REPORT

Report of the Fellow who availed Fellowship / Training under Human Resource Development for Health Research.

1	Name and designation of Fellow	:	**Dr. Chaturvedula B. Tripura Sundari** **Scientist**
2.	Address	:	CSIR-Centre for Cellular and Molecular Biology Uppal Road, Habsiguda, Hyderabad-500 007
3.	Type of Fellowship and period	:	Long Term fellowship training in Foreign Institute (30.03.2015 to 29.09.2015)
4.	Duration of fellowship	:	6 months
5.	Frontline area of research in which Training /research was carried out	:	Stem cell Research-EPIGENETICS
6.	Name & address of mentor and host institute	:	Prof. Dr. Andreas K. Nussler Siegfried Weller Institute, BG Klinik Universitat Tubingen, Schnarrenbergstrasse, Tubingen-72076 GERMANY
7.	Highlights of work conducted i) Technique/expertise acquired (Give in about 150 words)	:	Worked on two independent projects on the role of TET proteins in inducing changes in the epigenetic state of cells during liver development and disease.

Mouse liver tissue at different stages of fetal development and during adult liver fibrosis was analysed for changes in TET expression which govern the fate of the stem cells during liver development and regeneration. The role of induced TET expression, in triggering cell cycle arrest in liver carcinoma conditions (human HCC cell lines) was also investigated.

- Gained experience in the field of Epigenetics, in understanding the role of TET proteins in inducing the demethylation status of the genome for regulated transcription of specific genes.
- Learnt the use of epigenetic drugs such as 5-Azacytidine (5-AZA) in inducing subtle changes in the genome which could be further exploited for the differentiation of mesenchymal stem cells into hepatocyte like cells (other cell types of interest).
- The use of a combination of 5-AZA and vit C as a co-factor in improving the metabolic activity of hepatic cell lines was also learnt.
- Learnt isolation of primary human hepatocytes from human liver tissues and the set up of 3D cultures.

ii) Research results, including any papers, prepared/submitted for publication
(Give in about 300 words)

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- The project on mouse liver fibrosis is a continuation of the work initiated at CCMB. The aim of the study was to understand if the changes in the cellular composition during liver fibrosis supports, hematopoietic activity and if this has any implications in fibrosis progression or in liver regeneration. Using flow cytometry, *in vitro* hematopoietic CFU assays and immuno histochemical studies we have observed increased hematopoietic activity in fibrotic livers.

  It is now known that epigenetics plays a significant role in liver fibrosis, however the details of TET protein related changes or their specific role in liver fibrosis are not understood. Our preliminary experiments in this direction provide evidence that TETs undergo significant changes during liver fibrosis and are increased during regeneration phase of the liver. By Immuno-histochemistry, we have observed significant changes in the demethylation status in the fibrotic zones of the liver. Further analysis of the TET related changes in the individual cell types could throw a significant light on the epigenetic mechanism of liver fibrosis progression which in the recent times is gaining importance as a new target in the treatment of liver diseases.

  A manuscript entitled "**Reactivation of hematopoietic Niches during CCl4 induced chronic liver fibrosis and regeneration in mice"** is under preparation.

- 5-Azacytidine as a DNA methylation inhibitor has undergone several preclinical and clinical testing in cancer treatment. Prof. Nusslers lab has now shown that the DNA methylation inhibition activity of 5 AZA is through the increase in the expression of TET-2 and TET-3 proteins. In this study, when used along with Vitamin-C as a cofactor, 5-AZA showed an increase in the gene expression of TET-2 and TET-3 and caused enhanced cell death in HCC cell lines. It was known that 5-AZA induces cell death but the genes upregulated/downregulated was not known.

  In this study we have worked on the pathway for the cell cycle arrest and report that Snail, the master regulator for Epithelial to mesenchymal transition is down regulated primarily. The further sequence of events, leading to cell cycle arrest are elucidated.

  A manuscript for this part of work entitled "**Induction of active DNA demethylation by 5-AZA and Vitamin C induces cell cycle arrest by enhancing TETs and down regulating Snail expression"** is under preparation.

iii) Proposed utilization of the experience in the Parent Institute. (Please specify the project developed whether originally proposed/ new project)	:	Epigenetic changes in liver fibrosis progression/ regression is a relatively unexplored field which is now gaining significant importance for understanding the cause and the cure for targeted therapy. The experience gained from the current visit would be used for beginning a new study in addressing the role of specific TET proteins in liver fibrosis. **A project proposal on the same lines as proposed originally, but with a more specific aim to understand the changes in the liver mesenchymal cells-which are reported to be the cells supporting hematopoiesis in the adult liver- would be submitted.**

11.10.2015
Hyderabad

Signature of Fellow

Tasks accomplished during the fellowship:

1. Successfully completed the 6 month fellowship tenure with a positive outcome.
2. Had regular active discussions with the team members for continuation of the collaborative research.
3. Involved in discussions with a hepatology group in Belgium for a probable collaborative work in the field of liver fibrosis.
4. Two manuscripts are currently under preparation in collaboration with Prof. Nussler.
5. Based on the results, a project proposal is developed which would be submitted to DHR/ICMR.
6. The fellowship helped in laying a strong foundation for the proposed future long term Indo-German collaborative research grants for which the discussions are currently in progress.