REPORT

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

1. **Name and designation of ICMR- IF:** Dr. Showket Hussain

2. Address: Division of Molecular Oncology, National Institute of Cancer Prevention &Research, Indian Council Of Medical Research (NICPR-ICMR), Formerly, ICPO-ICMR, Department of Health Research (Ministry of Health & Family Welfare, Govt. of India), I-7, Sector-39, NOIDA (201301),INDIA.

3. Frontline area of research in which training/research was carried out: Cancer Biology

4. Name & address of Professor and host institute: Prof. Anil Kaul, M.D., DDS., MPH, Director, High-Complexity Clinical laboratories & Associate Professor, Obstetrics & Gynecology, OSU - Center of Health Sciences, 1111 West 17th Street, Tulsa, Oklahoma, U.S.A.

5. Duration of fellowship: 20th January to 10th July 2016 (06 Months)

6. Highlights of work conducted

i) Technique/expertise acquired: Our group has been working for the past ten years on Human papillomavirus (HPV) which is a well-established causative factor for the development of uterine cervical cancer. Cancer of the uterine cervix is a leading cause of cancer related deaths worldwide and the second most prevalent cancer in women after breast cancer. More than 80% of the cervical cancer burden is from developing countries like India, with the annual incidence of about 130,000 cases and 70-75,000 deaths.

Histologically, cervical cancer progresses from normal epithelium through a series of well-defined pre-cancer lesions referred to as cervical intraepithelial neoplasia (CIN). A large number of risk factors are known to contribute to high incidence of this disease. It has been well demonstrated, by various epidemiological studies, that infection with high-risk HPV (HR-HPV) is the main etiological factor in cervical cancer progression. But the fact that only a small fraction of HPV

infected cervical intraepithelial neoplastic lesions progress to invasive cancer, suggests that in addition to HPV, other host factors like weak immune response, aberrant expression of oncogenes, inhibition of the activity of tumor suppressor genes, play a pivotal role in transforming premalignant lesions into invasive cancer.

HPV manifests its oncogenic potential primarily through its two gene products, E6 and E7, which canonically interact with p53, pRB and other cell cycle components resulting in dysregulated cell cycle and tumorigenic transformation. Since, HPV does not code for its transcriptional machinery, the expression of viral oncogenes solely depends on the expression and activity of host transcription factors that control viral promoters through binding to their corresponding conserved cis-acting sites on upstream regulatory region (URR). Our recent investigations demonstrate potential carcinogenic role of transcription factors STAT3, NF- B, and AP-1 in the HPV-related malignancies, which may influence expression of viral oncogenes and subsequent carcinogenic events. Therefore, the proposed work was undertaken, using the expertise and guidance of US counterparts Prof. Anil Kaul & Prof. Rashmi Kaul, to investigate and understand the possible role of host complement regulatory proteins in cervical cancers.

The complement system plays a crucial role in host defense mechanisms against microorganisms and tumor cells to protect themselves from autologous complement mediated damage. Normal cells continuously express cell-membrane associated complement regulatory proteins (CRPs). To investigate the expression of these CRPs on cervical cancer cells, we examined the profile of complement regulatory proteins - membrane cofactor protein (MCP, CD46), Decay-accelerating factor (DAF, CD55) and protectin (MACIF, CD59) on HPV negative & HPV positive cervical cancer cell lines. In addition, we investigated the effect of chemo-preventive drug Sulfur Heteroarotinoid A2 (ShetA2), also called OK-1 (NSC-721689), developed by our collaborator Prof. Doris Benbrook, on CRPs in HPV positive/negative cervical cancer cell lines. SHetA2, was chosen as the lead Flex-Het because it exerts maximal cancer cell line cytotoxicity in comparison with other Flex-Hets, while not harming non transformed cells (Benbrook et al 2005, Liu et al 2007). In June 2016, a $2.2 million grant was awarded by the National Institutes of Health (NIH) to further investigate this drug for cervical cancer prevention in the United States. ShetA2 counteracts the effects of the HPV viral proteins on the tumor suppressors, thereby preventing cancer. Therefore, this study may allow us to understand the mechanisms to test the efficacy of this drug further in more experimental models and in clinical trials for HPV associated cancers, including cervical

cancer prevention and treatment. The proposed study demonstrates, for the first time, the differential expression of these CRPs in cervical cancer cells. We also observed a preferential dichotomy of CRPs between HPV positive & HPV negative cells. ShetA2 was found to be effective in down regulating CRPs & holds a great promise as a chemo-preventive drug for cervical cancer.

For these studies, I used BD LSR II flow cytometer system (BD Biosciences) and BD FACSDiva and/ or FlowJo softwares for data analysis including cell sorter system from BD Biosciences. Also, I used Leica TCS SPE confocal microscope system designed for confocal recording (laser scanning images) of fluorescence-marked living and fixed specimens as well as for quantitative measurements. In addition, I learnt and used the technique of invasion assay for detecting and quantitating intracellular bacterial counts; Western blotting; Immunohistochemistry and other relevent molecular biology techniques used in cancer research.

Further, futuristic aspects of the above leads are being tested & analyzed to see the functional significance of CRPs & HPV onco-proteins for translational cancer research in cervical cancer. In addition, I have gained an expertise in the field of latest molecular diagnostic techniques in communicable diseases, using a probe based Transcription Mediated Amplification (TMA) technology to detect the multiple infections (Chlamydia trachomatis, Neisseria gonorrhoeae, etc. including HPV infection from a single clinical sample) using the Panther system (Hologic Inc.). Panther is a fully automated sample-to-answer instrument, which eliminates the need for batch processing and automates all aspects of nucleic acid testing on a single, integrated platform. It provides unprecedented control of workflow, driven by randomaccess and continuous loading of molecular samples, reagents and consumables. This system combines true walkaway freedom with intuitive design for ease of use and this technology would be excellent thrust area to be explored or implemented in India for women health.

In addition, I was regularly taking part in weekly lab meetings to present my work progress including taking part in Lab Journal club(s) and institutional seminars.

I was also invited to deliver a scientific talk/ seminar at the Stephenson Cancer Center, University of Oklahoma in Oklahoma City.

Prior to start of project work, I have sucssfully passed/ completed all required training necessary for working in laboratorry and these included the biosafety training, blood-borne pathogen training and the HIPAA training.

ii) Research results, including any papers, prepared/submitted for publication: Two manuscripts are anticipated from this study which is under preparation for publication. In addition, both US based counterparts are planning to extend this project into a joint Indo- US program on translational research in cervical cancer.

iii) Proposed utilization of the experience in India: Translational cancer research is one of the main important mandates of National Institute of Cancer Prevention & Research, Indian Council of Medical Research (NICPR-ICMR) wherein other scientists are also contributing in addition to ICMR- International Fellow. Therefore, the newly acquired skills by me in cancer research will give further impetuous to the mandate of the NICPR and other cancer research institutes of the country.

Signature of ICMR-IF

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