REPORT

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

1. Name and designation of ICMR- IF : Dr. Gyanendra Singh, Scientist 'C'

2. Address : Department of Toxicology, ICMR-National Institute of Occupational Health, Ahmedabad 380016

3. Frontline area of research in which training/research was carried out : Heavy metal toxicity

4. Name & address of Professor and host institute : Dr. Udai Pandey, Associate Professor Department of Pediatrics, Human Genetics and Neurology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

5. Duration of fellowship with exact date* : Dec 15, 2019 to June 15, 2020*

6. Highlights of work conducted :

   i) Technique/expertise acquired :
   - Immuno fluorescence/Immuno blotting
   - Confocal Microscopy
   - Transfections in HEK293T cells
   - Experiments in mouse neuroblastoma cell lines
   - Drosophilla toxicity model development

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

   Amyotrophic lateral sclerosis (ALS) is a devastating motor neuron disease for which no effective therapies available and very little is known about the disease mechanisms. There are several animal model and human ALS patient-based studies that link heavy metal exposure with ALS susceptibility and disease process. Cytoplasmic mislocalization of FUS carrying a mutation and its subsequent accumulation into cytoplasmic SGs is one of the hallmarks of ALS. Stress Granule (SGs) formation is believed to be a conserved and a general protective mechanism against various cell stresses. These events are causally associated with the mutant FUS protein toxicity and progressive motor neuron death in humans as well as in animal models. Therefore, we hypothesized that by treating with these heavy metals in mammalian cells would increase the FUS incorporation into SGs, thereby augmenting toxicity and cell death. To test this, we used human embryonic kidney 293 cells (HEK293T) for exploring changes in the FUS distribution in WT and with different FUS mutants (FUS-R518K and FUS-R521C) with respect to formation of SGs in the cytoplasm.

We have performed the toxicity experiments in HEK293T cells in-vitro with the three chosen heavy metals viz. chromium, lead and arsenic individually as well in the combination with different time points and concentrations. In parallel, recovery experiment was also set up to see the growth pattern of HEK293T cells in response to the different metals treatment. The cell survival was found to be lower in the combination group as compared to the individual treatment groups.

Untransfected HEK293T cells were treated with the lead, arsenic and chromium (individually as well as in the combination) for induction of cytoplasmic positive SGs. After successful validation of expression of WT-FUS and the mutants (R518K and FUS-R521C) using immunoblotting in HEK293T cells, we also visualized the distributions of WT FUS, FUS-R518K and FUS-R521C in transiently transfected HEK293T cells. We observed that in the absence of heavy metals treatment, WT- FUS remained localized in the nucleus and was not incorporated into SGs. In contrast, FUS-R518K and FUS-R521C mislocalized to the cytoplasm and incorporated into the formation of positive SGs. The presence of SGs in the untreated cells in cells expressing mutants (FUS-R518K, and FUS-R521C) suggests that ALS-associated mutations of FUS are sufficient for SG formation, even without additional cellular stimuli. Though sodium arsenite, lead acetate and potassium dichromate induced SG formation in all the treatment groups, the formation of SGs was higher in the combination group treatment as compared to the other treatment groups.

To test whether heavy metals exposure modifies FUS toxicity in mammalian neurons, we used mouse neuroblastoma cells as a model for FUS-associated toxicity studies. The formation of cytoplasmic SGs was confirmed by confocal microscopy. In cells transfected with FUS alone, WT FUS primarily localized to the nucleus, whereas FUS-R518K and FUS-R521C mislocalized to the cytoplasm as expected. Similar to the co-transfections of WT FUS or mutant FUS-R518K and FUS-R521C in HEK293T cells, these heavy metals induced G3BP stress granule assembly factor 1 (G3BP1)-positive SGs in mouse neuro cells when treated with different metals The impact is even higher in the combination group treatment. The recovery experiments were also performed in parallel with all the treatment groups. These results suggest that above heavy metals augments FUS toxicity by altering its cellular homeostasis thereby formation of SGs in the cytoplasm.

A manuscript would be submitted to the relevant journal after data completion (analysis could not be completed due to COVID-19 pandemic). A collaborative project is likely to be developed soon in the field of mutual interest.

Seminars attended:

- Addressing Treatment Challenges in Zellweger Spectrum Disorders (Speaker: Nancy Braverman, McGill University)
- A funny thing happened on the way to the periphery: Controlling self-reactivity through redox and energy regulation (Speaker: Jon D. Piganelli, UPMC Children's Hospital of Pittsburgh)
- DNA damage and immunogenicity in Ewing sarcoma (Speaker: Kelly Bailey, UPMC Children's Hospital of Pittsburgh)

- Kidney susceptibility during development and disease (Speaker: Sunny Sims-Lucas, UPMC Children's Hospital of Pittsburgh)
- Overlapping genetic architecture of type 2 diabetes and cystic fibrosis-related diabetes (Speaker: Scott Blackman, Johns Hopkins University)
- Terminal differentiation of heart muscle cells (Speaker: Dr. Bernhard Kuhn and Dr Lu Han, UPMC Children's Hospital of Pittsburgh)
- Hold the sugar, pass the salt: A novel and conserved role for the O-GlcNAc transferase OGT in the response to stress (Speaker: Todd Lamitina, UPMC Children's Hospital of Pittsburgh)
- How Chemokines, Receptors and Glycosaminoglycans Work Together to Facilitate Cell Migration (Speaker: Tracy M. Handel, University of California)
- Chewing the Fat about PAI-1 and inflammation in obesity induced Non-Alcoholic Fatty Liver Disease (NAFLD) (Speaker: Jon D. Piganelli, UPMC Children's Hospital of Pittsburgh)
- Manipulating metabolism to modulate T cell-mediated immunity (Speaker: Craig Byersdorfer, UPMC Children's Hospital of Pittsburgh)

iii) Proposed utilization of the experience in India:

The expertise acquired during this 'ICMR-DHR International Fellowship' will be very useful for the research projects being carried out in the area of heavy metal toxicity at my institute. The research experience gained at the University of Pittsburgh- Medical Center, Pittsburgh, USA will be helpful in developing new project proposals in the area of heavy metals induced neurogenerative diseases at ICMR-NIOH.

Signature of ICMR-IF

ICMR Sanction No. INDO/FRC/452(Y- 58y201 9-20-IHD

*Repatriated earlier due to COVID-19 pandemic.