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Identification and Characterization of Mitochondrial Genome Encoded microRNAs

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Introduction

Mitochondria being the powerhouse of the cell, also involved in maintaining cellular homeostasis, by apoptosis, ROS production, calcium signaling etc. Being a semi-autonomous organelle, mitochondria requires various nuclear encoded proteins, non-coding RNAs like rRNAs, tRNAs, including regulatory RNAs for functioning of various biochemical pathways. It is reported that nuclear genome encoded miRNAs translocate to mitochondria and modulate their functions through anterograde signaling, while mitochondria in return respond by producing signaling molecules like ROS and metabolites which is known as retrograde signaling. Recently, there has been various reports on mitochondria harboring various non-coding RNAs along with miRNAs. Our study reports mitochondrially encoded miRNAs for the first time regulating mitochondrial functions in breast cancer model system.

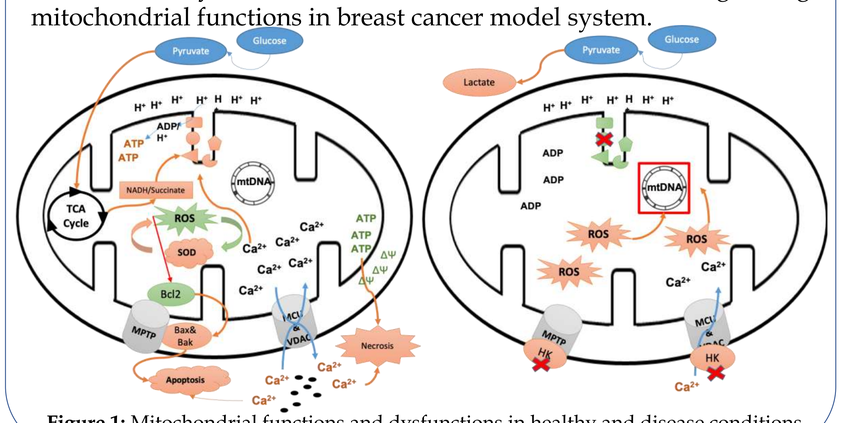
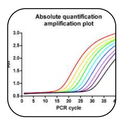


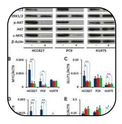
Figure 1: Mitochondrial functions and dysfunctions in healthy and disease conditions

Aim and Objectives

To identify and functionally validate novel mitochondrial genome encoded microRNAs targeting mitochondrial function for comprehensive understanding of their expression, mechanism of action and study their possible role as novel biosignatures for the early diagnosis of breast cancer.



Expression analysis of mitochondrially encoded miRNAs targeting mitochondria in breast cancer cell lines and tissue specimens.



Functional Characterization of and mitochondrially encoded miRNAs in breast cancer cell lines.

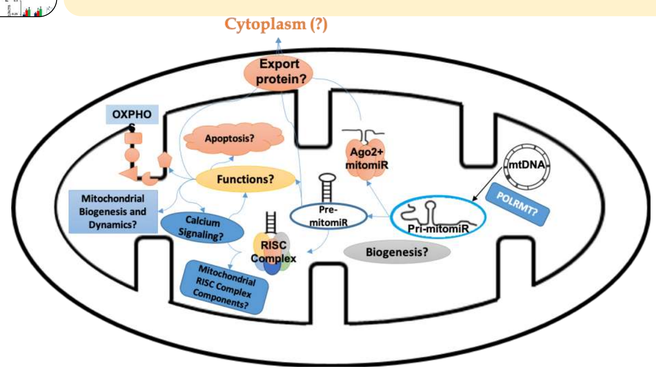


Figure 2: Possible mechanism of mitomiRs biogenesis and functions in mitochondria

Methodology

***In-silico* prediction of mitomiRs**
- Prediction by secondary structure
- Homology and Conservation based prediction

Expression Analysis
- Small RNA sequencing
- Cell line and clinical tissue specimens
- mtDNA-less cells
- Ago2-IP

Functional Analysis
- Target gene prediction
- Luciferase Assay
- Target gene expression validation
- Functional studies

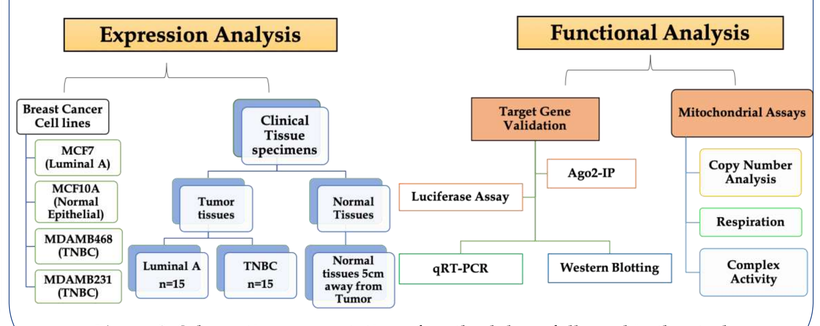


Figure 3: Schematic representation of methodology followed in the study

Results

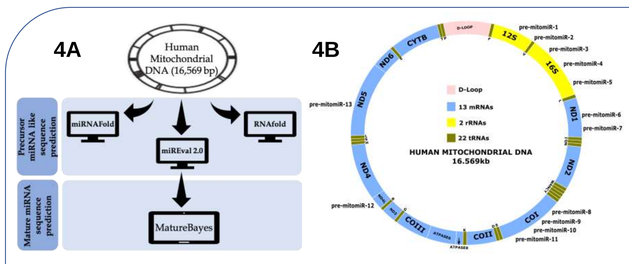
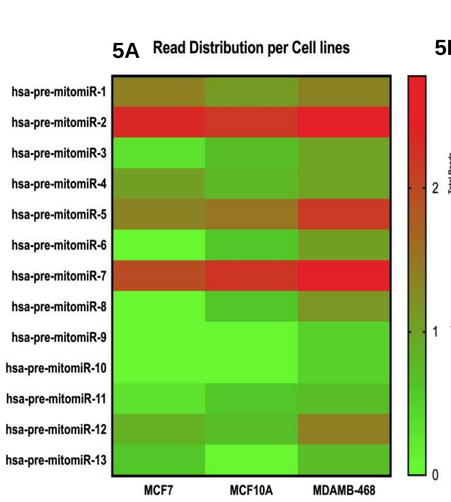


Figure 4- *In-silico* identification of mitomiRs:
A: MitomiR prediction strategy; B: Localization of predicted precursor mitomiR candidates on mitochondrial genome.



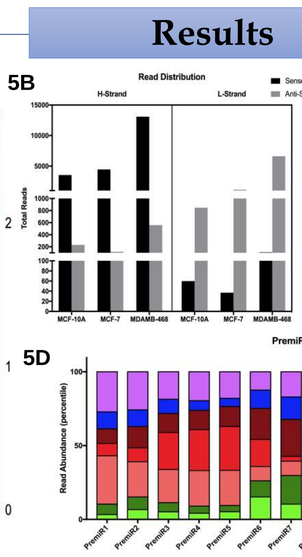


Figure 5- Small RNA sequencing:
A: Read distribution of 13 pre-mitomiRs in breast cancer cell lines; B: Read distribution on H & L strands of mtDNA; C: Read distribution on various mitochondrial genes; D: Read distribution of mitomiRs on various breast cancer cell lines (public dataset).

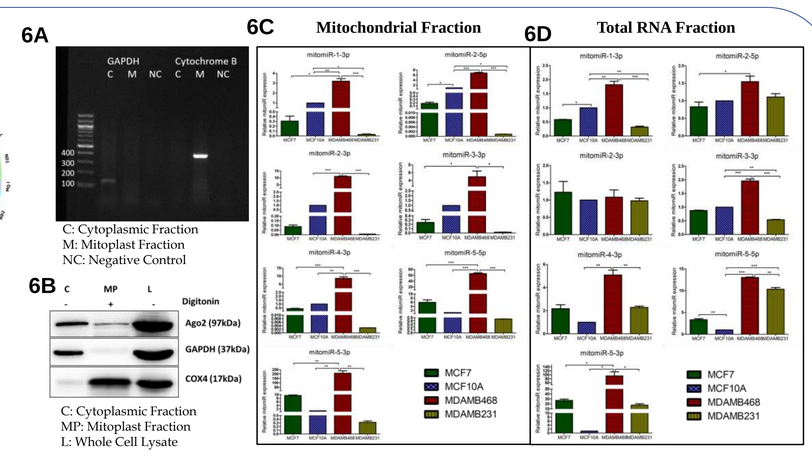


Figure-6 Expression profile of mitomiRs in breast cancer cell line mitochondria
A & B: Purity of isolated mitochondria fraction (RNA & Protein);
C & D: mitomiR expression profile in breast cancer cell lines (MCF10A: reference; 5S rRNA: endogenous control).

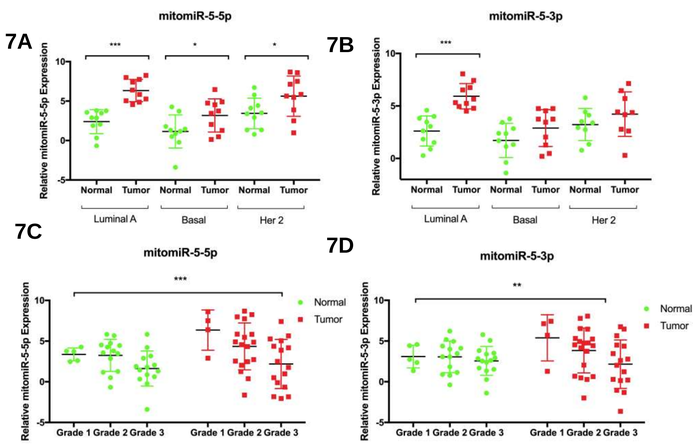


Figure 7- Expression profile of mitomiRs in luminal A, basal and Her2 positive breast cancer tissue samples:
(A&B): Hormonal classification; (C&D) Tumor grade-based classification in n=40 samples.
Relative quantification of mitomiR-5-5p and mitomiR-5-3p in luminal A (n=15), basal (n=15) and Her2 positive (n=10) breast cancer specimens with matched controls and RNU6B as endogenous control;

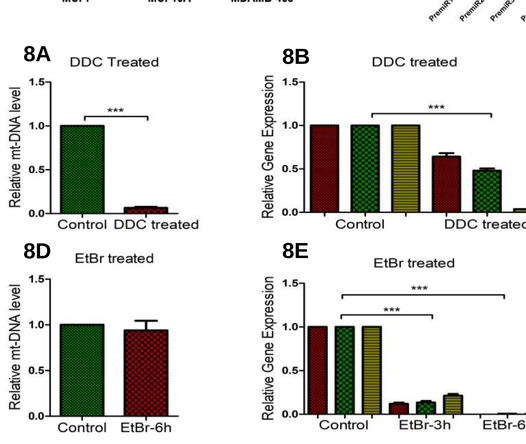


Figure 8- mitomiR-5 is encoded by mitochondrial genome:
mtDNA less cells in MCF7: 2, 3-Dideoxycytidine (DDC-2µM) treated: mtDNA replication inhibition; and Ethidium Bromide (EtBr-50ng/ml) treated: mtDNA transcription inhibition. (A&D) Copy Number Variation, (B&E) Gene Expression and (C&F) mitomiR & Nuclear miRNA expression validation was done by qRT-PCR using the formula $2^{-\Delta\Delta Ct}$.

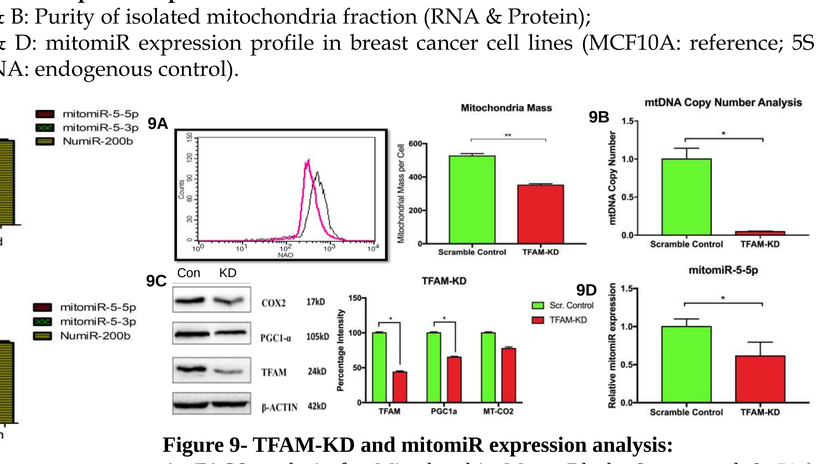


Figure 9- TFAM-KD and mitomiR expression analysis:
A: FACS analysis for Mitochondria Mass; Black: Scr. control & Pink: TFAM-KD.
B: mtDNA copy number analysis in TFAM-KD.
C: Immunoblotting analysis showing TFAM levels in TFAM-KD cells.
D: mitomiR-5-5p expression in TFAM-KD cells through qRT-PCR analysis.

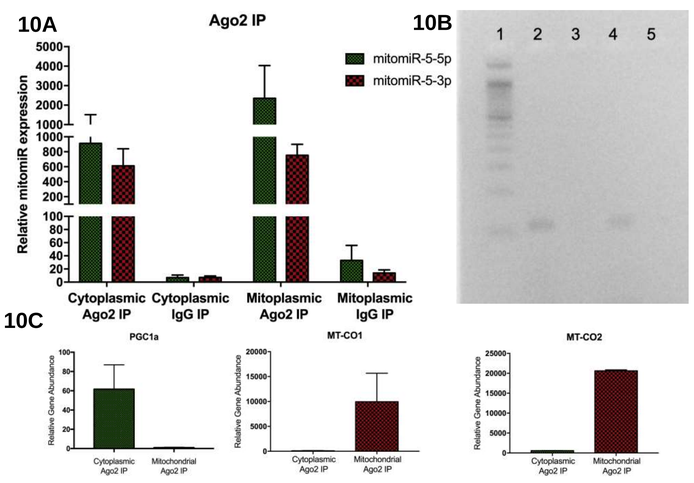
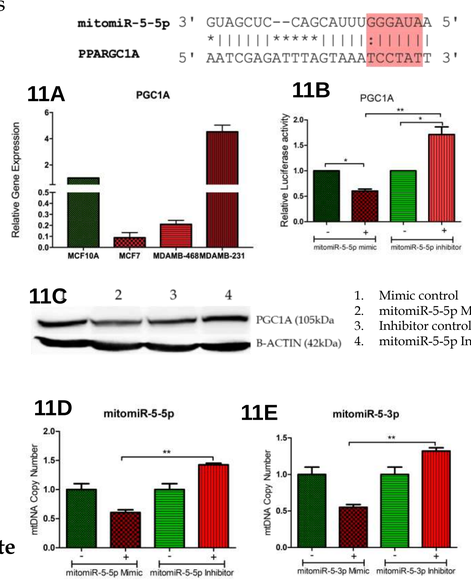


Figure 10- mitomiR-5-5p and mitomiR-5-3p associates with Argonaute 2 inside mitochondria:
A: mitomiR-5-5p and mitomiR-5-3p abundance in MDA-MB-468 cells. Rabbit IgG: experimental control, uncoated empty beads: negative control, formula $2^{-\Delta Ct}$. B: Agarose gel electrophoresis: Ago2-IP & IgG-IP in 2&3: cytoplasmic, 4&5 mitochondria fraction. C: Target gene abundance in Ago2-IP elute from cytoplasmic & mitochondria fraction.



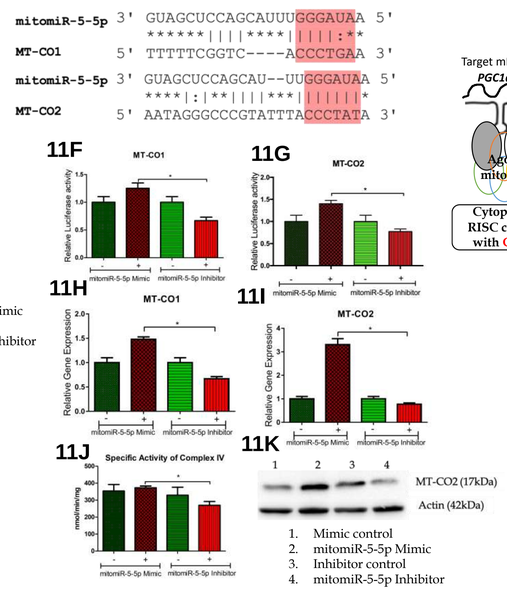
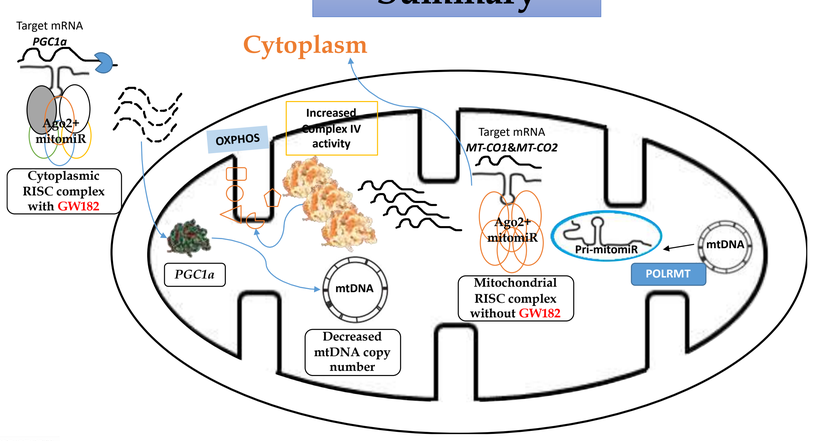


Figure 11- Target gene validation of mitomiR-5:
A-E: *PGC1a* target gene validation in MDA-MB-468 breast cancer cell lines with mitomiR-5-5p mimic & inhibitors.
F-K: *MT-CO1* and *MT-CO2* target gene validation in MDA-MB-468 breast cancer cell lines with mitomiR-5-5p and mitomiR-5-3p mimic & inhibitors.

Summary



- ❖ mitomiR-5 is encoded from 16S rRNA of mtDNA by the mitochondrial transcription machinery comprising of *POLRMT* and *TFAM*.
- ❖ mitomiR-5 expression levels are significantly enhanced in Luminal A, Triple Negative Breast Cancer (Cell lines & Tissue samples).
- ❖ mitomiR-5 trans-locates to cytoplasm to regulate nuclear encoded target gene PGC1a after associating with Ago2-RISC complex in mitochondria.
- ❖ mitomiR-5 regulates mitochondrial complex activity by altering the levels of mitochondrial complex proteins MT-CO1 and MT-CO2 in Triple Negative Breast Cancer cells.

Acknowledgement

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