TOUR REPORT

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

1. Name and Designation of ICMR IF : **Dr. A. Venkateshwari,**
   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: **Six months (24th October to 24th April 2016)**

6. Highlights of work conducted:

I was involved in the following two projects

i) Molecular functions of BRIP1/FANCJ in regulation of FA-BRCA tumor suppressor pathway in response to genotoxic DNA damage in lung cancer cell lines.
ii) *In vitro* evaluation of chemopreventive and anticancer properties of natural compound Andrographolide in platinum sensitive and platinum resistant ovarian cancer models.

Introduction

Breast cancer is one of the leading cause of death in women globally. It is the second largest cause of cancerous deaths among Indian women after cervical cancer. Germline mutations in the breast cancer early onset genes BRCA1 and BRCA2 predispose individuals to cancer development (primarily breast and ovarian cancer), with an autosomal dominant mode of inheritance. Several studies suggested that BRCA1 and BRCA2 closely associate with Fanconi anemia (FA) DNA repair pathway proteins in processing of DNA lesions and maintain genome/chromosomal stability. Mutations in some of the FA genes are also known to predispose to Breast and Ovarian cancers with a low penetrance. On the other hand, biallelic mutations in BRCA1/FANCS and BRCA2/FANCD1 genes also cause Fanconi anemia. The aim of the present study is to evaluate the role and molecular functions of BRIP1 also known as FANCJ in the FA-BRCA tumor suppressor pathway.

Ovarian cancer is a serious problem worldwide and is the most deadly 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 45.6%. This highlights the need to find new therapeutic options for patients with ovarian cancer. The aim of the present study is to evaluate the chemopreventive and anticancerous role of andrographolide in platinum sensitive and resistant ovarian cancer cell lines in *in vitro*.

i) Technique/expertise acquired:

The following studies were undertaken in several human breast (MCF-7, MDA-MB-231), ovarian (A2780, SKOV3, OV90), lung cancer (A549, H1299) cell lines and in the normal immortalized cells (HDF, MCF10A). The technical expertise gained from these projects are culturing of human cancer and normal cells, transfection of plasmids, siRNAs, preparation and isolation of proteins, RNA, DNA experiments and the western blot analysis of several DNA damage response and repair signaling proteins. Further downregulation and ectopically complement several DNA damage response and repair proteins in the FA-BRCA tumor suppressor pathway is one of the upgradation of my skills; apart from their effects on cell responses to different genotoxins induced damage by Western blot analysis. Immunoflouroscence using confocal microscopy and fluorescently tagged proteins or using antibodies, single cell comet assay to assess single and double stranded DNA breaks, cell cycle analysis and apoptosis assay using Flow cytometry was also part of the skills. Furthermore, stem cell signaling mechanisms were evaluated in tumor cell spheroid cultures and invasion assays.

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

Knock down studies were carried out in cultured human cancer and normal cell lines to understand the role of BRIP1/FANCJ on the DNA damage response and repair proteins involved in repair of DNA double strand breaks. DNA repair proteins such as BRCA1, BRCA2/FANCD1, Rad18 and Rad51 were found to be destabilized when FANCJ was depleted. Furthermore, the study revealed that FANCJ does not alter transcription of these proteins, but protects them from degradation by proteases. It is also observed that deficiency in FANCJ abrogrates DNA damage checkpoint signaling mediated by ATM and ATR kinases, and these cells show hypersensitivity to replication-associated DNA damage induced by chemotherapeutic agents like campothecin and hydroxyurea. Diminished activation of ATM, ATR, CHK1, and CHK2 and phosphorylation of their downstream targets was revealed. In addition of the aforementioned replication blockers, DNA crosslinking agents such as Mitomycin C and carboplatin as well as ultraviolet radiation were also tested wherein similar results were observed with hydroxyurea and campothecin. And

finally, through expression of a mutant FANCJ lacking a functional DNA helicase, it is observed that the enzymatic activity is not required to stabilize the other DNA repair proteins and maintain a proper DNA damage response. These observations suggest that FA and BRCA genes work in a common DNA repair pathway to maintain genome/chromosomal stability.

Andrographolide exhibited concentration dependent cytotoxic effects in ovarian cancer cell lines with and without combination of carboplatin. The cell cycle analysis showed inhibition of proliferation of human ovarian cancer cells through induction of G2/M arrest by andrographolide. Additionally, andrographolide decreased the activity of stem cell spheroid formation in a concentration dependent manner. Western blot analysis revealed induction of DNA damage repair pathway as demonstrated by up-regulation of the expression of phos- Chk 1, phos-Chk 2, γ H2AX, FANCD2, P53 etc in ovarian cancer cells treated with andrographolide. Comet assay revealed the genotoxic efficiency of andrographolide in cancer cells.

In conclusion, the present study revealed anti-cancerous nature of andrographolide in ovarian cancer cell lines, thereby suggesting that the combination of andrographolide and carboplatin could be used as an effective therapy for ovarian cancer.

Manuscripts Communicated/to be communicated

1. Ananthapur Venkateshwari, David W. Clark, Pratibha Nallari, Cingeetham Vinod, Thangaraj Kumarasamy, Alla Goverdhan Reddy , Akka Jyothy, Malladi Vijay Kumar, Ramaiyer Raghu Raman and Komaraiah Palle. Mutation analysis of FANCJ/BRIP1 in a family with history of male and female breast cancer in India: a case study. **Clinical Breast Cancer Journal** (2016) (Communicated).
2. Kaushlendra Tripathi, Chinnadurai Mani, Ranganatha R. Somasagara, David W. Clark, Venkateshwari Ananthapur, Kambappa Vinay, Komaraiah Palle Detection and evaluation of estrogen DNA-adducts and their carcinogenic effects incultured human cells using biotinylated estradiol. **Carcinogenesis (2016)** (Communicated).

3. Ananthapur Venkateshwari, Kaushalendra Tripathi, Ranganatha Somasagara, David W. Clark, Chinnadurai Mani, Pratibha Nallari, B. Shashidhar Rao and Komariah Palle. In vitro evaluation of anticancer properties of natural compound Andrographolide in platinum sensitive and platinum resistant ovarian cancer models. (Manuscript in preparation).

iii) Proposed utilization of the experience in India:

The training programme on the breast and ovarian cancer research reveals the novel mechanisms involved in the FA- BRCA tumor suppressor pathway deficiency leading to carcinogenesis, which has provided me ample opportunities to get an appraisal to ongoing clinical and translational research at USA Mitchell Cancer Institute. Personalized and targeted therapies are shown to dominate current traditional chemo and radiation therapies in future based on the molecular and genetic information of the patient and population structure. Since such studies are meager from Indian population, the studies on the genes involved in FA-BRCA tumor suppressor pathway are essential in understanding the role of these genes in the etiopathogenesis of breast and ovarian cancer. Based on the training acquired, two project proposals are planned to be prepared and submitted to funding agencies to carry out mutation variant analysis in high penetrant and moderate/low penetrant genes in the etiology of breast cancer as well as ovarian cancer. The acquired training also help in setting up the advanced diagnostic techniques at the Institute which will be useful in the mutation screening of breast cancer patients and their family members for early detection of the disease and offer appropriate genetic counseling strategies.

The project on the anticancer properties of andrographolide in ovarian cancer cell lines help in formulating new projects to further evaluate the role of andrographolide in breast cancer cell lines and also to understand the role of other andrographolide analogues in the ovarian and breast cancer cell lines.

Considering my previous experience of work at the Institute 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 screening of mutations in the high risk and moderate/low risk penetrant genes in the etiology of breast cancer and ovarian cancer with the expertise acquired from the

present training program. The knowledge gained will help in the development of new research initiatives and strengthening of ongoing programs of the Institute.

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Dr. A. Venkateshwari
Signature of ICMR IF