ICMR REPORT

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

1. Name and designation of ICMR- IF : **Dr.Dibyendu Banerjee**

2. Address : CSIR-CDRI, Lucknow, India

3. Frontline area of research in which training/research was carried out :To study the differential expression of DNA repair proteins in cancer cells for novel therapeutic strategies using CRISPR/CAS9.

4. Name & address of Professor and host institute :**Dr. Muralidhar L. Hegde**
Associate Professor
Houston Methodist Research Institute (HMRI)
6550 Fannin St, Smith 8-05
Houston, TX 77030, USA

5. Duration of fellowship : Six (6) months

6. **Highlights of work conducted** : The expression of DNA repair proteins in colon cancer in the presence and absence of drug and radiation stressors was studied in order to discover potential new targets for combination therapy of drug resistant colorectal cancers. An inducible DLD1-CAS9 cell line was made during the fellowship period. This cell line can be induced with doxycycline to express CAS9 and sgRNA against various DNA repair proteins can then be transfected to produce the gene knock-out cells. In addition, RT2 profiler RNA array screening studies were carried out to study the mRNA expression level expression of 84 DNA repair proteins in a 96 well plate format. These studies were conducted in the presence of Topo1 and Lig1 inhibitors and compared with control untreated cells. Reverse phase protein array (RPPA) studies were also conducted to study the drug response of DLD1 cancer cells in presence of Top1 and Lig1 inhibitors for about 400 proteins and their phosphorylated forms to characterize the activation of signaling pathways. This provides information about the expression level of the genes at the protein level upon drug stress. Finally, PLA (Proximity Ligation Assay) was conducted to study the protein-protein interactions between some important DNA repair proteins. In summary, we studied a part of the DNA Repairosome of DLD1 cells in the presence of different stresses (Topoisomerase1 inhibitor, DNA Ligase1 inhibitor and Radiation), in order to understand the response of the DNA repair mechanism in colon

cancer. The idea is to discover new gene targets that can sensitize drug resistant colon cancers to available treatments such as radiation and chemotherapy.

i) **Technique/expertise acquired** : CRISPR/Cas9 mediated gene knock-out technique; RT^2 profiler gene array assay; RPPA (Reverse phase protein array); PLA (Proximity Ligation Assay), Western blotting, RT-PCR, etc.

ii) **Research results, including any papers, prepared/submitted for publication** : The research work will be completed in the next six months and results will be published in peer reviewed journals. In addition, this visit could lead to a collaborative research agreement/MOU between HMRI and CSIR-CDRI, which has been initiated.

iii) **Proposed utilization of the experience in India:** The techniques learnt will be incorporated into the experiments conducted in my laboratory in India. Students will be personally trained to conduct the experiments and get faster and more accurate results. These techniques will immensely help both in the execution of existing projects and in the formulation of future projects.

D Banerjee

ICMR Sanction No. INDO/FRC/452(Y-43)/2018/2019-IHD

Signature of ICMR-IF