REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1. Name and designation of ICMR- IF : **Dr. Pallavi Shukla**
   Scientist C

2. Address: **Department of Molecular Endocrinology,**
   Indian Council of Medical Research-
   National Institute for Research in Reproductive Health (ICMR-NIRRH),
   Mumbai

3. Frontline area of research in which training/research was carried out : **Molecular Genetics, Genomics and cell biology**

4. Name & address of Professor and host institute **Dr. Keshav Singh,Professor of Genetics, Pathology and Environmental Health at Department of Genetics, School of Medicine, University of Alabama Birmingham, Alabama, USA**

5. Duration of fellowship : **1 year (31st Jan 2020-31st Jan 2021)**

6. Highlights of work conducted:

   i) Technique/expertise acquired : **CRISPR/CAS9 technique**
   SRB assay for cell proliferation study
   Cell Migration Assay
   Cell Invasion Assay
   mtDNA copy number analysis
   TCGA/CPTCA data analysis
   Subcellular fractionation (for nuclear And mitochondrial separation)

   ii) Research results, including any papers, prepared/submitted for publication

Title of the study: **Analysis of Abundance of Nuclear Mitochondrial DNA Segment (NUMT) and Role of *Yme1L1* Gene in Endometrial Cancer**

Yme1L1 is described to be the first numtogenesis suppressor protein. Yme1L1 (YME1 Like 1 ATPase) is a gene that encodes a protein which is the human ortholog of yeast mitochondrial AAA metalloprotease, Yme1p. It is localized in the mitochondria and plays an important role in mitochondrial protein metabolism. Further it also regulates mitochondrial structure and maintain normal cristae morphology. It's role in ensuring cell proliferation, proper assembly of respiratory complexes, mitophagy and apoptosis has also been recognized. Singh and group describe that loss of Yme1l1 leads to migration of mitochondrial DNA segments to nuclear DNA, process known as Numtogenesis. Increased abundance of nuclear mitochondrial DNA segments (NUMTs) may cause genomic instability and may play a role in cancer development. Yme1l1 is known to be involved in mitochondrial pathologies but its role in cancer is sparsely studied. Moreover, its role in racial disparity has not been reported. The research study was taken to study the role of Yme1L1 in endometrial cancer. The role of Yme1L1 in cancer is has not been elucidated yet.

Material Methods: We analyzed Clinical Proteomic Tumor Analysis Consortium (CPTAC) databases (https://proteomics.cancer.gov/programs/cptac) to study the expression of YME1L1 protein in endometrial cancer. The functional role of Yme1L1 was analysed in AN3CA cell line (endometrial cancer Type II) using CRISPR/CAS9 knockout followed by functional genomics techniques.

We identified many novel findings.

CPTAC data analysis

CPTAC data analysis showed that Yme1L1 protein expression is increased in uterine corpus endometrial carcinoma (UCEC) samples compared to normal samples (Fig. 1a). Yme1L1 protein expression is increased in all stages of UCEC samples compared to normal samples (Fig. 1b). Yme1L1 protein expression is significantly increased in all grades 1 of UCEC Samples compared to normal samples (Fig. 1c). Grade 3 is significantly increased as compared to grade 2 UCEC samples ($p<1E-12$). Yme1L1 expression is increased significantly in endometrial carcinoma, serous carcinoma UCEC samples and others compared to normal samples (Fig. 1d).

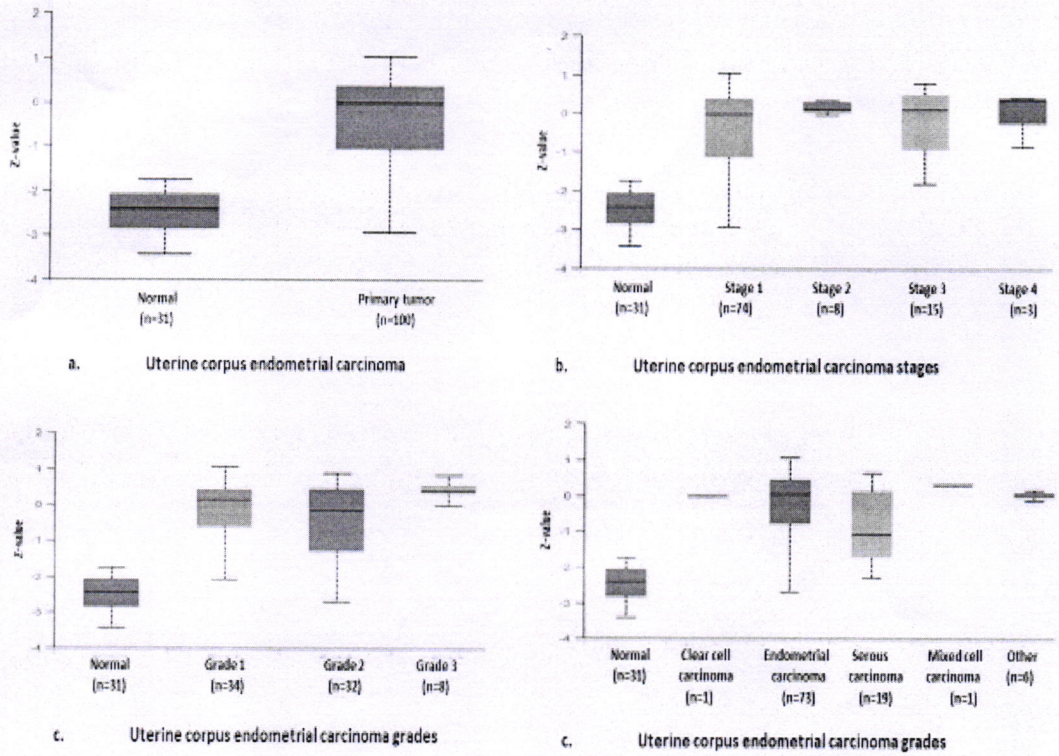
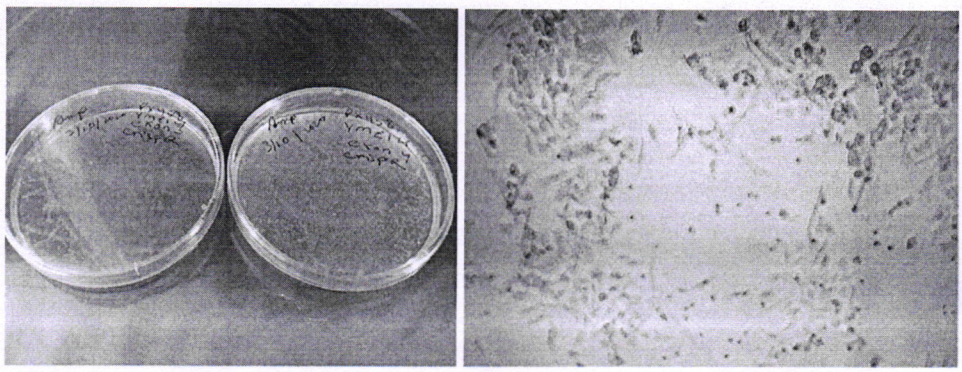


Fig. 1 CPTAC data analysis of Yme1L1 in Uterine corpus endometrial carcinoma

CRISPR/CAS9 knockout

Yme1L1 was knocked out in AN3CA cancer cells (Type II endometrial cancer) using CRISPR/CAS9 technique (Fig.2a,b) using PAX458 vector.



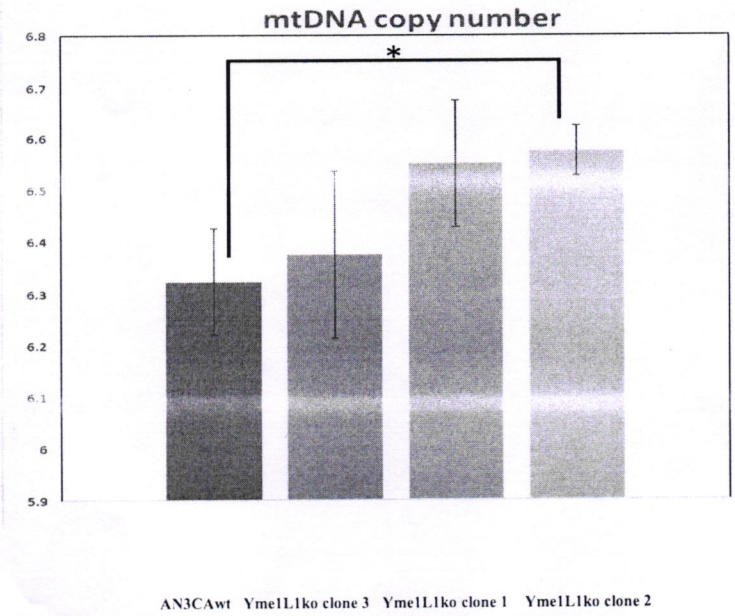
PAX458 Crispr/cas9 Yme1L1Ko clones AN3CA cell line

Functional analysis
Results

1. Loss of YME1L1 promote numtogenesis

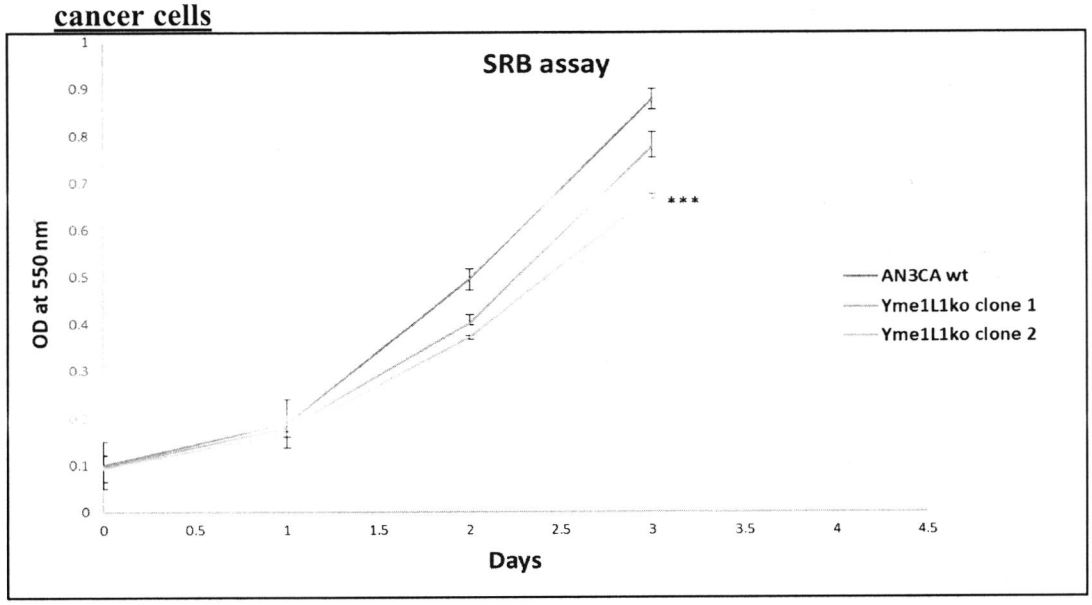
mtDNA copy number analysis

Nuclear separation was done as per protocol. DNA quantification done by nanometer. mtDNA copy number analysis was performed by Real time PCR



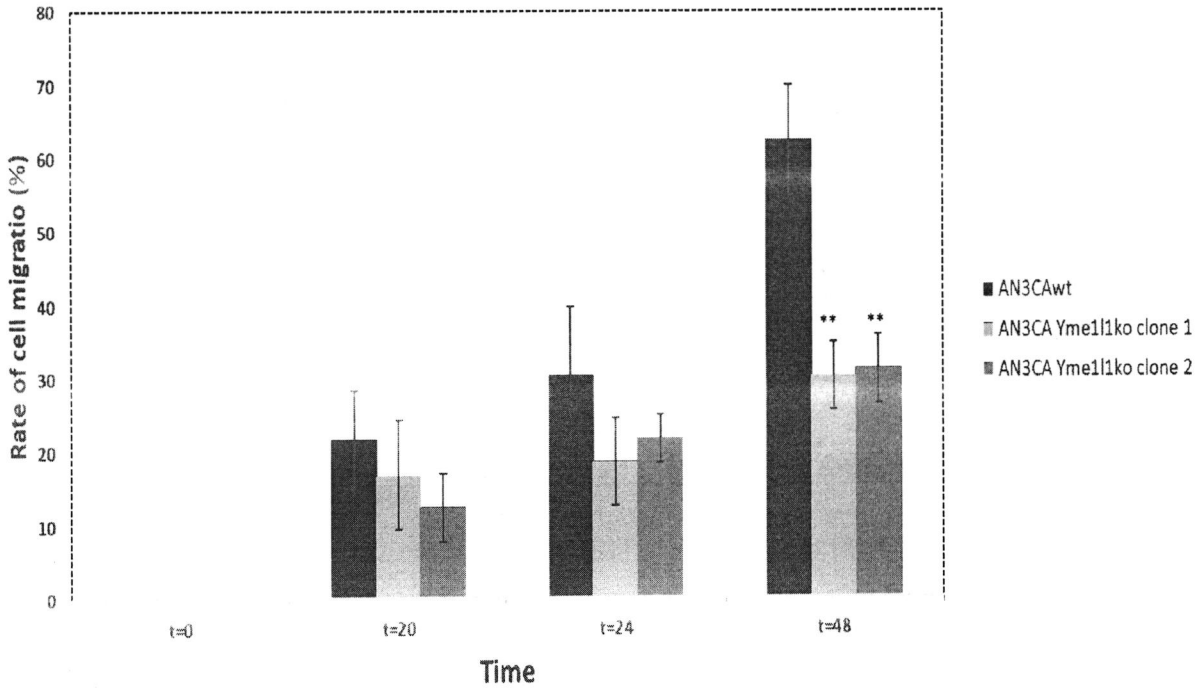
Yme1L1 Ko shows increase in mtDNA copy number with increase in KO efficiency in AN3CA cell line and found to be increased in all clones and significantly increased in one of the clone ($p=0.0469$).
Finally clone 1 and 2 were chosen for further functional analysis.

2. **Loss of *Yme1L1* leads to decreased cell proliferation in Endometrial cancer cells**



*Yme1L1*Ko clones 1, 2 were found to have decreased proliferation compared to AN3CAwt

3. **Loss of *Yme1L1* leads to decreased cell migration in Endometrial cancer cells**



Lectures attended

1. Genetics and genomics seminar series lecture by Dr. Girish C Melkani entitled **"Pathophysiological basis and mitigation of cardiometabolic and Genetic disorders"** at UAB, USA on 15 Jan 2021

2. Lecture by Dr. Scott M. Williams entitled **"The Genetics of Infectious Disease: Disrupted Host-Pathogen Co-Evolution"** on 7th August 2020.

Training in Animal Courses

Certificates were obtained for the following online courses conducted by animal house at UAB

- Working with Mice in Research
- Using Animals for Teaching Testing and Research
- Tumor guidelines
- Fighting mouse guidelines
- Rodent Surgery

Publications

1. **Shukla P***, Singh KK. Uncovering Mitochondrial Determinants of Racial Disparities in Ovarian Cancer. Trends Cancer. 2021 Feb;7(2):93-97. **(1F=11).**

2. Chiang JL, **Shukla P**, Pagidas K, Ahmed NS, Karri S, Gunn DD, Hurd WW, Singh KK. Mitochondria in Ovarian Aging and Reproductive Longevity. Ageing Res Rev. **2020** Nov;63:101168. **(1F=11).**

3. **Pallavi Shukla** and Keshav K Singh, The Mitochondrial Landscape of Ovarian Cancer: Emerging insights (Submitted in "Carcinogenesis" journal).

4. **Pallavi Shukla** and Keshav K Singh, Numtogenesis Suppressor *Yme1L1* Gene Expression in African Americans Cancer Patients (manuscript in preparation).

5. **Pallavi Shukla** and Keshav K Singh. Loss of Yme1L1 promotes numtogenesis, perturbs mitochondrial dynamics and leads to decreased cell proliferation and cell migration in endometrial cancer cells. (manuscript in preparation).

iii) Proposed utilization of the experience in India

Various technical experience gained at University of Alabama (UAB), USA will be utilized in India in many ways. Projects will be initiated based on CRISPR/CAS9 technique to study the novel functional role mitochondrial genes in gynecological cancers. Further utilization of CPTCA data will be done to make novel hypothesis in reproductive cancers. Animal courses training will help in designing studies on animal models to study the role of desired gene in-vivo. Translational projects will also be initiated in future to study the expression and mechanism in women with cancers. NUMT analysis will be sought to examine as a diagnostic/prognostic marker in women with cancer.

Furthermore, the fellowship has given an excellent opportunity to build an international collaboration with the mentor in designing Indo-US collaborative project in near future.

Signature of ICMR-IF

ICMR Sanction No. INDO/FRC/452(Y- 9y201 9-20-lHD