TOUR REPORT

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

1. Name and Designation of ICMR IF : **Dr. Annamaneni Sandhya, Assistant Professor**

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3. Frontline Area of Research in which training/ research was carried out: **Human Genetics**

4. Name and Address of Professor and host Institute:
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5. Duration of Fellowship: **One year (1st November 2019 to 31st October 2020)**

6. Highlights of work conducted:

The focus of the project was on Synthetic lethality based therapeutic approaches to treat metastatic breast cancer and Ovarian Cancer, mainly associated with chemoresistance and design of novel therapeutics. Following are the brief highlights of the work conducted.

- Identified Haspin Kinase as a therapeutic target for triple negative breast cancer and chemoresistant ovarian cancer.
- Explored DNA damage response signaling pathways in cancer and identified novel function for Haspin Kinase in the regulation of FA-BRCA tumor suppressor pathway.
- Evaluated therapeutic efficacy of Haspin Kinase in platinum sensitive and resistant ovarian cancer models.
- *In vitro* and *in vivo* evaluation of anti-cancer compounds in the development of novel combinationatorial therapies for chemosistant breast and ovarian cancers
- Generation and establishment of primary cell lines from breast and ovarian cancer tissues of patients.
- Establishment of patient derived tumor organoids for *ex vivo* pharmacological screening

Introduction/ Background of the project

Breast and ovarian cancers are the most common heterogeneous malignancies in women accounting to worldwide incidence of 25% and 4% respectively. Breast cancer is the second largest cause of cancerous deaths among Indian women. The prognosis for patients without metastatic disease is very good with a 5-year relative survival rate over 95%. However, for metastatic tumors, the survival rate decreases to 85% for patients with regional lymph node metastases and 22% when there are distant metastases. The treatment is ineffective in many due to drug resistance and relapse. Similarly, ovarian cancer is a serious problem worldwide and is the most lethal gynecological malignancy in women. It is typically asymptomatic during early development and thus is frequently detected at late stages with poor prognosis and a five year survival rate being 40-50%. Tumor heterogeneity, dynamic tumor microenvironment, metastatic potential, drug resistance and recurrence are major hurdles for effective therapy in these cancers. Therapeutic efficacies of PARPi are only 10-15% for breast cancer patients who have BRCA mutations or homologous recombination deficient (HRD) tumors, in which they exert therapeutic activity by synthetic

lethality. Current clinical trials although have shown remarkable success for PARPi in treating a small subset of metastatic breast cancer patients with BRCA mutations and advanced ovarian cancers with defective repair, other potential synthetic approaches need to discovered for the benefit of majority. Better understanding of cellular and molecular mechanisms that promote metastasis and chemoresistance is required to develop novel effective treatment strategies. This highlights the need to find new therapeutic options and in view of this, our goal is to identify key regulators of these complex networks in tumors and develop rotationally designed therapeutic combinations for effective therapy. The present study was aimed to evaluate the role of novel target, haspin kinase in the DNA damage and repair pathway in breast and ovarian cancers and pharmacologically test the therapeutic efficacy of haspin kinase inhibitors.

i) Technique/expertise acquired:

The projects undertaken during the training broadly focus on discovery and development of novel therapeutics for cancer therapy, and involved basic to advanced technical as well a scientific approaches useful for translational and early pre-clinical research. Particularly, the emphasis was on DNA damage response signaling pathways in breast and ovarian cancers and involved handling of several cell lines that included human breast (MCF-7, MDA-MB-231 and MDA-MB-453), ovarian (Ovcar3, Ovcar4, Ovcar5, Ovcar8, OV90, ES-2, SKOV3 and A2780) cell lines and the normal immortalized cells (HDF, FTEC). Besides basic cell culture techniques, through *in vitro* and *in vivo* evaluation of the identified molecular target, Haspin Kinase in cancer, I could gain expertise in advanced techniques such as isolation of proteins, RNA and DNA from cell lines; Gene expression analysis; Western blot analysis; transfection of plasmids; siRNA transfection; Immunohistochemistry and Immmuno florescence assays for colocalisation studies; Comet assay for assessment of DNA damage; Chromatin Immunoprecipitation assays to explore protein-protein interactions for identifying haspin interactors; and cancer phenotype analysis (Migration, Invasion and Spheroid assays). Further, I could get immense exposure to applications of Flow cytometry through cell cycle analysis, expression of cell markers and Dr-GFP DNA repair reporter assay to assess proficiency of homologous recombination. Additionally, leant specialized techniques like DNA Fiber assays to evaluate the role of the target protein in DNA replication dynamics and repair. Upgrading the skills in generation of primary cell cultures, patient derived organoids and *in vivo* evaluation of therapeutic efficacy of compounds, enhanced my research strengths to independently plan and execute complex *in vivo* studies in future in the area of cancer therapeutics. Overall, by analysis of several DNA damage

response and repair proteins in the Homologous Recombination Repair pathway for their effects on cell responses to different DNA damaging agents/drugs in haspin inhibited cells, the training could provide in-depth expertise on not only how to identify and validate the function of a target protein but also imparted immense knowledge on designing of novel therapeutic approaches to address challenges in the management of aggressive and chemoresisant cancers. Further, being in highly motivated and interactive team and assisting in other lab projects, gained exposure on molecular techniques like RNA sequencing, site-directed mutagenesis, specific enzyme assays and mitosox assays.

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

Haspin kinase is an atypical serine/threonine protein kinase that specifically phosphorylates H3T3 and has an established role in chromosome assembly and spindle formation during cell division. Analysis of the cancer genome atlas (TCGA) data and recent reports revealed significant overexpression of haspin in certain cancers suggesting it as potential therapeutic target for cancer therapy. However, molecular and biological functions of Haspin and its regulation in cancers are poorly studied. In view of this, we aimed to explore the role of Haspin kinase on the DNA damage response and repair pathway in DNA damage induced cells in breast and ovarian cancer. Primarily, found elevated haspin gene expression and protein levels in various breast and ovarian cancer cells implying the probable role of haspin in cancer development. To understand its role, various cancer cell lines treated with selective haspin inhibitor were compared with untreated cells upon DNA damage using several agents/drugs like Camptothecin, Tototecan, Olaparib and Cisplatin, and examined the effect on DNA damage response proteins of different pathways. Not only pharmacological inhibition but also siRNA mediated down regulation of haspin showed significant reduction in FANCD2 ubiquitination, phospho-RPA32 levels and gamma H2AX levels indicating the role of haspin in DNA double strand break repair pathway. The cell cycle analysis also revealed S-phase delay and G2 arrest in haspin inhibited cells. Consistent with these observations, Dr-GFP analysis indicated significant dose-dependent decrease of homologous recombination in haspin inhibited. Also, enhanced DNA damage with decreased FANCD2 and pRPA foci formation in treated indicated impair DNA repair altogether pointing a novel function for Haspin in DNA damage and repair. From our studies, it is evident that haspin inhibition leads to replication stress and DNA damage in tumor cells. Further comparison of A2780 and CP70 isogenic ovarian cancer cells revealed

enhanced therapeutic efficacy of haspin for chemoresistant cancers. Cancer phenotype studies revealed reduced cancer cell migration, invasion and spheroid formation in haspin inhibited cells. Results of clonogenic survival assays indicated effective cancer cell death treated with haspin inhibitor alone and in combination with DNA damaging agents/platinum based drugs. Not only in several ovarian cancer subtypes but also in aggressive TNBC subtype haspin inhibition demonstrates significant influence on chemosensitisation via downregulation of FA-BRCA pathway mediated DNA double strand break (DSB) repair. As deficiency/inhibition of Haspin abrogrates DNA damage checkpoint signaling via FANCD2 and pRPA and cells show hypersensitivity to replication-associated DNA damage induced by chemotherapeutic agents like campothecin based therapeutic analogue Topotecan. Interestingly, the results were reproducible in patient derived ovarian cancer cells also strongly indicating the role of haspin inhibition in effective cancer therapy. Nevertheless *in vivo* studies using transgenic mouse also revealed similar trend. Further validation and confirmation of results is ongoing. Further, deciphering specific checkpoints and its downstream targets implicated in haspin regulation during cancer development need to be evaluated. Currently, work is in progress to address this and also to identify protein substrates or regulators of haspin in order to understand the detailed molecular mechanisms of haspin regulation in cancer.

In conclusion, the present study reveals novel function for haspin kinase in DNA damage response signaling in ovarian and breast cancer and suggests its potential therapeutic target, particularly for chemoresistant cancers.

Pertaining to this project, the results have been documented and the following manuscripts are drafted for communication in peer- review journals.

Manuscripts Communicated/to be communicated

1. Sandhya Annamaneni, Karunakar Saamarthy, Chinnadurai Mani, Shireesha Jonnalagadda and Komariah Palle. Novel function of Haspin Kinase in DNA damage response signaling in high grade serous ovarian cancer (To be communicated).

2. Sandhya Annamaneni, Karunakar Saamarthy and Komariah Palle. Haspin Kinase as a potential target for chemoresistant cancers- A Review (Manuscript under preparation).

iii) Proposed utilization of the experience in India:

The training programme on the breast and ovarian cancer research helped in deciphering novel mechanisms involved in the DNA damage and repair pathways in cancer, which has provided me ample opportunities to get an appraisal to ongoing clinical and translational research at Texas Tech University at Lubbock, Texas. Given rising incidence of breast and ovarian cancer in India, also the molecular and clinical heterogeneity in the origin, progression and relapse of several sub-types, personalized and targeted therapies are shown to dominate current traditional chemo and radiation therapies in future. In this direction, we plan to focus on identification of novel molecular targets from cancer pathways so as to plan and develop effective therapeutic strategies for patients with distinct profiles.

With the scientific and technical expertise gained, I feel empowered to improve and upgrade academic as well as research programs for PG and PhD students to impart better training and skills by introducing advanced practical training and methodologies in the existing curriculum. Further, workshops and conferences will be planned in the area of cancer genetics and advanced molecular therapeutics to expose students to ongoing scientific challenges and research. Needless to mention, the exposure gained in academic and research activities during the training period will help in formulating new guidelines and policies at faculty level. Considering my previous experience of work at the Department of Genetics, Osmania University in genetic studies in cancers, it is worthwhile to carry out a scientific program and establish the facility for the extensive molecular screening and therapeutics lab. Having gained international exposure in cancer research, I aim for high quality research by executing innovative research ideas and projects. To achieve this, would like to plan and establish national and international collaborations at multidisciplinary level to carry out impactful scientific projects and foster high end basic and translational research.

The knowledge gained will help in the development of new research initiatives and strengthening of ongoing programs at our Department. The acquired training in cutting edge research can help in setting up the advanced molecular biology facility for genomic and proteomic analysis at the Department of Genetics, Osmania University which will be useful not only in the ongoing screening and analysis of cancer patients but also in the validation of identified molecular mechanisms in cancer origin and progression. Particularly, next focus is to identify the genetic and epigenetic signaling factors that regulate DNA repair genes (BRCA) in metastatic breast

cancer. Also, to identify patients at risk for metastasis and relapse based on which appropriate treatment regimen can be followed so as to improve the survival rate of breast and ovarian cancer patients.

Overall ICMR-DHR has given an excellent opportunity to the encourage and support me to take up state-of art advanced research training with Dr. Komaraiah Palle at the host institute, TTUHSC a Lubbock, Texas to pursue my goals in career and for upgradation of skills. This transformation will definitely help me in achieving academic excellence and develop basic to advanced translational research at our University by adopting innovative research ideas and thereby impart better training to students. Further, contribute to growth of molecular medicine in reducing cancer burden in India.

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Forwarded

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