# 5. Association of small ncRNA with mitochondria: A Systematic approach

Mitochondria are one of the central regulators of many cellular processes beyond its well established role in energy metabolism. The inter-organellar crosstalk is critical for the optimal function of mitochondria. Many nuclear encoded proteins and RNA are imported to mitochondria. The translocation of small RNA (sRNA) including miRNA to mitochondria and other sub-cellular organelle is emerging and still not clear. Here, sRNA including miRNA association with human mitochondria is systematically characterized by cellular fractionation and deep sequencing approach.

### 5.1. Isolation and Analysis of Mitochondrial small RNA

To analyse the presence of microRNA in mitochondria, a highly purified population of mitochondria was isolated from HEK293 cells. The purity of mitochondrial fraction was confirmed by western blotting (Figure 5.1A). It was observed that NDUFS2, a subunit of mitochondrial complex-I, is highly enriched in purified mitochondrial fraction whereas RPS9 was only detectable in total cell and not in purified mitochondrial fraction. Ribosomal protein S9 (RPS9) is S4P family protein, a component of 40S ribosomal subunit, which is localized mainly in nucleolus and cytoplasmic ribonucleic component (Lindstrom MS et. al, 2008) but not in mitochondria. Hence, RPS9 was used to determine nuclear and cytosolic contamination. This evidence strongly suggests that purified mitochondrial fraction was free from nuclear as well as polysomal contamination. The purity of mitochondria was also confirmed by PCR using specific primers for genomic and mitochondrial DNA. The mitochondrial preparations were devoid of nuclear genomic markers, while both mitochondrial and nuclear markers were present in total cellular counterparts (Figure 5.1B).

RNA was isolated from total cells and purified mitochondria from both HEK293 and HeLa. The quality of RNA was determined by RNA integrity number (RIN values). The total cellular RNA showed clear peak of 28S and 18S rRNA at 50 sec and 43 sec with RIN values of 8.5 and 10 for HEK293 and HeLa respectively (Figure 5.1C). The microfluidic electrophoresis of mitochondrial RNA showed several peaks (Figure 5.1D). It suggests

presence of sRNAs other than 28S and 18S therefore traditional RIN may not be valid for mitochondrial RNA integrity.

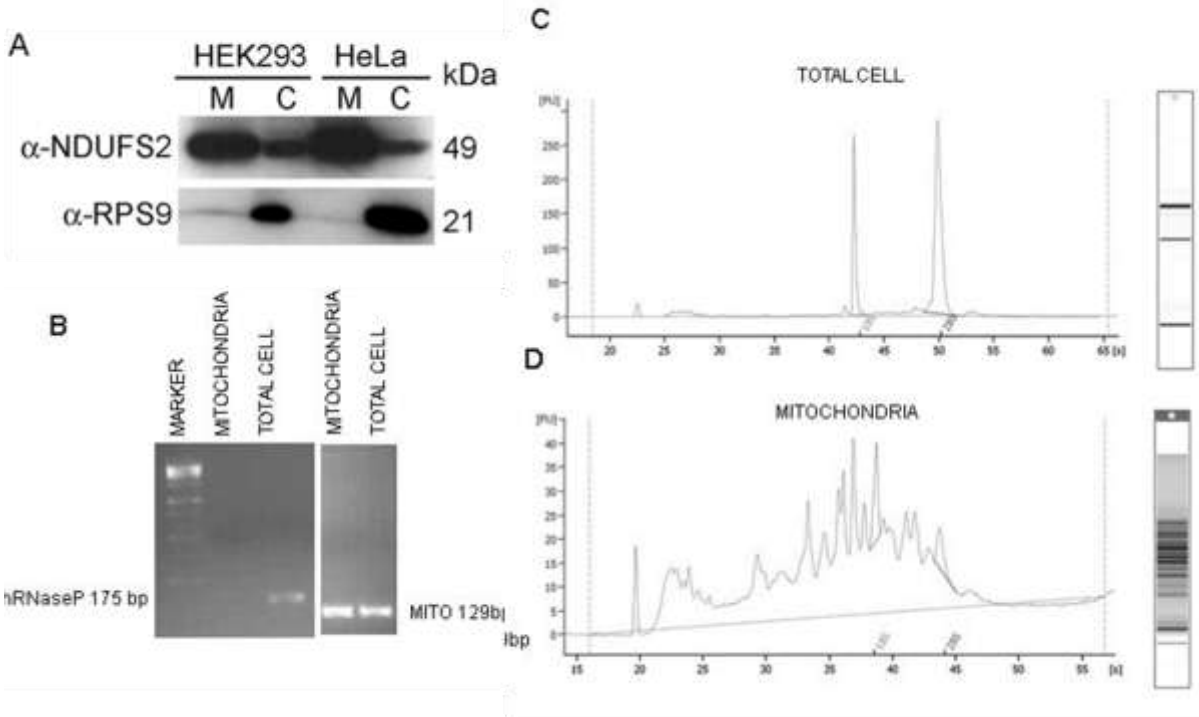


***Figure 5.1. Quality assessment of mitochondrial RNA.*** *The mitochondria were isolated and purified from HEK293 and HeLa. (A) The protein contents of whole cell lysate (C), and purified mitochondria (M) were normalized, resolved on 12.5% SDS-PAGE, transferred to PVDF membrane and probed with NDUFS2 and RPS9 antibody (B) lane labelling PCR of cellular fractions. The DNA was extracted from mitochondria and cell. Lane 2, 3 were amplified using hRNaseP primer. Lane 4, 5 were amplified using primer specific for mitochondrial genome. It clearly shows the absence of genomic impurities in mitochondrial fraction. The integrity and quality of RNA fractions was checked using the Agilent 2100 Bioanalyzer. Electrophoretic images of (C) cytosolic RNA from HEK293 with RIN value 10 (D) mitochondrial RNA from HEK293 with numerous small peaks and undetermined RIN values.*

The purity of mitochondrial RNA was also analysed at RNA level by RTPCR of β-actin, nuclear encoded gene and ND4, mitochondrial encoded gene. The nuclear encoded

mRNA, β -actin was only detected in the RNA from total cells and was absent in the mitochondrial fraction (Figure 5.2A) whereas ND4 was detected both in mitochondrial as well as total cellular RNA (HEK293 and HeLa) as expected. This suggests that there is no non specific association of nuclear encoded RNA with mitochondria. The mitochondrial RNA purity was further analyzed by qPCR for cytosolic RNA contamination by analyzing the presence of two nuclear encoded mRNA (TRIM4 and MITA) and two mitochondrial DNA encoded (ND4 and CYB) mRNA were taken as positive controls. The mitochondrial encoded mRNA was enriched significantly in mitochondrial fraction as compared to cytosolic mRNA (Figure 5.2B). These two experiments also suggest that mitochondrial RNA further analyzed by deep sequencing, is free from nonspecific association of nuclear encoded RNA.

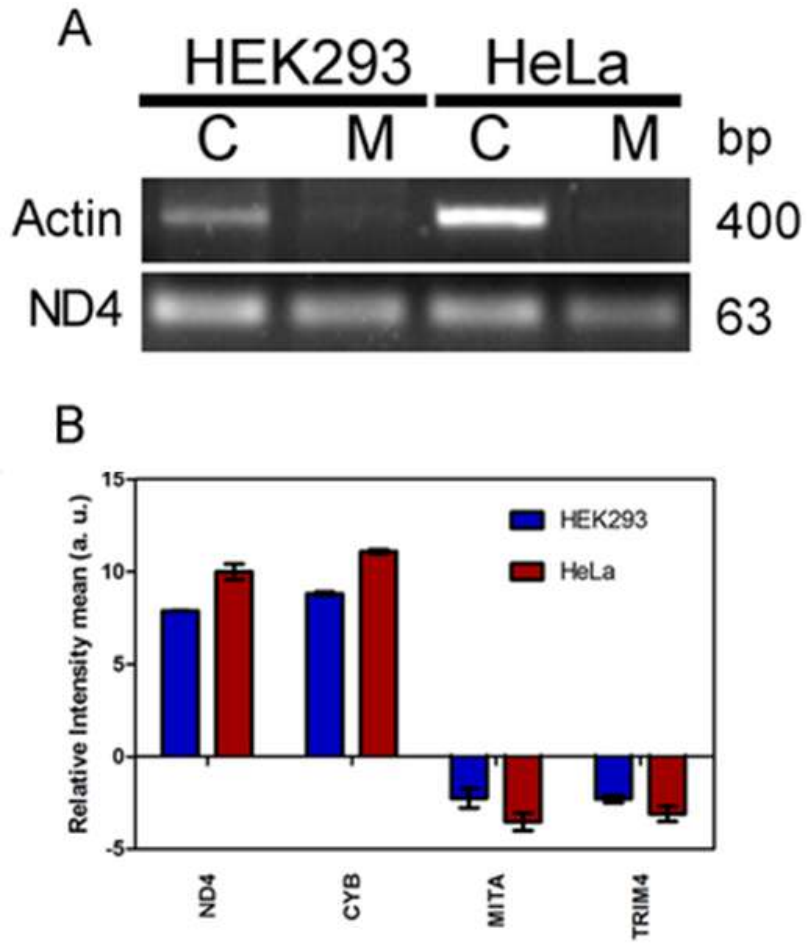

***Figure 5.2. Analysis of purity of RNA isolated from mitochondria.*** *The mitochondria were isolated and purified from HEK293 and HeLa. (A) RNA was isolated from purified mitochondria and total cell. The subsequent cDNA was used for PCR amplification of mitochondrial encoded ND4 and cytosolic/nuclear specific b-actin. M: mitochondrial fraction; C: cellular lysate. (B) RNA was isolated from mitochondria. The nuclear RNA contamination in mitochondrial RNA was assessed by checking relative enrichment of mitochondrial encoded RNA (ND4, CYB) and nuclear encoded mRNA (TRIM4, MITA) by RT-qPCR.*

## 5.2. Analysis of sRNA libraries associated with human mitochondria

The small RNA (18-30 nucleotides) were isolated, library generated and sequenced using Illumina high-throughput sequencing platform. The sequencing generated 19580503 and 17743919 raw sequencing reads from mitochondria of HEK293 and HeLa respectively. The dataset was deposited into NCBI Gene Expression Omnibus [accession No: **GSM797669 and GSM797670** for sRNA from mitochondria of HEK293 and HeLa respectively]. After removing tags 19089819 and 17312962 sequences were annotated from HEK293 and HeLa libraries respectively. The further analysis of sRNA sequence library showed that 95% of total sequences (31686782 reads) corresponding to 10.52% unique sequence (104064 reads) were common in both HEK293 and HeLa. While 3.87% total sequences (1290120 reads) correspond to 63.3% unique sequences (626229 reads) and 1.04% total sequences (347483 reads) corresponding to 26.18% unique sequences (258992 reads) were specific to mitochondria of HEK293 and HeLa respectively (Figure 5.3A, Figure 5.3B).

The length of miRNA, piRNA and siRNA are generally 21-22, 30 and 24 nucleotides respectively [20]. Thus, the length distribution analysis helps to categorize the sRNA. The length of 85.3% (14349816) and 91.2% (15070360) sequences from HEK293 and HeLa respectively were between 20-27 nucleotides. The highest number of sequences were of 25 nucleotide in length (17% and 27% sequences from HEK293 and HeLa respectively) (Figure 5.3C) clearly indicating the abundance sRNA other than miRNA and piRNA.

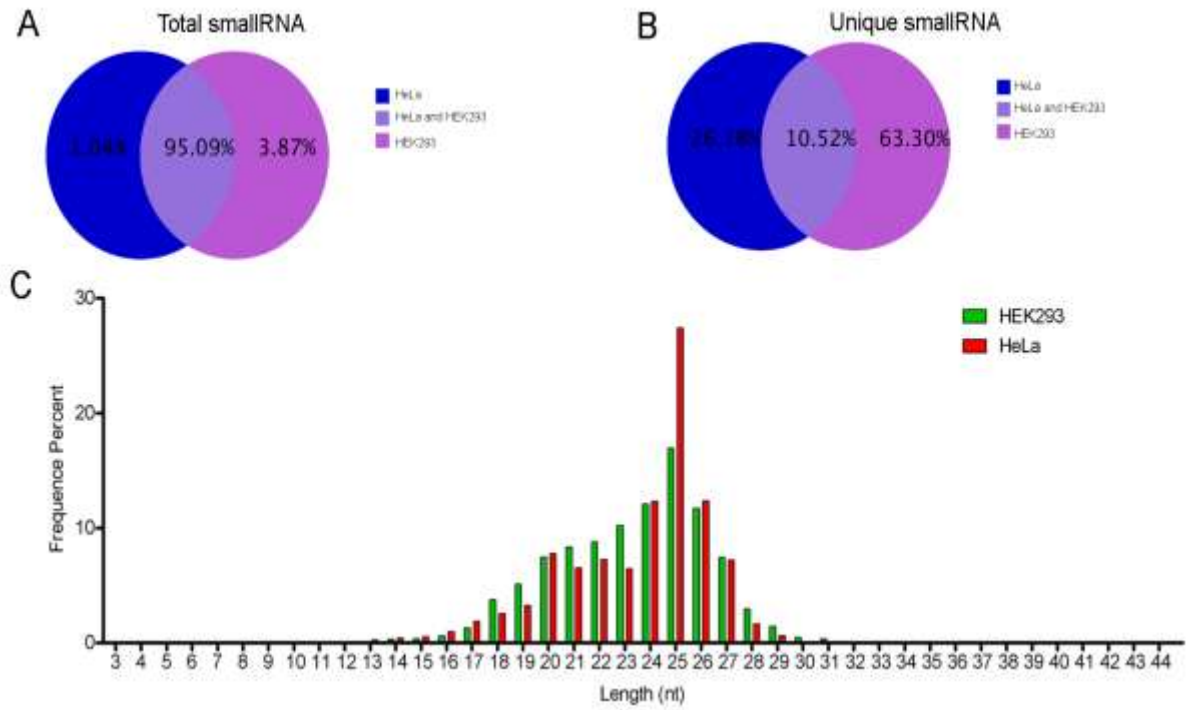


***Figure 5.3. Generation and analysis of sRNA sequences from mitochondria.*** *sRNA library generated from mitochondria from HEK293 and HeLa were sequenced using Illumina Hiseq 2000 platform that generated 19089819 and 17312962 clean sequence respectively. (A) Venn diagram showing distribution of common and specific sRNA total sequence reads amongst the two libraries. (B) Venn diagram showing distribution of common and specific sRNA unique sequence reads amongst the two libraries. (C) Length distribution and frequency percent of sequences in HEK293 and HeLa mitochondrial sRNA libraries.*

The sRNA sequences were mapped across the latest UCSC release hg19 genome browser assembly to determine their origin and distribution. Around 87.58% (14728844 reads) and 92.67% (15297190 reads) aligned sense/antisense orientation to the human genome from HEK293 and HeLa, respectively. Majority of the sRNA reads aligned to the sense strand of uncharacterized region of genome (chrUn_g1000220) followed by antisense stands of chromosome 8 and 2 (Figure 5.4A, Figure 5.4B). The sRNA sequences were annotated according to their overlap with sequences of known RNA in Genbank and Rfam. As expected, the most abundant sRNA classes in the both the libraries were rRNA (78.11% in HEK293 and 91.76% in HeLa), the unannotated sRNA (8.47% in HEK293 and in 3.27% in HeLa) and tRNA (3.96% in HEK293 and in 0.88% in HeLa).

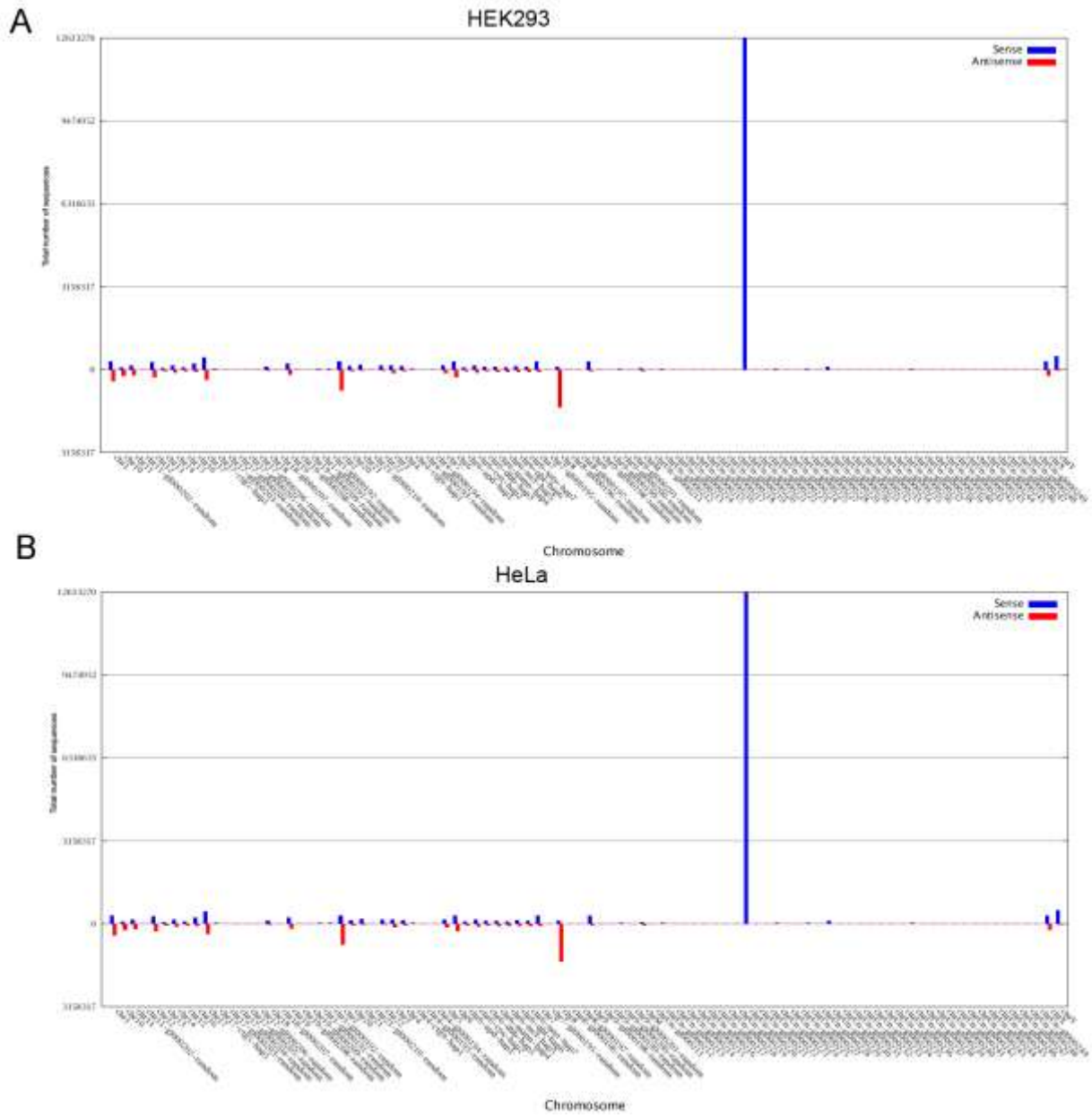


***Figure 5.4 The genomic mapping of mitochondria associated sRNAs.*** *The clean sequence reads were mapped onto human reference genome (UCSC hg19) to determine the genomic locations of small RNA (sRNA) using SOAP. (A) The number of sRNA tags associated with mitochondria of HEK293 mapped at specific chromosomal position. (B) The number of sRNA tags associated with mitochondria of HeLa mapped at specific chromosomal position. The area above 0 is the number of small RNAs (sRNAs) on sense strand of chromosome, shown in blue whereas area below 0 is the number of sRNAs on the antisense strand of the chromosome, shown in red.*

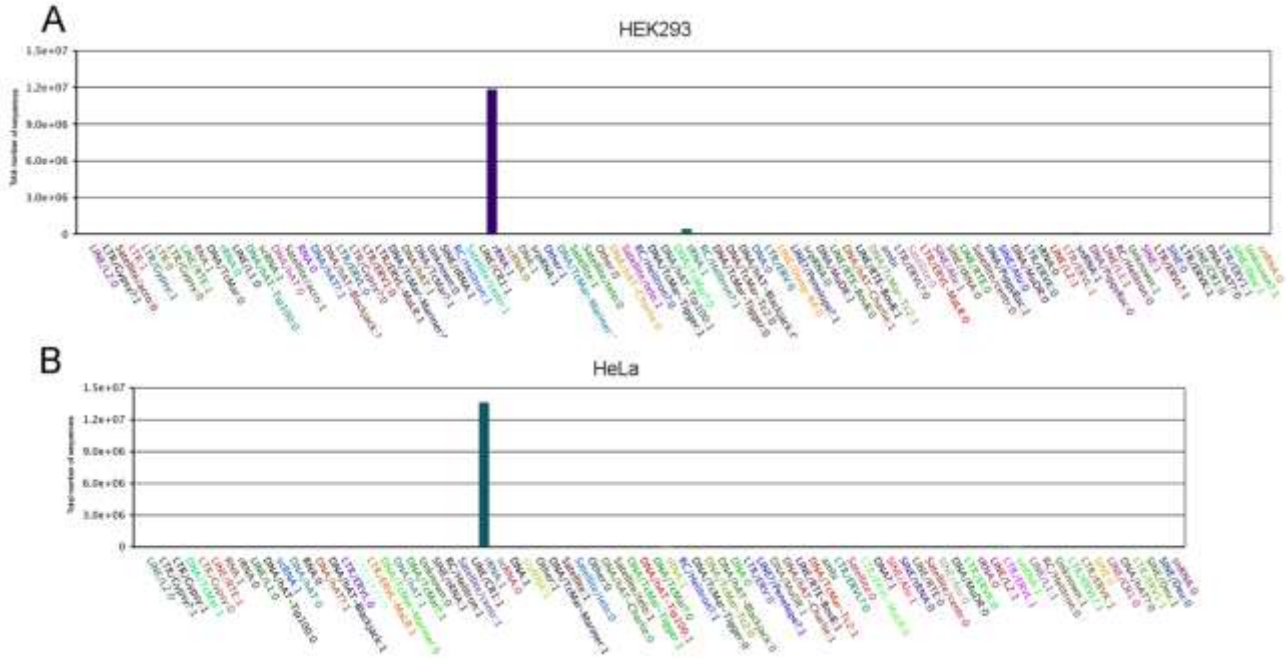


***Figure 5.5. Classification of repeat associated reads from mitochondria-associated sRNA libraries****. The total sequence reads classified as repeat associated elements were further categorized to determine the levels of each repeat associated RNAs in graphical format. (A) Detailed clustering of repeat-associated RNAs from mitochondria-associated sRNA library of HEK293. (B) Detailed clustering of repeat-associated RNAs from mitochondria-associated sRNA library of HeLa.*

The sequences corresponding to exons/introns, snRNA were low (1.21% in HEK293 and in 0.11% in HeLa). This strongly suggests that the library and sequencing was devoid of degraded mRNA products. The other categories of sRNA included snRNA (1.21% in HEK293 and 0.11% in HeLa), piRNA (0.01% in HEK293 and HeLa), srpRNA (0.17% in HEK293 and 0.01% in HeLa) and snoRNA (0.21% and 0.03% in HEK293 and HeLa respectively) and repeat associated sRNA. The top five abundant classes of repeats were rRNA (47.32% and 99.26% HEK293 and HeLa respectively), tRNA (1.63% and 0.36% HEK293 and HeLa respectively), LINES (0.104%and 0.057% HEK293 and HeLa respectively), SINE/Alu (0.15% and 0.028% HEK293 and HeLa respectively) and snRNA (0.32% and 0.072% HEK293 and HeLa respectively) in mitochondria of both cell lines. Low levels of LTR/ERVL (0.015% and 0.007% HEK293 and HeLa respectively), LTR/ERV1 (0.015% and .007% HEK293 and HeLa respectively), LTR/ERVL-MaLR

(0.015%1 and 0.006% HEK293 and HeLa respectively) were also found in the mitochondria of both cell lines (Figure 5.5, Table 5.1).

### 5.3. Analysis of known miRNA associated with mitochondria from HEK293 and HeLa

Our major interest was to investigate the presence of miRNA specifically in mitochondria, hence we focused our analysis on miRNA. A total of 2249 unique tags (4.21% or 710742 sequence reads) of HEK293 mitochondria and 1584 unique tags (2.58% or 426907 sequence reads) of HeLa mitochondria were categorized as miRNA (Table 5.2). The counts of miRNA varied from 1 to 85000.The presence of 428 and 327 mature miRNA (1539 mature miRNA in miRBase 17.0) was observed in mitochondria of HEK293 and HeLa respectively. According to miRBase 17.0 we also found 65 and 60 miRNA* in the mitochondria of HEK293 and HeLa respectively.

### 5.4. Analysis of differential association of miRNA with mitochondria

The differentially enriched miRNA in mitochondria of both HEK293 and HeLa has been summarized in Table 5.3. The expression pattern of 35 miRNAs was similar in the mitochondria from both the cell lines (Figure 5.6A). The expression levels ranged from less than 10 to more than 100,000 counts (Figure 5.6B). The most abundant miRNA in mitochondria of HEK293 and HeLa were hsa-miR-423-5p, hsa-miR-320a and let-7 family members (let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7h and let-7i) followed by hsa-miR-103b, hsa-miR-140-3p, hsa-miR-744, hsa-miR-107 (Figure 5.6C & 5.6D). hsa-miR-10a, hsa-miR-128, hsa-miR-1307, hsa-miR-140-3p, hsa-miR-185, hsa-miR-196a, hsa-miR-25, hsa-miR-320a, hsa-miR-330-3p, hsa-miR-340, hsa-miR-423-5p, hsa-miR-629 and hsa-miR-744 enriched significantly in the mitochondria of HEK293. Similarly let-7i, hsa-miR-181b, hsa-miR-21, hsa-miR-23a, hsa-miR-29a, hsa-miR-30a, hsa-miR-31 and hsa-miR-452 enriched specifically in mitochondria of HeLa.

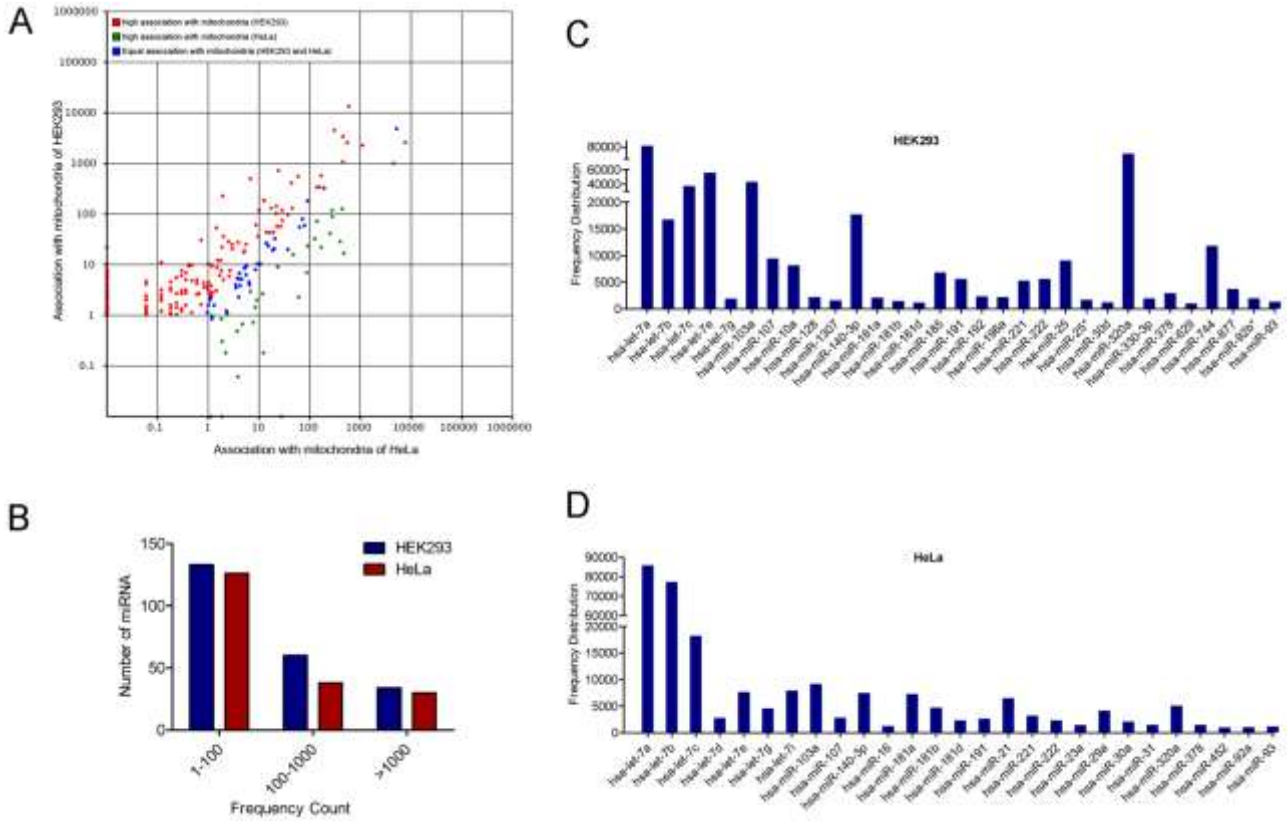


***Figure 5.6. Analysis of differential association of miRNAs to mitochondria from HEK293 and HeLa.*** *(A) Scatter Plot depicting the differential association of miRNAs from libraries of HeLa and HEK293. The X and Y axis shows association level of miRNAs with mitochondria from two cell lines. Red points represent miRNA with ratio.2; Blue points represent miRNA with 1/2,ratio,2; Green points represent miRNA with ratio,1/2. Ratio = normalized association of the HEK293/HeLa. (B) Distribution of known miRNAs: Numbers of sequence reads are taken as miRNA levels and the values are represented in the form of range of values in both HEK293 and HeLa sRNA libraries. (C) The frequency of highly associated miRNAs (.10,000 counts) with mitochondria of HEK293. (D) The frequency of highly associated miRNAs (.10,000 counts) with mitochondria of HeLa.*

## 5.6 Biological processes regulated by miRNA associated with mitochondria from HEK293 and HeLa

An individual miRNA may regulate several mRNAs in a pathway thus fine-tuning the cellular processes. Hence, we determined the targets of all the miRNA enriched in

mitochondria of both cell lines. Majority of target prediction tools gives many false positive results therefore, it has to be validated by many other tools. We used Starbase v.2.0 that has been developed based on recent studies of CLIP (Cross-linking immunoprecipitation) and RNA degradome sequencing experiments. The false positives targets can be minimized further through intersections of targets from 5 target prediction tools based on Clip-Seq results. Therefore, the results obtained from this analysis may give reliable and verified data mimicking closely to the physiological conditions.

The analysis of targets was classified on the basis of miRNA count expressed both in HEK293 and HeLa: highly expressed miRNA (>5000) and less expressed (<5000). These targets were further analyzed using DAVID for GO and KEGG to functionally annotate the identified genes into smaller and biologically meaningful groups. Gene Ontology (GO) clustering was done which broadly covered the following aspects of physiology: molecular function, cellular component and biological process. The targets of highly enriched miRNA populated many GO categories with significant enrichment (>1.0) and p-Value (<0.05). As miRNA is known to regulate transcription, GO terms related to regulation of transcription (e.g. GO:0045449, GO:0010629) were significantly enriched. The other significantly enriched categories included GO:0051301~cell division, GO:0007049~cell cycle, GO:0016568~chromatin modification, GO:0035195~gene silencing by miRNA, GO:0007389~pattern specification process, GO:0001701~in utero embryonic development, GO:0042921~glucocorticoid receptor signaling pathway (Table 5.4). To analyze the role that miRNAs play in the regulatory networks, we assigned putative miRNA targets into KEGG pathways, and observed that ubiquitin mediated proteolysis pathway was significantly enriched (p-value <0.05) which is important for all the biological processes (Table 5.5). The GO analysis of the targets of low counts of miRNA present in mitochondria (<5000) showed significant enrichment of GO terms related to regulation of transcription (GO:0045449, GO:0010629, GO:0006357, GO:0051252) and the regulation of biosynthetic processes (GO:0031327/28, GO:0009891). To further analyze the networks we also used KEGG and found that pathways in cancer (solid tumors) and chronic myeloid leukemia were significantly enriched ($p<0.05$). Similarly TGF, Wnt and cell cycle pathway genes were also found to be significantly enriched ($p<0.05$) (Table 5.6).

### 5.7 Validation of known miRNA associated with mitochondria of HEK293 and HeLa

The experiments were done to validate the association of miRNAs with mitochondria. The non specific association of miRNA was excluded by analyzing controls based on previous experiments. U6 snRNA and 5S rRNA was taken as endogenous and positive controls respectively due to their known association with mitochondria (Bain et al., 2010, Bandiera et al., 2011). We also observed high levels of U6 in our sRNA microarray analysis of mitochondrial RNA from HEK293 (data not published). Our microarray results also demonstrated that hsa-miR-145 was not detected in mitochondrial fraction whereas predominantly present in the total cell suggesting that it is not associated with mitochondria, hence was taken as negative control. The analysis by real time PCR showed that 5S rRNA was significantly associated whereas no association of has-miR-145 was observed in the RNA fraction isolated from purified mitochondria (Figure 5.7A). This experiment here as well as results from previous experiments clearly showed that specific miRNA associate with mitochondria and there is no non-specific association. The miRNAs with high count from both libraries (let-7b, let-7g, hsa-miR-107, hsa-miR-181a, hsa-miR-221 and hsa-miR-320a) were considered for the analysis for their association with mitochondria.

The miRNAs assessed by RT-qPCR followed similar pattern of association (Figure 5.7B) supporting the sequencing results. Argonaute proteins bind to guide strand of mature miRNA and regulate the process of translation. The association of miRNA to mitochondria also suggests that Ago proteins may also be localized to mitochondria hence we analyzed the subcellular localization of Ago2/3 proteins. We observed that Ago2 colocalized with mitochondria (Figure 5.8) which was in consonance with earlier observations (Bain et al., 2010, Bandiera et al., 2011). Ago3 also colocalized with mitochondria whereas GFP vector (negative control) showed no co-localization with mitochondria. We hypothesized that if these miRNAs and miRISC components are associated with mitochondria, the target mRNAs may also be associated to the outer surface of mitochondria. We analyzed the targets of 3 miRNAs (let-7b: STAT3; hsa-miR-107: MFN2; hsa-miR-320a: XIAP) by StarBase using intersection of 3 computational

tools and checked their association with mitochondria. To check the association of miRNA/target mRNA with outer membrane the levels of target mRNAs were analyzed by qPCR in both mitochondria and mitoplast.

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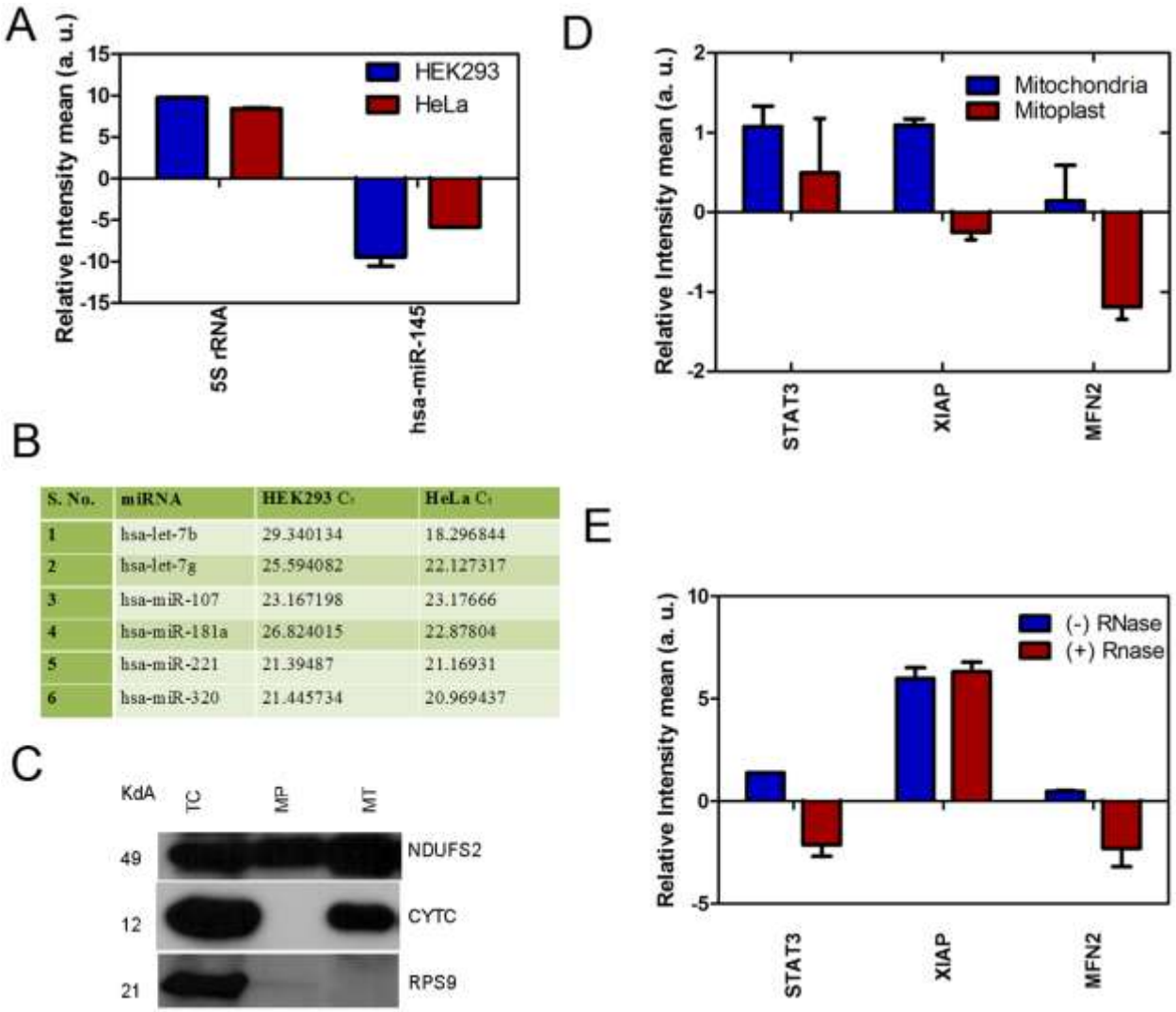


| S. No. | miRNA | HEK293 $C_T$ | HeLa $C_T$ |
|---|---|---|---|
| 1 | hsa-let-7b | 29.340134 | 18.296844 |
| 2 | hsa-let-7g | 25.594082 | 22.127317 |
| 3 | hsa-miR-107 | 23.167198 | 23.17666 |
| 4 | hsa-miR-181a | 26.824015 | 22.87804 |
| 5 | hsa-miR-221 | 21.39487 | 21.16931 |
| 6 | hsa-miR-320 | 21.445734 | 20.969437 |

***Figure 5.7. Validation of miRNAs and their targets associated with mitochondria.*** *The enrichment of target mRNA of mitochondria associated miRNAs with outer mitochondrial membrane were validated. (A) The association of 5S rRNA and negative control (hsa-miR-145) to mitochondria was analyzed. The relative enrichment of 5S rRNA and hsa-miR-145 was determined by qPCR as described in methods. (B) The miRNAs (frequency .1000) which were common in both the libraries were selected for validations by RT-qPCR. RNA was prepared from purified mitochondria and cDNA synthesized. The mean CT values of*

*miRNAs (with CT ,30 and distinct melt curve) from the mitochondria of both HEK293 and HeLa are listed. (C) The mitoplast was prepared from HEK293 as described in method section. The purity of mitoplast preparation was assessed by western blotting by probing with antibody against NDUFS2 (mitochondrial inner membrane protein) and cytochrome c (inter membrane space protein). TC: total cell; MP: mitoplast; MT: mitochondria. The targets of 3 miRNAs associated with mitochondria (let-7b: STAT3; hsa-miR-107: MFN2; hsa-miR-320a: XIAP) were determined by Starbase and validated by qPCR. (D) Validation of target mRNA associated with mitoplast as compared to mitochondria from HEK293. (E) Validation of target mRNA associated with RNase A treated mitochondria from HEK293.*

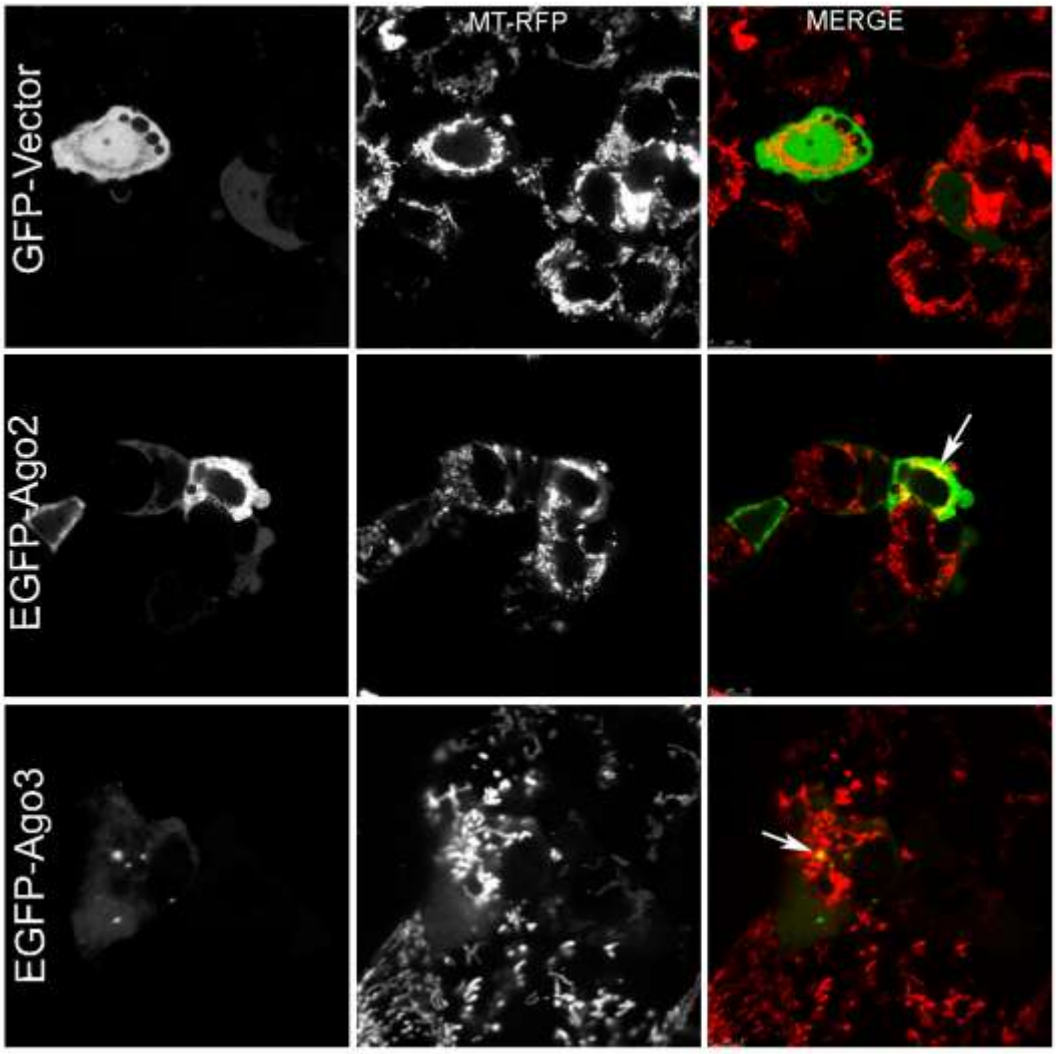


***Figure 5.8 Association of human Argonaute with mitochondria. Argonaute proteins co-localizes with mitochondria****. HEK293-MTRFP cells were transfected with pEGFPC1-Ago2, pEGFPC1-Ago3 and*

*pAcGFP-N1. After 24 hrs of transfection, cells were stained with Hoechst and analyzed by confocal microscopy*

The mitoplast preparation was checked prior to isolation of RNA by western blotting. The inner membrane localized protein, NDUFS2 was significantly enriched in mitoplast whereas, the inter membrane space protein cytochrome c was absent in mitoplast (Figure 5.7C). This was also confirmed by analyzing the association of mRNAs with mitochondria after RNase A treatment. RNase A treatment resulted in significant decrease in levels of target mRNAs (STAT3 and MFN2) whereas the levels of XIAP remained unchanged (Figure 5.7E). This may be due to association of XIAP mRNA with RNP complexes or localization site in mitochondria which may not be accessible by RNase A for degradation. These evidences suggest that the subunits of RISC complex like Ago proteins localize to the outer membrane may bind to target mRNA and may serve as platform for assembly of site-specific regulation of mRNA turnover and protein levels.

**5.8 Discussion**

The import of nuclear encoded tRNAs and rRNAs into the mitochondria has been demonstrated (Entelis et al., 2001, Tarassov et al., 2007). This raised an interesting question of possible import of small non-coding RNA across the mitochondria which may fine tune target proteins and assist mitochondrial transcription machinery under various physiological conditions. The deep sequencing of mitochondrial small RNA confirmed the presence of diverse population of sRNAs: snRNA, srpRNA and snoRNA. The transport of sRNAs including snRNA and snoRNA was reported from cytoplasm to nuclear regions (Matera et al., 2007). There is strong evidence that suggests that snoRNA are processed in similar way like miRNA (Matera et al., 2007) and may perform functions similar to miRNA other than traditional role in ribosomal biogenesis. The presence of snoRNA in mitochondria needs to be further studied for their role in mitochondrial ribosome assembly, RNA modifications or miRNA like functions. The presence of srpRNA also suggests the translational of the proteins and its transport to mitochondria. This is further strengthened from the recent study describing the mitochondrial

transcriptome where snRNA, snoRNA and srpRNA were observed to be enriched in mitochondria but depleted in mitoplast (Mercer et al., 2011). This suggests unique crosstalk occurs at the outer membrane of mitochondria to assist yet unknown functions. Interestingly piRNA were present in mitochondria of both HEK293 and HeLa cells. Although it is known that piRNAs are expressed in germ line tissues however emerging evidences suggest the presence of piRNA (like sRNA) in different somatic tissue (Yan et al., 2011). The expression of piRNA was observed in 17 out of the 40 mouse somatic tissues and cell types. The observation of piRNA in mitochondria of HEK293 and HeLa in this study suggests either mitochondria are involved in biogenesis of piRNA or it is postranscriptionally translocated like miRNA. The role of mitochondria in biogenesis of piRNA has been further supported by recent studies. Two groups have clearly demonstrated the role of mitochondrial protein known as MitoPLD/zuc (*Drosophila* homologue) in piRNA biogenesis and piRNA mediated silencing both in fly and mouse germlines (Huang et al., 2011b, Watanabe et al., 2011). This strongly suggests that mitochondria may be important player in piRNA pathways. Repeat associated RNA elements were also observed. The functional significance of these repeat associated sRNA needs to be further studied.

Interestingly, 2-5 % sRNA reads was categorized to be miRNAs which was well supported by contemporary evidence of existence of mitochondrial specific miRNAs (Bandiera et al., 2011, Barrey et al., 2011, Kren et al., 2009). The mitochondrial transcriptome analysis (Mercer et al., 2011) also demonstrated 3% of sRNA as miRNA. The targets of miRNA were highly enriched in mitochondria both from HEK293 and HeLa were determined and clustered. Interestingly GO terms related to RNA transcription were enriched. This suggests that mitochondria may regulate miRNA associated target gene at the level of transcription. Ago2 protein which is one of the important proteins in RISC complex involved in regulation and turnover of target mRNA has been demonstrated to be in association with mitochondria (Maniataki and Mourelatos, 2005). The pathway affected was predicted to be: apoptosis, cell cycle, kinase activity and proteins transport. Some of these miRNA target interactions have been observed previously. hsa-miR-103/107 highly enriched in mitochondria (as observed in our study) regulates cellular Acetyl-CoA and lipid levels (Ha, 2011). Similarly hsa-miR-181 (count =

2114 in HEK293 and 7329 in HeLa) targets multiple Bcl-2 family members and influences apoptosis (Ouyang et al., 2011). The transcription factor p53 has been also colocalized in the mitochondria during p53-dependant apoptosis and is a putative regulator of let-7 family and other miRNA (mir-107, mir-145, mir-134, mir-503, mir-21) were previously detected in the mitochondria of myotubes. These evidences strongly suggest that miRNA localized in mitochondria regulate the levels of protein that are implicated in mitochondrial related functions.

It has been demonstrated that Ago-2 is associated with outer mitochondrial membrane thus may bind to miRNA and associate with transcriptional machinery. This is further supported by recent study of mitochondrial transcriptome where depletion of outer membrane of mitochondria also results in selective loss of enrichment of miRNA (Mercer et al., 2011). Similarly, it has been observed that mitochondria also associate with P bodies (Huang et al., 2011a). These P bodies are cytoplasmic granules that are linked to mRNA decay, mRNA storage and RNA silencing (Adeli et al., 2011). This strongly suggests that mitochondria are important for miRNA mediated translational repression. Further study in this direction will help to understand many unanticipated role of mitochondria and associated miRNA in different physiological and pathological conditions.

In conclusion, the ncRNA, miRNAs, target mRNA and core component of miRISC are associated with mitochondria. However the systematic annotation of small RNA sequences remarkably elucidated some unannotated sequences. The Bioinformatic analysis further classified those sequences as potential putative miRNA like properties: ***the novel miRNAs.***

**LIST OF TABLES:**

**Table 5.1 Annotations of various classes of sRNA associated with mitochondria of HEK293 and HeLa**

| Cell Line | HEK293 | | | | HeLa | | | |
|---|---|---|---|---|---|---|---|---|
| **Category [1]** | **Unique sRNAs[2]** | **Percent(%)[3]** | **Total sRNAs[4]** | **Percent (%)[5]** | **Unique sRNAs[2]** | **Percent(%)[3]** | **Total sRNAs [4]** | **Percent (%)[5]** |
| **Exon antisense** | 3929 | 0.54% | 6968 | 0.04% | 1175 | 0.32% | 1500 | 0.01% |
| **Exon sense** | 130469 | 17.87% | 422936 | 2.51% | 49653 | 13.68% | 196076 | 1.19% |
| **Intron antisense** | 6242 | 0.85% | 11715 | 0.07% | 3130 | 0.86% | 3432 | 0.02% |
| **Intron sense** | 37754 | 5.17% | 72765 | 0.43% | 7664 | 2.11% | 8389 | 0.05% |
| **MiRNA** | 1956 | 0.27% | 708798 | 4.21% | 1474 | 0.41% | 426429 | 2.58% |
| **PiRNA** | 101 | 0.01% | 956 | 0.01% | 53 | 0.01% | 226 | 0.00% |
| **Rrna** | 140319 | 19.21% | 13135781 | 78.11% | 127189 | 35.03% | 15147302 | 91.76% |
| **Repeat** | 36828 | 5.04% | 81864 | 0.49% | 10010 | 2.76% | 12483 | 0.08% |
| **scRNA** | 2382 | 0.33% | 18443 | 0.11% | 764 | 0.21% | 2794 | 0.02% |
| **snRNA** | 8030 | 1.10% | 203999 | 1.21% | 2804 | 0.77% | 18411 | 0.11% |
| **snoRNA** | 2149 | 0.29% | 34650 | 0.21% | 961 | 0.26% | 4819 | 0.03% |
| **srpRNA** | 347 | 0.05% | 29186 | 0.17% | 128 | 0.04% | 1127 | 0.01% |
| **tRNA** | 29969 | 4.10% | 665393 | 3.96% | 13691 | 3.77% | 144813 | 0.88% |
| **Unannotated** | 329818 | 45.16% | 1423619 | 8.47% | 144360 | 39.76% | 539511 | 3.27% |
| **Total** | 730293 | 100% | 16817073 | 100% | 363056 | 100% | 16507312 | 100% |

***Table 5.1 Annotations of various classes of sRNA associated with mitochondria of HEK293 and HeLa.*** *The total clean sequences obtained from the sRNA libraries were subjected to a series of sequence similarity searches using specific databases (rRNAs, tRNAs, sn/snoRNAs, miRNAs, other non-coding RNAs). The sequences that did not match with any known sequence were categorized as unannotated sequences. All annotations were summarized using tag2annotations software. An overview of sRNAs associated with mitochondria. 1 type of sRNA, 2 total number of unique sequences belonging to each category, 3 percentage of unique sequences belonging to each category, 4 total number of all sequences belonging to each category, 5 percentage of total sequences belonging to each category.*

**Table 5.2 Summary of known miRNAs from mitochondrial sRNA libraries**

| | miRNA | miRNA* | unique sRNAs matched to miRNA [1] | total sRNAs matched to miRNA [2] |
|---|---|---|---|---|
| Known miRNA in miRBase | 1539 | 194 | - | - |
| HEK293 | 428 | 65 | 2249 | 710742 |
| HeLa | 327 | 60 | 1584 | 426907 |

***Table 5.2. Summary of known miRNAs from mitochondrial sRNA libraries.*** *The clean sequence reads aligned to miRBase 17.0 to determine diversity and occurrence of miRNA in sRNA library. 1 count of unique sRNA sequences that matched to miRBase, 2 count of total sRNA sequences that matched to miRBase in HEK293 and HeLa.*

**Table 5.3 Pattern of miRNAs associated with mitochondria of HEK293 and HeLa**

| **miRNA[1]** | **Frequency count** | | **miRNA[1]** | **Frequency count** | | **miRNA[1]** | **Frequency count** | |
|---|---|---|---|---|---|---|---|---|
| | **HEK293[2]** | **HeLa[3]** | | **HEK293[2]** | **HeLa[3]** | | **HEK293[2]** | **HeLa[3]** |
| **hsa-let-7a** | 81018 | 85909 | **hsa-miR-18a** | 43 | 2 | **hsa-miR-3653** | 21 | 0 |
| **hsa-let-7b** | 16756 | 77340 | **hsa-miR-1908** | 13 | 8 | **hsa-miR-3654** | 146 | 25 |
| **hsa-let-7c** | 37408 | 18274 | **hsa-miR-191** | 5644 | 2694 | **hsa-miR-3662** | 20 | 2 |
| **hsa-let-7d** | 361 | 2801 | **hsa-miR-192** | 2344 | 375 | **hsa-miR-3687** | 84 | 6 |
| **hsa-let-7e** | 55978 | 7672 | **hsa-miR-193a-5p** | 45 | 11 | **hsa-miR-374a** | 42 | 18 |
| **hsa-let-7f** | 43062 | 127719 | **hsa-miR-193b** | 17 | 39 | **hsa-miR-374b** | 139 | 86 |
| **hsa-let-7g** | 1936 | 4591 | **hsa-miR-193b*** | 439 | 230 | **hsa-miR-375** | 133 | 0 |
| **hsa-let-7i** | 278 | 7960 | **hsa-miR-194** | 10 | 2 | **hsa-miR-378** | 2979 | 1531 |
| **hsa-miR-1** | 44 | 11 | **hsa-miR-196a** | 2184 | 753 | **hsa-miR-378c** | 99 | 43 |
| **hsa-miR-100** | 0 | 471 | **hsa-miR-196b** | 605 | 33 | **hsa-miR-3908** | 16 | 0 |
| **hsa-miR-101** | 946 | 384 | **hsa-miR-196b*** | 40 | 1 | **hsa-miR-3917** | 27 | 12 |
| **hsa-miR-103a** | 43274 | 9216 | **hsa-miR-19b** | 66 | 59 | **hsa-miR-3928** | 16 | 20 |
| **hsa-miR-106a** | 16 | 5 | **hsa-miR-200c** | 15 | 1 | **hsa-miR-421** | 73 | 17 |
| **hsa-miR-106b** | 599 | 160 | **hsa-miR-20a** | 172 | 177 | **hsa-miR-423-3p** | 730 | 230 |
| **hsa-miR-106b*** | 307 | 87 | **hsa-miR-20b** | 23 | 0 | **hsa-miR-423-5p** | 222791 | 9970 |
| **hsa-miR-107** | 9483 | 2834 | **hsa-miR-20b*** | 15 | 0 | **hsa-miR-424** | 83 | 10 |
| **hsa-miR-10a** | 8188 | 114 | **hsa-miR-21** | 472 | 6575 | **hsa-miR-424*** | 204 | 29 |
| **hsa-miR-10a*** | 24 | 0 | **hsa-miR-21*** | 1 | 64 | **hsa-miR-425*** | 11 | 6 |
| **hsa-miR-10b** | 162 | 8 | **hsa-miR-210** | 57 | 59 | **hsa-miR-4446-3p** | 21 | 0 |
| **hsa-miR-1180** | 20 | 2 | **hsa-miR-2110** | 158 | 20 | **hsa-miR-4467** | 47 | 0 |
| **hsa-miR-122** | 129 | 5 | **hsa-miR-22** | 11 | 78 | **hsa-miR-4485** | 19 | 16 |
| **hsa-miR-1224-5p** | 172 | 0 | **hsa-miR-221** | 5331 | 3235 | **hsa-miR-4488** | 90 | 64 |

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|---|---|---|---|---|---|---|---|---|
| **hsa-miR-124** | 13 | 0 | **hsa-miR-221*** | 133 | 141 | **hsa-miR-449a** | 12 | 0 |
| **hsa-miR-1246** | 14 | 30 | **hsa-miR-222** | 5647 | 2342 | **hsa-miR-449c** | 92 | 4 |
| **hsa-miR-1250** | 95 | 0 | **hsa-miR-224** | 3 | 207 | **hsa-miR-452** | 38 | 1036 |
| **hsa-miR-1254** | 45 | 20 | **hsa-miR-23a** | 116 | 1468 | **hsa-miR-4525** | 67 | 1 |
| **hsa-miR-1255a** | 33 | 18 | **hsa-miR-23b** | 44 | 195 | **hsa-miR-4687-3p** | 26 | 0 |
| **hsa-miR-1255b** | 18 | 3 | **hsa-miR-23b*** | 76 | 105 | **hsa-miR-4728-5p** | 21 | 1 |
| **hsa-miR-125a-5p** | 63 | 72 | **hsa-miR-24** | 262 | 794 | **hsa-miR-4745-5p** | 16 | 0 |
| **hsa-miR-125b** | 24 | 142 | **hsa-miR-25** | 9081 | 989 | **hsa-miR-4750** | 21 | 0 |
| **hsa-miR-1262** | 29 | 4 | **hsa-miR-25*** | 1685 | 364 | **hsa-miR-4787-3p** | 10 | 0 |
| **hsa-miR-1266** | 34 | 1 | **hsa-miR-26a** | 541 | 347 | **hsa-miR-483-5p** | 361 | 0 |
| **hsa-miR-1268** | 323 | 313 | **hsa-miR-26b** | 726 | 324 | **hsa-miR-486-5p** | 32 | 2 |
| **hsa-miR-1268b** | 343 | 334 | **hsa-miR-27a** | 33 | 152 | **hsa-miR-499-5p** | 3 | 0 |
| **hsa-miR-1269b** | 39 | 0 | **hsa-miR-27b** | 49 | 114 | **hsa-miR-500a*** | 44 | 5 |
| **hsa-miR-1270** | 35 | 0 | **hsa-miR-27b*** | 3 | 37 | **hsa-miR-501-3p** | 11 | 2 |
| **hsa-miR-1272** | 28 | 0 | **hsa-miR-28-3p** | 51 | 24 | **hsa-miR-502-3p** | 51 | 3 |
| **hsa-miR-1273d** | 22 | 1 | **hsa-miR-29a** | 673 | 4213 | **hsa-miR-503** | 369 | 31 |
| **hsa-miR-1273f** | 18 | 1 | **hsa-miR-29b** | 20 | 38 | **hsa-miR-505*** | 102 | 5 |
| **hsa-miR-1275** | 91 | 4 | **hsa-miR-29c** | 201 | 26 | **hsa-miR-532-3p** | 57 | 3 |
| **hsa-miR-128** | 2187 | 289 | **hsa-miR-30a** | 535 | 2131 | **hsa-miR-532-5p** | 169 | 17 |
| **hsa-miR-1285** | 26 | 17 | **hsa-miR-30a*** | 150 | 398 | **hsa-miR-548h** | 53 | 2 |
| **hsa-miR-1291** | 44 | 4 | **hsa-miR-30c** | 14 | 20 | **hsa-miR-574-3p** | 11 | 10 |
| **hsa-miR-1292** | 43 | 10 | **hsa-miR-30c-2*** | 38 | 66 | **hsa-miR-574-5p** | 26 | 6 |
| **hsa-miR-1293** | 17 | 4 | **hsa-miR-30d** | 1242 | 483 | **hsa-miR-584** | 2 | 12 |
| **hsa-miR-1301** | 897 | 24 | **hsa-miR-30e** | 82 | 33 | **hsa-miR-589** | 59 | 14 |
| **hsa-miR-1306** | 76 | 3 | **hsa-miR-30e*** | 22 | 3 | **hsa-miR-589*** | 17 | 3 |
| **hsa-miR-1307** | 1587 | 628 | **hsa-miR-31** | 389 | 1532 | **hsa-miR-598** | 184 | 2 |
| **hsa-miR-130a** | 158 | 97 | **hsa-miR-3127-5p** | 36 | 2 | **hsa-miR-615-5p** | 137 | 0 |
| **hsa-miR-130b** | 421 | 50 | **hsa-miR-3177-3p** | 12 | 0 | **hsa-miR-629** | 1001 | 145 |
| **hsa-miR-138** | 13 | 5 | **hsa-miR-3192** | 46 | 6 | **hsa-miR-641** | 17 | 1 |
| **hsa-miR-139-3p** | 14 | 1 | **hsa-miR-3198** | 20 | 1 | **hsa-miR-660** | 50 | 5 |
| **hsa-miR-140-3p** | 17763 | 7511 | **hsa-miR-32** | 15 | 4 | **hsa-miR-663** | 72 | 14 |
| **hsa-miR-146b-5p** | 338 | 50 | **hsa-miR-320a** | 74410 | 5130 | **hsa-miR-664*** | 154 | 20 |
| **hsa-miR-148a** | 115 | 6 | **hsa-miR-320b** | 413 | 94 | **hsa-miR-7** | 88 | 62 |
| **hsa-miR-148b** | 50 | 17 | **hsa-miR-320c** | 59 | 16 | **hsa-miR-708** | 78 | 1 |
| **hsa-miR-149*** | 66 | 0 | **hsa-miR-324-5p** | 47 | 20 | **hsa-miR-744** | 11832 | 410 |
| **hsa-miR-151-3p** | 86 | 73 | **hsa-miR-330-3p** | 1973 | 167 | **hsa-miR-760** | 509 | 12 |
| **hsa-miR-151-5p** | 63 | 19 | **hsa-miR-339-3p** | 33 | 1 | **hsa-miR-765** | 17 | 0 |
| **hsa-miR-152** | 709 | 274 | **hsa-miR-339-5p** | 24 | 4 | **hsa-miR-877** | 3759 | 32 |

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|---|---|---|---|---|---|---|---|---|
| **hsa-miR-155** | 5 | 31 | **hsa-miR-33a** | 34 | 18 | **hsa-miR-9** | 43 | 0 |
| **hsa-miR-15a** | 86 | 75 | **hsa-miR-340** | 454 | 64 | **hsa-miR-92a** | 908 | 1070 |
| **hsa-miR-15b** | 171 | 156 | **hsa-miR-342-3p** | 291 | 85 | **hsa-miR-92a-1*** | 469 | 42 |
| **hsa-miR-16** | 977 | 1315 | **hsa-miR-342-5p** | 79 | 8 | **hsa-miR-92a-2*** | 23 | 0 |
| **hsa-miR-17** | 387 | 255 | **hsa-miR-346** | 22 | 0 | **hsa-miR-92b** | 333 | 624 |
| **hsa-miR-17*** | 105 | 93 | **hsa-miR-34a** | 128 | 30 | **hsa-miR-92b*** | 1961 | 476 |
| **hsa-miR-181a** | 2114 | 7329 | **hsa-miR-34c-5p** | 34 | 0 | **hsa-miR-93** | 1331 | 1220 |
| **hsa-miR-181b** | 1461 | 4729 | **hsa-miR-3605-5p** | 21 | 1 | **hsa-miR-940** | 15 | 4 |
| **hsa-miR-181c** | 66 | 111 | **hsa-miR-361-5p** | 22 | 5 | **hsa-miR-941** | 110 | 68 |
| **hsa-miR-181c*** | 13 | 7 | **hsa-miR-362-5p** | 13 | 13 | **hsa-miR-95** | 17 | 1 |
| **hsa-miR-181d** | 1179 | 2359 | **hsa-miR-363** | 57 | 0 | **hsa-miR-96** | 13 | 4 |
| **hsa-miR-182** | 102 | 28 | **hsa-miR-363*** | 12 | 0 | **hsa-miR-98** | 12 | 128 |
| **hsa-miR-184** | 10 | 0 | **hsa-miR-3647-3p** | 71 | 10 | **hsa-miR-99a** | 148 | 89 |
| **hsa-miR-185** | 6819 | 747 | **hsa-miR-3648** | 17 | 3 | **hsa-miR-99b** | 939 | 457 |
| **hsa-miR-186** | 38 | 19 | **hsa-miR-365*** | 154 | 7 | **hsa-miR-99b*** | 128 | 45 |
| **hsa-miR-187** | 15 | 0 | **hsa-miR-3651** | 25 | 1 | | | |

***Table 5.3 Pattern of miRNAs associated with mitochondria of HEK293 and HeLa.*** *The miRNAs associated with mitochondria from both cell lines and their respective frequency count. 1 name of miRNA according to miRBase 17.0, 2 and 3 total sequences reads that matched to particular miRNA from mitochondria-associated sRNA library of HEK293 and HeLa respectively.*

**Table 5.4 The GO term of predicted targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to high frequency count category (count>5000).**

| **Cluster [1]** | **Term [2]** | **No. of target genes [3]** | **P-Value [4]** | **Benjamini [5]** |
|---|---|---|---|---|
| **Cluster 1 (ES : 7.5)** | GO:0045449~regulation of transcription | 86 | 8.47E-11 | 1.19E-07 |
| **Cluster 2 (ES : 2.75)** | GO:0010629~negative regulation of gene expression | 22 | 1.18E-04 | 0.027259 |
| **Cluster 3 (ES : 2.46)** | GO:0051301~cell division | 14 | 0.001452 | 0.1849 |
| | GO:0007049~cell cycle | 24 | 0.005737 | 0.201385 |
| **Cluster 4 (ES : 2.42)** | GO:0016568~chromatin modification | 13 | 0.002312 | 0.195174 |
| **Cluster 5 (ES : 2.13 )** | GO:0035195~gene silencing by miRNA | 4 | 0.002665 | 0.19814 |
| **Cluster 6 (ES : 2.04)** | GO:0007389~pattern specification process | 13 | 0.001859 | 0.196039 |
| **Cluster 7 (ES : 1.95)** | GO:0001701~in utero embryonic development | 10 | 0.002906 | 0.193893 |
| **Cluster 8 (ES : 1.60 )** | GO:0042921~glucocorticoid receptor signaling pathway | 3 | 0.005569 | 0.211877 |

***Table 5.4 The GO term of predicted targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to high frequency count category (count>5000).*** *The targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to high frequency count category were determined by StarBase and clustered into GO terms using the DAVID gene annotation tool. 1 Number of cluster and enrichment score (ES) .1.05, 2 The gene annotation term, 3 The number of target genes which belonged to GO cluster, 4 Fisher exact p-value representing the degree of enrichment of the GO term, 5 Benjamini correction value for each category.*

**Table 5.5 KEGG pathways enriched for targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to high frequency count category (count > 5000)**

| Term [1] | No. of target genes [2] | P-Value [3] | Benjamini [4] |
|---|---|---|---|
| **hsa04120: Ubiquitin mediated proteolysis** | 6 | 0.03358 | 0.93495 |
| **hsa05200: Pathways in cancer** | 9 | 0.06473 | 0.93123 |
| **hsa04110: Cell cycle** | 5 | 0.08169 | 0.89696 |
| **hsa05222: Small cell lung cancer** | 4 | 0.09821 | 0.8735 |

***Table 5.5 KEGG pathways enriched for targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to high frequency count category (count > 5000).*** *The targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to high frequency count category were determined by StarBase and clustered into KEGG pathways using the DAVID gene annotation tool. 1 KEGG pathway and its ID, 2 the number of target genes, which belong to the pathway, 3 Fisher Exact p-value representing the degree of enrichment, 4 Benjamini correction value for each category.*

**Table 5.6 The GO term of predicted targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to low frequency count category (count<5000).**

| Cluster [1] | Term [2] | No. of target genes [3] | P-Value [4] | Benjamini [5] |
|---|---|---|---|---|
| **Cluster 1 (ES : 8.32)** | GO:0045449~regulation of transcription | 278 | 9.54E-27 | 2.78E-23 |
| | GO:0051252~regulation of RNA metabolic process | 175 | 1.40E-11 | 8.14E-09 |
| **Cluster 2 (ES : 9.02)** | GO:0006357~regulation of transcription from RNA polymerase II promoter | 93 | 1.68E-12 | 1.63E-09 |
| | GO:0010629~negative regulation of gene expression | 71 | 1.39E-11 | 1.01E-08 |
| | GO:0031327~negative regulation of cellular biosynthetic process | 74 | 1.11E-10 | 4.62E-08 |

| | | | | |
|---|---|---|---|---|
| | GO:0045934~negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | 65 | 8.38E-09 | 1.75E-06 |
| **Cluster 3 (ES : 7.03)** | GO:0010628~positive regulation of gene expression | 68 | 9.13E-08 | 1.27E-05 |
| | GO:0016563~transcription activator activity | 53 | 1.48E-07 | 1.69E-05 |
| | GO:0009891~positive regulation of biosynthetic process | 76 | 2.30E-07 | 3.04E-05 |
| | GO:0031328~positive regulation of cellular biosynthetic process | 75 | 2.67E-07 | 3.39E-05 |

***Table 5.6 The GO term of predicted targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to low frequency count category (count<5000).*** *The targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to low frequency count category were determined by StarBase and clustered into GO term using the DAVID gene annotation tool. 1, 2, 3, 4, 5 same as table 5.4.*

**Table 5.7 KEGG pathways enriched for targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to low frequency count category (<5000).**

| **Term [1]** | **No. of target genes [2]** | **P-Value [3]** | **Benjamini [4]** |
|---|---|---|---|
| **hsa05200: Pathways in cancer** | 43 | 7.65E-08 | 1.06E-05 |
| **hsa05220: Chronic myeloid leukemia** | 16 | 8.15E-06 | 5.66E-04 |
| **hsa04110: Cell cycle** | 19 | 1.12E-04 | 0.00519 |
| **hsa04310: Wnt signaling pathway** | 20 | 4.40E-04 | 0.01516 |
| **hsa04350: TGF-beta signaling pathway** | 14 | 7.06E-04 | 0.01943 |
| **hsa04114: Oocyte meiosis** | 16 | 7.57E-04 | 0.01738 |
| **hsa05212: Pancreatic cancer** | 12 | 0.0015 | 0.02929 |
| **hsa05210: Colorectal cancer** | 13 | 0.00168 | 0.02882 |
| **hsa04115: p53 signaling pathway** | 11 | 0.00325 | 0.04901 |
| **hsa04360: Axon guidance** | 16 | 0.00381 | 0.05168 |
| **hsa05222: Small cell lung cancer** | 12 | 0.00517 | 0.0634 |
| **hsa05214: Glioma** | 10 | 0.00626 | 0.07015 |
| **hsa04120: Ubiquitin mediated proteolysis** | 16 | 0.0067 | 0.06938 |
| **hsa05223: Non-small cell lung cancer** | 9 | 0.00784 | 0.07519 |
| **hsa05215: Prostate cancer** | 12 | 0.008 | 0.07177 |
| **hsa04010: MAPK signaling pathway** | 25 | 0.00904 | 0.07583 |
| **hsa05221: Acute myeloid leukemia** | 9 | 0.01199 | 0.09395 |
| **hsa04722: Neurotrophin signaling pathway** | 14 | 0.01583 | 0.11591 |

| | | | |
|---|---|---|---|
| **hsa04012: ErbB signaling pathway** | 11 | 0.01816 | 0.12548 |
| **hsa04510: Focal adhesion** | 19 | 0.02311 | 0.14997 |
| **hsa05219: Bladder cancer** | 7 | 0.02406 | 0.14888 |
| **hsa04910: Insulin signaling pathway** | 14 | 0.0298 | 0.17398 |
| **hsa05211: Renal cell carcinoma** | 9 | 0.03392 | 0.18825 |
| **hsa05218: Melanoma** | 9 | 0.03651 | 0.1938 |
| **hsa04916: Melanogenesis** | 11 | 0.04025 | 0.2042 |
| **hsa04810: Regulation of actin cytoskeleton** | 19 | 0.04135 | 0.2021 |

***Table 5.7 KEGG pathways enriched for targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to low frequency count category (<5000).*** *The targets of miRNAs associated with mitochondria (HEK293 and HeLa) belonging to low frequency count category (count ,5000) from were determined by StarBase and clustered into KEGG pathways using the DAVID gene annotation tool. 1, 2, 3, 4 same as table 5.5.*