INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	KHYATI RAINA
Roll Number	:	176106110
Programme of Study	:	Ph.D.

Thesis Title: **Investigating the role of human UTF1 in reprogramming, self-renewal and differentiation using a CRISPR/Cas toolbox**

Name of Thesis Supervisor(s)	:	**Dr. RAJKUMAR P. THUMMER**
Thesis Submitted to the Department/ ~~Center~~	:	**BIOSCIENCES AND BIOENGINEERING**
Date of completion of Thesis Viva-Voce Exam	:	**20/09/2024**
Key words for description of Thesis Work	:	**Undifferentiated embryonic cell transcription factor 1, human induced pluripotent stem cells, human fibroblasts, CRISPR/Cas9, gene knockout, knockdown, spontaneous differentiation**

SHORT ABSTRACT

Stem cells are self-renewing cells present at the apex of the lineage hierarchy and, therefore, serve as the founder cells during organismal development. Embryonic Stem Cells (ESCs) are pluripotent cells that can differentiate into all the cell types belonging to the three germ layers: ectoderm, mesoderm, and endoderm. However, human ESCs are not considered ideal for cell therapy applications because of ethical issues and their inability to be used for autologous therapy. Circumventing these limitations, a ground-breaking study was published in 2006, in which pluripotency was induced in terminally differentiated cells (fibroblasts) by the introduction of a cocktail of transcription factors, namely OCT3/4, SOX2, KLF4, and c-MYC (popularly called Yamanaka factors) in mouse fibroblasts to generate induced Pluripotent Stem Cells (iPSCs). Subsequently, the first human iPSCs were reported from fibroblasts using retroviral and lentiviral transduction of reprogramming factors. Since its inception, various reprogramming approaches and combinations of reprogramming factors have been explored to generate iPSCs with higher efficiency and quality. Among these, a pluripotent cell-specific transcription factor Undifferentiated embryonic cell Transcription Factor 1 (UTF1) was believed to be a promising factor for the generation of quantitatively and qualitatively better human iPSCs due to its high expression in pluripotent stem cells. Therefore, our aim was to elucidate the role of human UTF1 in the generation and maintenance of human iPSCs.

Firstly, the thesis delves into the generation and characterization of a human fibroblast-derived iPSC line IITGi-001A by transfection of oriP/EBNA-1 based episomal plasmids expressing OCT3/4, SOX2, KLF4, L-MYC, LIN28 and a p53 shRNA. This iPSC line expressed core pluripotency markers, maintained normal karyotype, and showed trilineage differentiation potential. Further, genomic PCR confirmed the absence of episomal plasmid integration in this iPSC line, which indicated that the cell line generated was indeed integration-free. In addition, DNA fingerprinting of fibroblasts and iPSCs DNA by microsatellite analysis confirmed the genetic identity of this cell line. This iPSC line was free from mycoplasma contamination.

Secondly, the thesis focuses on establishing the importance of human UTF1 in reprogramming by generating a *UTF1* knockout toolbox using the CRISPR/Cas9 technology. Previously, murine studies have successfully generated iPSCs from *UTF1* knockout or UTF1 knockdown fibroblasts with typical pluripotency features, and only one study reported a significant reduction in reprogramming efficiency. However, the essentiality of UTF1 in human iPSC generation remained unexplored to date. Here, the generation of human iPSCs from *UTF1* knockout fibroblasts revealed that the targeted deletion of the human *UTF1* gene exhibited a significant decline in the reprogramming efficiency of human iPSCs. Moreover, the few iPSC clones that did emerge in the absence of UTF1 showed instability upon expansion, hinting at the importance of UTF1 in maintaining pluripotency.

Thirdly, the thesis explores the effect of *UTF1* knockout in iPSCs. Various studies have deleted the mouse *Utf1* gene in vivo and observed a spectrum of outcomes, ranging from developmental arrest to embryonic lethality or developmental delay result- ing in death within two days of birth. These outcomes indicated that UTF1 is crucial for proper murine embryonic development at different stages. Here, we established that, the absence of human UTF1 protein resulted in a loss of viability of iPSCs due to the induction of apoptosis.

Lastly, the thesis delves into understanding the effect of reduced UTF1 levels in human iPSCs. To achieve this, a shRNA against UTF1 was expressed in iPSCs. The decline in UTF1 levels was slower than UTF1 levels observed in UTF1 knockout iPSCs. Also, the effects were not as pronounced as observed upon UTF1 knockout iPSCs. These iPSCs did not undergo apoptosis but showed spontaneous differentiation of human iPSCs.

In this thesis, we report the generation and characterization of a human iPSC line named IITGi001-A, derived from a human fibroblast cell line, using a non-integrative reprogramming method. Subsequently, we explored the involvement of human UTF1 in both the reprogramming of human fibroblasts to iPSCs and their maintenance. This investigation of the importance of human UTF1 was carried out by loss of function studies. This was achieved by using a CRISPR/Cas9 gene editing tool to generate human *UTF1* knockout fibroblasts and iPSCs, and gene silencing was achieved using a shRNA targeting human UTF1. The thesis collectively indicates that human UTF1 plays a vital role in the generation and maintenance of human iPSCs.