REPORT

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

1.	Name and designation of ICMR- IF	: Yeguvapalli Suneetha Assistant Professor
2.	Address	: Department of Zoology, Sri Venkateswara University, Tirupati- 517502
3.	Frontline area of research in which training/research was carried out	: Health Sciences
4.	Name & address of Professor and host institute	: Jamboor K. Vishwanatha, Ph.D. Regents Professor and Vice President Principal Investigator, National Research Mentoring Network Director, Texas Center for Health Disparities Center for Diversity and International Programs University of North Texas Health Science Center 3500 Camp Bowie Blvd Fort Worth, TX 76107
5.	Duration of fellowship with exact date	: 3 months (1-3-2020 to 31-5-2020)
6.	Highlights of work conducted	: Enclosed Annexure I
	i) Technique/expertise acquired	:
	ii) Research results, including any papers, prepared/submitted for publication	
	iii) Proposed utilization of the experience in India	:

Y. Suneetha

Signature of ICMR-IF

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Annexure I

Highlights of work conducted

i) Technique/expertise acquired:

Bioinformatics analysis

In silico analyses were performed to determine the putative miRNAs that are key for breast cancer. The software programs used included miRANDA; a comprehensive modeling of microRNA targets to predict functional non-conserved and non-canonical sites (Betel et al., 2010), PicTar; a combinatorial microRNA target predictions server (Krek et al., 2005), miRbase; annotating high confidence microRNAs using deep sequencing data (Kozomara and Griffiths-Jones, 2014), TargetScan; a conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets (Lewis et al., 2005), all of which used the 3'UTR as the target region to determine miRNA recognition elements and provided scores to determine predictive values, PITA; an algorithm for site accessibility in microRNA target recognition (Kertesz et al., 2007), RNAhybrid; a server that make microRNA target prediction easy, fast and flexible (Kruger and Rehmsmeier, 2006), miRU; an automated plant miRNA target prediction server (Zhang, 2005), DIANA-microT; a server to predict functional microRNA targets in protein coding sequences (Reczko et al., 2012), EIMMo; a server to infer miRNA targets using evolutionary conservation and pathway analysis (Gaidatzis et al., 2007) and RNA22 tool, which identifies putative target sites (target islands) independently of the conservation status and a pattern-based method for the identification of microRNA binding sites and their corresponding heteroduplexes (Miranda et al., 2006). The human disease association for respective miRNAs were performes using HMDD V2.0 database (Li et al., 2014), a database that covers 5430 known miRNA-disease associations between 495 miRNAs and 383 diseases. Due to the extreme COVID-19 situation in the USA, expertise/training on some of the experimental procedures/techniques mentioned were acquired remotely.

Cell lines and culture

The human epithelial breast cancer cell line MDA-MB-231 was obtained from the American Type Culture Collection (Manassas, VA, USA). The cell lines were authenticated by STR analysis with the Promega PowerPlex Fusion V1.0. All three cell lines tested negative for mycoplasma infection when tested with MycoAlert PLUS from Lonza (Basel, Switzerland). The cell lines were confirmed to be mycoplasma free prior to use. All cell lines were cultured in DMEM high-glucose (HyClone) supplemented with 10% FBS, 4.05mM glutamine, 100IU penicillin, 100IU streptomycin and 0.25ug/ml Amphotericin B. Cultures were maintained in a humidified incubator at 37°C with 5% CO2.

siRNA, miRNA and plasmid transfections

The cell lines were authenticated according to "Authentication of Human Cell Lines: Standardization of STR Profiling" using GenePrint® 10 System (Promega); all cell lines and their passages exhibited >80% match to the initial cell line STR profile provided by ATCC. The smart pool siRNAs were obtained from Dharmacon (Thermo Fisher Scientific), while the precursor and inhibitor miRNA oligos (Pre- and Anti-miR) were purchased from Ambion (Life Technologies). The final concentration of the miRNA oligos used for transfection was determined by preliminary concentration-dependent studies and remained constant for all the experiments. Plasmid transfections were performed using Lipofectamine 2000 while Lipofectamine RNAiMAX was used for RNAi transfections, performed according to the manufacturer's protocols (Life Technologies).

Antibodies and reagents

The following antibodies and reagents were used: mouse monoclonal and mouse polyclonal genes (Abnova; antibody specificity tested and proven in previous studies (Dasgupta et al., 2009), rabbit polyclonal genes (Life Technologies; antibody specificity tested in previous studies, mouse monoclonal GAPDH (Santa Cruz Biotechnology), rabbit monoclonal pNF-κB p65 S536 and rabbit polyclonal MMP-9 (Cell Signaling Technology), mouse monoclonal VEGF and uPA (R&D Systems), mouse monoclonal Alexa Fluor 594 conjugated Phalloidin (Life Technologies), mouse monoclonal E-cadherin (BD Biosciences), Vimentin (supernatant developed in mouse and tested against human antigen, Developmental Studies Hybridoma Bank), anti-mouse and anti-rabbit IgG (Promega), AlexaFluor 488 goat anti-mouse IgG and AlexaFluor 594 goat anti-mouse IgG (Life Technologies) sheep anti-DIG-AP antibody and NBT-BCIP ready-to-use tablets (Roche), sheep serum (Jackson ImmunoResearch), rabbit IgG, BSA, levamisole hydrochloride, Tris-HCl (pH 7.4),

nuclease free water, SSC buffer, Xylene, Tween-20, Nuclear Fast Red, Hematoxylin and Eosin (Sigma-Aldrich) and Permount and PBS (Thermo Fisher Scientific).

qPCR

Total RNA was isolated from the cell lines using TRIzol (Life Technologies) and quantified. Equal amount of RNA was used for the one-step or two-step qPCR performed using the Superscript III SYBR Green qRT-PCR kits, according to manufacturer's instructions (Life Technologies). For miRNA, PCR was performed using NCode VILO miRNA cDNA Synthesis and EXPRESS SYBR GreenER miRNA qRT-PCR Kits (Life Technologies), according to the manufacturer's protocol. The primers (sequences provided in the Supplementary materials and methods; Additional file 7) were designed using Primer 3 (Koressaar and Remm, 2007) and synthesized by Integrated DNA Technologies (Coralville, IA). PCR was performed using Realplex2 Mastercycler ep gradient S thermal cycler (Eppendorf).

Western blotting

Western blotting was performed according to standard protocols. Briefly, total protein was isolated using NP-40 lysis buffer and estimated using the standard Micro BCA Protein Assay Kit (Pierce Biotechnology). NuPAGE® Novex® 4-12% Bis-Tris Gels were used and the samples were transferred onto nitrocellulose membranes using an iBlot (Life Technologies). Membranes were blocked in 5% non-fat dry milk or 1% BSA prior to antibody subjection. The chemiluminescent reaction was captured by the AlphaImager (ProteinSimple) and bands were analyzed using ImageJ software (Schneider et al., 2012).

Northern blotting

Northern blotting was performed using miRNA Northern Blot Assay Kit and custom ordered biotin-labeled miRNA and control probes (Signosis) with one microgram of total RNA from each cell line, according to manufacturer's instructions.

RNA stability assay

Cells were transfected with the precursor oligomiRs and 48 hours after transfection, treated with 10 µg/ml Act-D (Sigma-Aldrich). RNA was isolated at several time points and quantified. Equal amounts of RNA were used to run qPCR to determine gene levels.

Luciferase reporter assay

Cells were transfected with 3′UTR luciferase constructs (Origene) - Empty Vector (Vec) or 3′UTR-MIEN1 (MIEN1WT / MIEN1Mut) and miR-940 or miR-NT in duplicate. Luciferase assay was

performed using the Luciferase Assay System (Promega) according to manufacturer's instructions and luminescence read using Synergy2 Alpha Microplate Reader (BioTek).

Statistical analyses

The results were calculated as mean ± S.E.M of independent experiments. The p-value was calculated according to Student's t-test when comparing two groups using GraphPad P-value calculator. The differences were considered significant if p-value was at least ≤ 0.05.

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

Datamining of breast cancer miRNAs

PubMed and EMBASE was searched using a highly sensitive and highly specific search strategy, which was "(breast cancer [MeSH Terms] OR carcinoma OR breast OR cancer OR disease) AND (miRNA [MeSH Terms] OR miRNA OR miR OR mircoRNA)." Search was updated to April 2020. Our results showed 83 miRNAs that are related to breast cancer. List of miRNAs that are known to be regulated in breast cancer is shown in the Table 1 given below.

Table 1. List of miRNAs that are regulated in breast cancer

Disease name:	**Carcinoma, Breast**	
miRNA_name	**PMID**	**Description**
hsa-mir-106b	27519168	down-regulation of miR-106b increased the expression of FUT6 and resulted in an obvious decrease of cell migration, invasion, and proliferation in MDA-MB-231 cells.
hsa-mir-124	27748910	MicroRNA-124 inhibits cell proliferation and migration by regulating SNAI2 in breast cancer.
hsa-mir-1254	30132526	MicroRNA-1254 exertsÂ oncogenic effects by directly targeting RASSF9 in human breast cancer.
hsa-mir-125a	30076753	MiR-125a-5p functions as a tumour suppressor in breast cancer by downregulating BAP1.
hsa-mir-125b	30177391	miR-125b-5p inhibits breast cancer cell proliferation, migration and invasion by targeting KIAA1522.
hsa-mir-130a	29384218	microRNA-130a suppresses breast cancer cell migration

		and invasion by targeting FOSL1 and upregulating ZO-1.
hsa-mir-130a	29746865	MiR-130a-3p inhibits migration and invasion by regulating RAB5B in human breast cancer stem cell-like cells.
hsa-mir-130b	28163094	miR-130b-3p inhibits cell invasion and migration by targeting the Notch ligand Delta-like 1 in breast carcinoma.
hsa-mir-140	30032164	miR-140-5p inhibits the proliferation and enhances the efficacy of doxorubicin to breast cancer stem cells by targeting Wnt1.
hsa-mir-142	26657485	microRNA miR-142-3p Inhibits Breast Cancer Cell Invasiveness by Synchronous Targeting of WASL, Integrin Alpha V, and Additional Cytoskeletal Elements. HrC-9698
hsa-mir-142	29620260	MicroRNAâ€'142â€'5p modulates breast cancer cell proliferation and apoptosis by targeting phosphatase and tensin homolog.
hsa-mir-145	28349828	Silencing of bach1 gene by small interfering RNA-mediation regulates invasive and expression level of miR-203, miR-145, matrix metalloproteinase-9, and CXCR4 receptor in MDA-MB-468 breast cancer cells.
hsa-mir-145	28393176	miR-145 inhibits proliferation and migration of breast cancer cells by directly or indirectly regulating TGF-Î²1 expression.
hsa-mir-146a	29915929	Identification of miR-146a is Associated with the Aggressiveness and Suppresses Proliferation via Targeting CDKN2A in Breast Cancer.
hsa-mir-151	27930738	miR-151-3p Targets TWIST1 to Repress Migration of Human Breast Cancer Cells.
hsa-mir-15a	27596816	miR-15a/miR-16 induces mitochondrial dependent apoptosis in breast cancer cells by suppressing oncogene BMI1.
hsa-mir-16	27596816	miR-15a/miR-16 induces mitochondrial dependent apoptosis in breast cancer cells by suppressing oncogene BMI1.
hsa-mir-181	28224609	miR-181 elevates Akt signaling by co-targeting PHLPP2 and INPP4B phosphatases in luminal breast cancer.
hsa-mir-183	27476679	Overexpression of miR-183-5p significantly enhanced the

		cell proliferation and inhibited cell apoptosis in MCF-7 and MDA-MB-231 cells.
hsa-mir-185	27651238	RKIP suppresses the proliferation and metastasis of breast cancer cell lines through up-regulation of miR-185 targeting HMGA2.
hsa-mir-185	30015912	miRâ€'185â€'5p inhibits Fâ€'actin polymerization and reverses epithelial mesenchymal transition of human breast cancer cells by modulating RAGE.
hsa-mir-191	30084985	Amplification of Hsa-miR-191/425 Locus Promotes Breast Cancer Proliferation and Metastasis by Targeting DICER1.
hsa-mir-193b	30320920	MORC4 is a novel breast cancer oncogene regulated by miR-193b-3p.
hsa-mir-199b	30250555	miR-199b-5p inhibits triple negative breast cancer cell proliferation, migration and invasion by targeting DDR1.
hsa-mir-19b	30038508	miR-19b serves as a prognostic biomarker of breast cancer and promotes tumor progression through PI3K/AKT signaling pathway.
hsa-mir-200b	28972876	miR-200b regulates epithelial-mesenchymal transition of chemo-resistant breast cancer cells by targeting FN1.
hsa-mir-200c	30209363	Phosphodiesterase 7B/microRNA-200c relationship regulates triple-negative breast cancer cell growth.
hsa-mir-203	28349828	Silencing of bach1 gene by small interfering RNA-mediation regulates invasive and expression level of miR-203, miR-145, matrix metalloproteinase-9, and CXCR4 receptor in MDA-MB-468 breast cancer cells.
hsa-mir-205	27468619	Knock-up of miR-205 expression by transfection with its mimics promoted MDA-MB-468 cells apoptosis (P=0.006 1).
hsa-mir-206	29886033	Down-regulation of NAMPT expression by mir-206 reduces cell survival of breast cancer cells.
hsa-mir-21	28067096	Differential response of normal and transformed mammary epithelial cells to combined treatment of anti-miR-21 and radiation.
hsa-mir-210	30188754	Up-regulation of miR-210 induced by a hypoxic microenvironment promotes breast cancer stem cells

		metastasis, proliferation, and self-renewal by targeting E-cadherin.
hsa-mir-216b	27720715	miR-216b suppresses breast cancer growth and metastasis by targeting SDCBP.
hsa-mir-217	27916422	PGC-1 alpha interacts with microRNA-217 to functionally regulate breast cancer cell proliferation.
hsa-mir-221	30110679	Our findings provide strong evidence that miR-9 and miR-221 can enhance the generation of cancer stem cells to yield an invasive phenotype and that overexpression of these miRNAs predicts a poor outcome for breast cancer patients
hsa-mir-23a	30007957	Effect of the LncRNA GAS5-MiR-23a-ATG3 Axis in Regulating Autophagy in Patients with Breast Cancer.
hsa-mir-26a	27517917	MiR-26a overexpression resulted in a reduction in cell viability that was partially recovered by inhibiting it.
hsa-mir-26b	29620147	lncRNA PVT1 promotes the angiogenesis of vascular endothelial cell by targeting miRâ€‘26b to activate CTGF/ANGPT2.
hsa-mir-27a	28099945	In vivo and in vitro effects of microRNA-27a on proliferation, migration and invasion of breast cancer cells through targeting of SFRP1 gene via Wnt/Î²-catenin signaling pathway.
hsa-mir-27b	30012170	Suppression of PDHX by microRNA-27b deregulates cell metabolism and promotes growth in breast cancer.
hsa-mir-29a	29021023	Knockdown of microRNA-29a Changes the Expression of Heat Shock Proteins in Breast Carcinoma MCF-7 Cells.
hsa-mir-29a	29435304	Knockdown of microRNA-29a regulates the expression of apoptosis-related genes in MCF-7 breast carcinoma cells
hsa-mir-301b	30269739	MicroRNA-301b promotes cell proliferation and apoptosis resistance in triple-negative breast cancer by targeting CYLD.
hsa-mir-3178	30333478	miR-3178 inhibits cell proliferation and metastasis by targeting Notch1 in triple-negative breast cancer.
hsa-mir-322	28404630	miR-424(322)/503 is a breast cancer tumor suppressor whose loss promotes resistance to chemotherapy.

hsa-mir-328	29620238	miR-328-5p inhibits MDA-MB-231 breast cancer cell proliferation by targeting RAGE.
hsa-mir-330	28419078	Targeting of CCBE1 by miR-330-3p in human breast cancer promotes metastasis.
hsa-mir-340	30300682	LGR5 acts as a target of miR-340-5p in the suppression of cell progression and drug resistance in breast cancer via Wnt/Î²-catenin pathway.
hsa-mir-346	27913185	MiR-346 promotes the biological function of breast cancer cells by targeting SRCIN1 and reduces chemosensitivity to docetaxel.
hsa-mir-34a	27524218	MiR-34a expression was remarkably down-regulated in BC tissues and cell lines compared with normal tissues and cell lines.
hsa-mir-34a	27813227	MiR-34a modulates ErbB2 in breast cancer.
hsa-mir-365	27906431	Overexpression of microRNA-365 inhibits breast cancer cell growth and chemo-resistance through GALNT4.
hsa-mir-375	28075453	miR-375 inhibits cancer stem cell phenotype and tamoxifen resistance by degrading HOXB3 in human ER-positive breast cancer.
hsa-mir-381	28012397	miR-381 inhibited breast cancer cells proliferation, epithelial-to-mesenchymal transition and metastasis by targeting CXCR4.
hsa-mir-384	29693185	MicroRNA-384 inhibits the progression of breast cancer by targeting ACVR1.
hsa-mir-3908	28327197	Lipid raft-mediated miR-3908 inhibition of migration of breast cancer cell line MCF-7 by regulating the interactions between AdipoR1 and Flotillin-1.
hsa-mir-409	28459205	MicroRNA-409-5p is upregulated in breast cancer and its downregulation inhibits cancer development through downstream target of RSU1.
hsa-mir-410	30016800	MiR-410 Acts as a Tumor Suppressor in Estrogen Receptor-Positive Breast Cancer Cells by Directly Targeting ERLIN2 via the ERS Pathway.
hsa-mir-411	27572271	miRNA-411 acts as a potential tumor suppressor miRNA via the downregulation of specificity protein 1 in breast