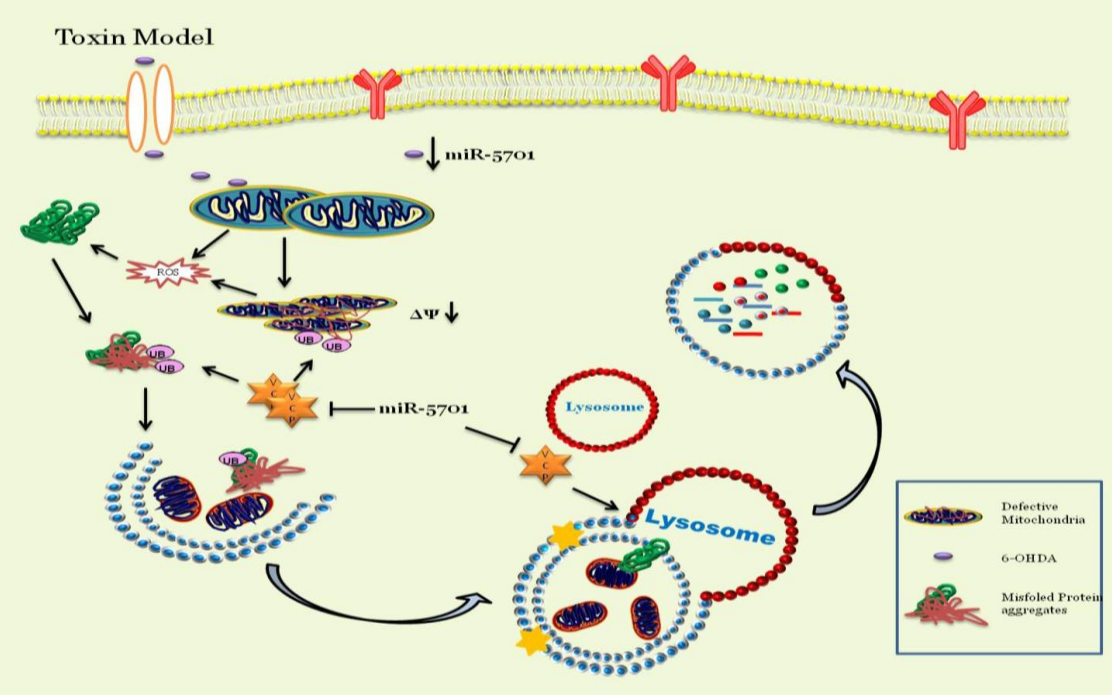

Figure 10.2: Summary of role of miR-5701 in toxin mediated PD stress condition

10.2 Conclusion:

Inflammatory mediated PD stress study provided evidence that increased levels of TNF-α decrease mitochondrial complex-I, leading to oxidative stress, activation of caspase cascade leading to mitochondrial dysfunction and neuronal cell death. TNF-α regulated miRNAs target nuclear encoded subunits of mitochondrial ETC. The altered levels of miRNAs in the presence of TNF-α reduced the subunit of mitochondrial complex-I and complex-V, which leading to decreased levels of ATP. This study strongly suggests that TNF-α regulates miRNA targeting mitochondrial functions that may have important implication in the dopaminergic neuronal loss in PD.

In toxin base model of PD, It was identified signature patterns of miRNAs expression which may have a regulatory role in PD pathogenesis. Next, we identified novel miR-

5701, which was significantly down regulated in PD stress condition. Moreover, we also identified, VCP as a novel direct target of the miR-5701, which modulates autophagy-flux and mitochondrial quality control. The increased level of miR-5701 sensitized to 6-OHDA induced cell death. This study strongly suggests that miR-5701 regulate mitochondrial and lysosomal cross talk during PD stress conditions. This study further showed the miRNA can regulate inter organellar cross talk which is important for PD pathogenesis.

10.3 Limitation and Future perspective:

There is no standard biochemical test and treatment modality for PD, hence intense research in this area is further required.

Inflammation is one of the major cause progressive degeneration of neurons in PD as well as other neurodegenerative conditions. In the current study, inflammatory mediated cell model for PD was developed and concluded that TNF-α regulates miRNAs that modulate mitochondrial functions, induces oxidative stress and apoptosis in neuronal cells. Four miRNAs were further analyzed and showed the targets for nuclear encoded mitochondrial complex proteins. The experimental validation of one of the highest potential miR-155 showed that TNF-α regulates the expression of miR-155 that modulated neuronal cell death. The further to the characterization of other potential miRNAs for their role in mitochondrial function and neuronal cell death needs to be done. The identified miRNAs needs to be further validated in the brain tissue or CSF obtained from PD patients having increased levels of TNF-α in serum and CSF and showing the symptoms of Parkinsonism.

In the toxin base model of PD, top five up and down regulated miRNAs were identified. However, the role of miR-5701 in the regulation of mitochondrial function and autophagy flux was further characterized. Thus, functional characterization of other miRNAs will need to be done in cell model system. The expression of identified miRNAs should be further done in PD patients CSF or brain tissue.

The long-term goal is to identify miRNAs are endogenous modulators of PD pathogenesis and use of modified oligonucleotides for targeting endogenous miRNAs as possible therapeutics in PD.