1. Introduction

1.1 Mitochondria: emerging role beyond bioenergetics.

Mitochondria are double membrane bound organelle present in all eukaryotic cells. The cells with high energy demand like muscles, neurons and hepatic cells are evolved with numerous and highly specialized three dimensional mitochondrial networks. The mitochondrion is dynamically involved in numerous physiological processes from generation of ATP by oxidative phosphorylation, generation and detoxification of reactive oxygen species (ROS), apoptosis, calcium homeostasis, synthesis and catabolism of metabolites and the transport of the organelles themselves to distant locations within the cell (Chatterjee et al., 2011; Jeppesen et al., 2011; Nakagawa et al., 2007; Tschopp, 2011)

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Mitochondria also plays critical role in specialized functions like urea cycle, γ - aminobutyric acid cycle, amino acid metabolism, one-carbon metabolism, FeS protein synthesis, heam synthesis, fatty acid metabolism, fission and fusion (Adeva et al., 2012; Hertz, 2011; Schöneich, 1975; Stemmler et al., 2010). Its involvement in multiple processes makes it crucial part of the cell.

Abnormalities in any of these processes are cause and consequences of mitochondrial dysfunction leading to numerous pathologies. Diseases such as Kearns-Sayre syndrome, Pearson's syndrome, and progressive external ophthalmoplegia are due to large-scale mtDNA rearrangements, whereas MELAS, LHON, MERRF are due to point mutations in mtDNA. Some mitochondrial associated disease like Friedreich's ataxia and Wilson's disease are consequence of defects in nuclear encoded genes (Schapira, 2012). The oxidative stress causes mitochondrial dysfunction in schizophrenia, bipolar disorder, dementia, Alzheimer's disease, Parkinson's disease, epilepsy, stroke, cardiovascular disease, retinitis pigmentosa, and diabetes mellitus (Greaves et al., 2012; Schon and Przedborski, 2011). This strongly suggest that dys-function of mitochondria is major cause of various pathologies like neurodegeneration, cancer, aging, infection and inflammation (Jeppesen et al., 2011; Tschopp, 2011).

The understanding of regulation of mitochondrial function is of immense importance to understand the various mitochondrial associated developmental and ageing related disorders.

1.2 New regulators of mitochondrial function: transport of ncRNA to mitochondria.

The mitochondrial genome encodes 13 proteins, 22 tRNAs and 2 rRNAs which is insufficient even for its optimal functioning (Andersson et al., 2003). Mitochondrial proteome had provided many newer aspects of regulation of mitochondrial functions. The mitochondrial proteome contains approximately 1500 proteins which are nuclear genome encoded and are transported to mitochondria for the optimal functioning. The transport of nuclear encoded RNA to mitochondria is also emerging as further mechanism to regulate mitochondrial function. The nuclear proteins and essential non coding RNAs are imported into mitochondria depending upon the, energy demand of the target cell (Calvo and Mootha, 2010).

The transport of nuclear encoded mRNA and ncRNA like 5S rRNA, RNaseP and RNase MRP are essentially imported into mitochondria for transcription and translation of its genome. Emerging evidence demonstrate the enrichment of mRNA and ribosome at close vicinity to mitochondria. The regulation of translation of mRNA transcript is now known to be regulated by non coding small RNA called as miRNA (Siomi and Siomi, 2009). They may regulate the target mRNA copy number and translation in narrow physiological range.

We hypothesized the association, import and transport of miRNA at and within mitochondria for site specific fine tuning of de novo synthesized protein within mitochondria or to shields of nascent mRNA transport.

1.3 miRNA in physiopathology

miRNA are endogenous RNAs highly conserved across the genomes of animals, plants, fungi and viruses. miRNAs account for ~ 1% of the human genome. The

miRNAs are transcribed from genomic DNA by RNA polymerase II as ~1000 nt long primary miRNA (pri-miRNA) with 5' cap and 3' tail. The pri-miRNA is then processed by Dorsha/Pasha complex into precursor miRNA (pre-miRNA) with a 2-nt overhang at 3' end. The 2-nt overhang of pre-miRNA is recognized by Exportin-5 and exported out of nuclei. In the cytosol, pre-miRNA is cleaved by Dicer into ~20 nt miRNA/miRNA* duplex. The guide strand of miRNA is loaded in miRISC complex, binds to the complimentary region of target mRNA and regulates translation repression (Yang and Lai, 2011). The guide strand bound Ago2 complex screens complimentary 3' UTR region in target mRNA and recriuts acessory proteins to fine tune the protein levels. The binding complementarity of seed sequence and mRNA regulates the fate of mRNA. The perfect complementarity between seed sequence (6-8 nt sequence beginning from first or second nt at 5') and target mRNA leads to mRNA degradation whereas imperfect complementarity causes translation repression or ribosome falling off from polysomes. The miRNA may bind to different cognate mRNAs and regulate numerous protein levels from same or different pathways.

Their aberrant expression link them with numerous diseases; including cancer, cardiovascular disorders, schizophrenia, renal function disorders, diabetes, Tourette's syndrome, muscular disorders, Fragile-X mental retardation syndrome, chronic hepatitis, AIDS and obesity by hampering critical cellular processes like proliferation, senescence, autophagy and both extrinsic and intrinsic cell death (Chatterjee et al., 2011; Jeppesen et al., 2011; Tschopp, 2011).

1.4 miRNA modulates mitochondrial function in cell death.

The mitochondrial dysfunction and aberrant miRNA expression is found in numerous physio-pathological process as both cause and consequence. The mitochondria and miRNA both regulate the cell death pathway in numerous ways. Apoptosis is important from development to adult body homeostasis.

Dysregulation of apoptosis leads to serious pathological disorders viz. neurodegeneration, diabetes (increased apoptosis) cancer, inflammatory diseases, autoimmune diseases (decreased apoptosis) (Susin et al., 1999; Verhagen et al., 2000).

The mitochondria are central executioner of apoptosis. It plays important role both in intrinsic and extrinsic pathway of apoptosis. The extrinsic pathway is initiated by the binding of death ligands, such as Fas ligand (FasL) or tumor necrosis factor α (TNF-α) to their corresponding death receptors which in turn oligomerizes and forms death-inducing signaling complex (DISC). The intrinsic pathway is initiated with the release of mitochondrial pro-apoptotic proteins such as Cytochrome-c, mitochondrial apoptosis-inducing factor (AIF) and Smac/Diablo (Cleland et al., 2011; Karbowski et al., 2006). Cytochrome-c forms a complex with Apaf-1 and procaspase-9 resulting in the activation of caspase-9. The intrinsic pathway is well regulated by the Bcl-2 family members (Gao et al., 2010).

The role of miRNA in intrinsic apoptotic pathway was first observed in chronic lymphotytic leukemia (CLL) patients. hsa- miR-15a and hsa- miR-16-1 were abnormal in patients (Akao et al., 2006). These miRNA targets Bcl-2, Mcl1 and impairs mitochondrial integrity in patients. hsa-miR-143 modulates cell proliferation by fine tuning ERK-5 levels. Its levels are found to be down regulated in colon cancers (Yu et al., 2008). The up-regulation of muscle specific miRNA, hsa-miR-1 results in impairment of mitochondrial membrane potential to release cytochrome-c (Karsy et al., 2012; Krichevsky and Gabriely, 2009). The same is true in experimental models, hsa-miR-21 modulates activation of caspase 3/ 7 in panel of cell lines (Karsy et al., 2012; Krichevsky and Gabriely, 2009).

These evidences suggest that miRNA regulates the cell death regulating proteins in narrow physiological range which are specifically localized on the outer mitochondrial membrane.

1.5 spatial localization of miRNA in cell: association with mitochondria.

The mitochondria and miRNA are not isolated and static, as previously believed but are dynamic in nature. The mitochondria undergo fusion-fission dynamics to maintain (Castanotto et al., 2009) its integrity, enhance OXPHOS, to remove impaired regions via coupled mitophagy. The organelle moves constantly in cellular milieu across

microtubule with help of protein complexes. The organelle may also stall at required regions to balance calcium levels, generate ATP or to balance redox requirements.

The localization of pre and mature miRNA and proteins involved in its biogenesis are not confined to site of their maturation, cytosol. The miRNA translocates to various sub-cellular regions: nucleus (Huang et al., 2011; et al., 2007), processing (P) bodies (Makarova et al., 2016), Multiple Vesicular Bodies (MVB) (Gibbings, 2011; Lee et al., 2002) and exosomes (Lesnik et al., 2015). Similarly, the mitochondria itself is found to have close contact with cytosolic ribosomes at its surface (Zhang et al., 2015), endoplasmic reticulum via mitochondria associated membrane and dynamically associates with P-bodies, exosomes (Zhang et al., 2015), stress granules and melanosomes.

Emerging evidences suggest enrichment of mitochondria, RNA binding proteins, translational apparatus and miRNAs at synapses, de-novo axonal branches, dendritic spines, soma, axon of neurons and lamellipodium of migrating cancer cells.

Hence we hypothesized systematic association and crosstalk of mitochondria and miRNA at particular cellular locations. miRNAs may regulate both nuclear DNA encoded mitochondrial targeted mRNA and mitochondrial DNA (mt DNA) encoded transcripts to modulate its function affecting cell death pathways in various physio-pathological conditions. The mitochondria on the other hand may either serve as novel transport machinery or localized fine tuning platform at distant cellular regions where the organelle stalls. The crosstalk of the miRNA and target mRNA may regulate critical cellular processes like axonal pruning, synaptic transmission, synaptic plasticity, cellular migration and cell death.

Here, we systematically characterized the association of miRNAs with mitochondria, to decipher the impact on mitochondrial function and cell death in human cells.