

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

1. Name and designation of ICMR- IF : **Dr. HARIPRASAD. G**  
Associate Professor
2. Address : Department of Biophysics  
All India Institute of Medical Sciences  
New Delhi
3. Frontline area of research in which Training/research was carried out : Effect of growth factor cytokines on cardiac stem cells
4. Name & address of Professor and host institute : Dr. PARAS KUMAR MISHRA  
Department of Cellular & Integrative Physiology  
University of Nebraska Medical Center  
601 S. Saddle Creek Rd, DRC1 Room 5047  
Omaha, Nebraska, USA68198-5850
5. Duration of fellowship : 3 months
6. Highlights of work conducted :

### **i) Technique/expertise acquired**

cardiac stem cell isolation, growth and maintenance of cardiac stem cell line, microRNA profiling, cDNA profiling, Immuno fluoro-cyto chemistry, creation of CRISPR/Cas9-Matrix Metalloproteinase-9 human cardiac stem cells

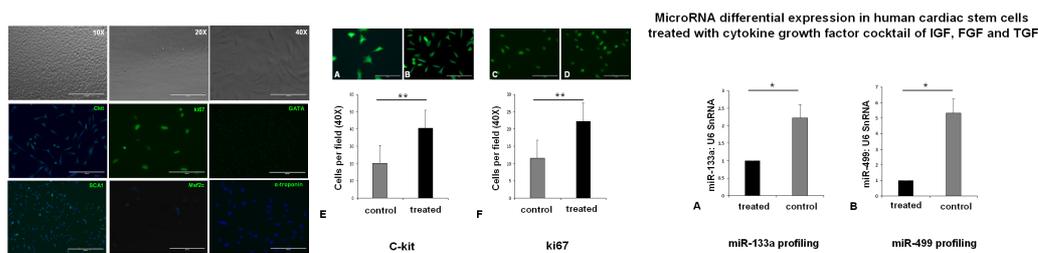
### **ii) Research results, including any papers, prepared/submitted for publication**

**Introduction:** Regenerative medicine has established the potential scope of stem cells as a therapeutic modality for myocardial infarction. Stem cell therapy has the potential to limit the extent of post-infarction cardiac damage by accelerating the normal process of healing, and potentially regenerating cardiac muscle. However, the efficacy of this approach is limited due to the hostile microenvironment of the acutely injured myocardium. We therefore hypothesized that a combination of cytokines could confer anatomical and physiological advantage to cardiac stem cells, thereby providing a therapeutic advantage.

**Objectives:** (1) Test the effect of growth factor cytokine cocktail on cardiac stem cells; (2) provide a rationale for the possible molecular mechanism for the observed experimental outcomes.

**Results:** Human cardiac stem cells were validated for the presence of C-kit, ki67, GATA, SCA-1, Mef2c, and the absence of cardiac troponin (**Fig. 1**). These were plated and grown on a six well

plate, with three of the wells treated with IGF, FGF and TGF. Cell medium was changed every 48 hours and maintained at appropriate growth conditions. Cells were harvested on the sixth day when they reached 90% confluency, and were taken for further experiments. Cell counter and C-kit and ki67-marker based grid counting experiments showed the treated cells to be having a higher degree of proliferation as compared to the control (**Fig. 2**). qPCR profiling for Nkx2.5, Mef2c, GATA, Nanog, SCA1 showed the treated cells to be having a higher amount of differentiation. This was further validated by miRNA-133a and miRNA-499 profiling in the two groups of cells (**Fig. 3**). Also, MMP9 showed a lower level of expression in treated cells prompting us to believe that the growth factor cytokines mediated their effect by modulating this enzyme. MMP9 gene was therefore knocked out in the cardiac stem cells using CRISPR/Cas9 method, and functional relevance was established by fluoro-immuno chemistry based intensity monitoring.



### Presentations/Talks:

Presented the work titled: **‘Evaluating the combined effect of IGF-1, TGF-β and FGF on cardiac progenitor stem cells: Implications in regenerative therapeutics of acute myocardial infarction’** at Nebraska Physiological Society on 15<sup>th</sup> October 2016

Presented the work titled: **‘Differential protein expression profiling as means to explain beneficial advantages of co-administering growth factor cocktail with cardiac stem cells for regenerative therapy’** at 8th Annual meeting of Proteomic Society, India; 3rd meeting of Asia Oceania Agricultural Proteomics Organization & International Conference on Functional and Interaction Proteomics: Application in Food and Health on 17<sup>th</sup> December 2016

### Publications:

Domada Ratna Kumar, Surabhi Swarnkar, Ashish Kumar Gupta, Mohd Imran Khan, Anupama Kakkar, Sujata Mohanty, Milind Hote, Pankaj Sharma, Subir Kumar Maulik, Senthil Kumaran, Sudhir Kumar Arava, **Gururao Hariprasad**. **Evaluating the combined effect of IGF-1, TGF-β**

**and FGF on rat cardiac stem cells: Implications in regenerative therapeutics of acute myocardial infarction:** To be communicated

**Gururao Hariprasad, Domada Ratna Kumar, Bishan Bhattarai, Hamid Shahshan, Shyam Kumar, Paras Kumar Mishra. Evaluating the combined effect of IGF-1, TGF- $\beta$  and FGF on human ventricular cardiac stem cells:** To be communicated

**iii) Proposed utilization of the experience in India:**

- Based on the results procured during this fellowship, a proposal titled ‘**Evaluating the effect of cytokine cocktail on cardiac stem cells: Implications in regenerative therapeutics of myocardial infarction**’ has been submitted for funding
  
- Knowledge gained in cardiac stem cell will be shared with fellow colleagues and clinicians at the institute to improve and fine tune upon the existing methodologies of **stem cell research and stem cell therapy**
  
- Based on the expertise gained, subject of **stem cell applications in medicine** will be added to the existing Biophysics curriculum for MBBS, MD and PhD students