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Analysis of Reprogramming Factors Engaged in Cell Transformation

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Abstract—*In the 21st century, a major problem of the developed and developing countries is liver transplantation. Liver transplantation refers to the replacement of a diseased liver with a healthy liver from another person. Liver transplantation has shown success rates and also significantly improved patient and graft survival has been observed over time but this method still has some threats that are being given a thought by the medical researchers. One method is to convert one adult cell type turn into a completely different cell type. This method is known as direct reprogramming. Induced pluripotent stem cells (iPSCs) and the cell reprogramming are providing powerful tools to generate patient-specific cells for research and therapeutic applications. We have made an attempt to study the different transcription factors present in liver that can be reprogrammed into another cell type by studying their various functions, pathways and how the expression of these factors in different tissues affects the reprogramming process. Around 20 transcription factors were studied and out of these 7 transcription factors were selected for further analysis. Different databases were utilized for the present study to understand the important functions of these small proteins. The localized expression of the specific transcription factors of liver were also checked in other tissues. In our present study, we found that the specific transcription factors like HNF1, HNF3A, HNF3B and HNF4 present in liver can be used to directly reprogram one cell type into target liver cell type. These transcription factors can play a very critical role in the regeneration of liver which is very important as it would help the liver to generate its lost part and no transplantation would be required.*

1. INTRODUCTION

The liver performs essential functions in the body by uniquely expressing hepatocyte-specific genes encoding plasma proteins, clotting factors and enzymes involved in detoxification, gluconeogenesis, glycogen synthesis, and glucose, fat, and cholesterol metabolism. People with either acute or chronic liver failure may need a liver transplant to survive. Due to the shortage of donor liver, many liver transplantation surgeries are not performed, and most of the patients undoubtedly suffer from the serious complications with the disease progression. Thus, exploring a new liver source is urgently needed.

To date, regenerative medicine is rapidly progressing, especially in the field of cellular reprogramming. It is now possible to directly convert fully differentiated mature cells into a variety of other cell types by controlling genes which the cell uses. Genes are expressed as products which often are proteins. The first major part of this process is transcription, in which the DNA for a gene is turned into mRNA and finally during translation the mRNA is then turned into protein (s). Transcription factors which regulate the transcription process binds directly to DNA and, based on where they bind the different genes are expressed in the cell. Transcription factors even regulate the expression of other transcription factors. Because different transcription factors are active in different cell types, different cell types make different proteins, and consequently have different identities. Transcription factors can be used to turn mature, adult human cells into cells that are like human embryonic stem cells (hESCs). Embryonic stem cells are derived from the blastocyst stage of early mammalian embryo. They are distinguished by their ability to differentiate into any cell type in the body and are referred to as pluripotent. These reprogrammed cells are labelled as induced pluripotent stem cells (iPSCs). Not only could the newly developed iPSCs turn into any adult cell type, like hESCs, but these new cells could also be patient specific. Making patient-specific cells may avoid some potential complications like immune rejection that can occur with tissue and organ transplants. However, the major hurdle in clinical application of ESCs and iPSCs is the risk of tumour formation.

Direct reprogramming, or turning one cell type directly into another type of cell, skips the need to have the iPSC middle man. By forced expression of one or a small number of key transcription factors, direct reprogramming results in a substantial phenotype switch between two distinct cell types. Cells generated by the process that does not pass through a pluripotent state are probably not tumorigenic, and may serve as an alternative for cell replacement therapy. Direct reprogramming skips the complex steps of iPS generating process, directly produces patient specific reprogrammed cells, and eliminates the possibility of immune rejection and as well as ethical issues that means a patient could have some cells, such as skin cells, removed; these cells could then be directly made into the cell type the patient needs, such as liver cells for a liver transplant [1]. All of this research is part of the rapidly developing field of regenerative medicine, which develops technologies that may be used to regenerate tissues and whole organs.

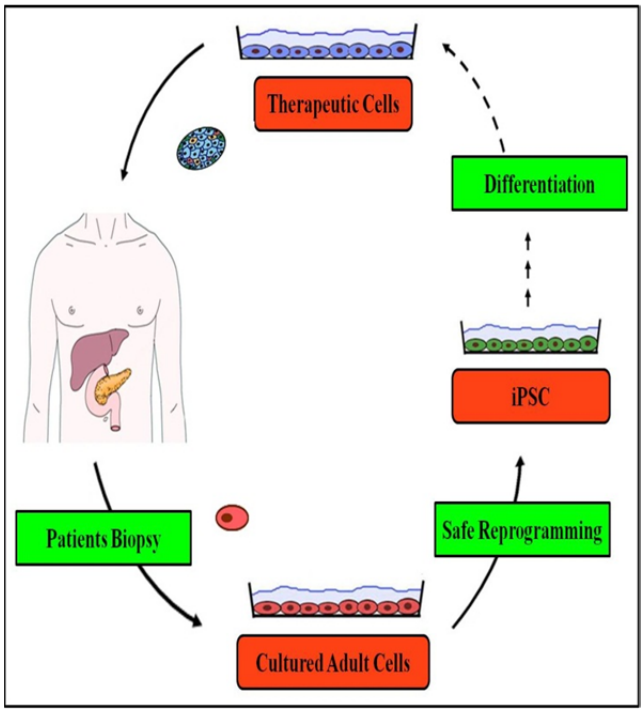

Fig. 1: Induced Pluripotent Stem Cells (iPSCs)

2. METHODOLOGY

A tissue type (liver) was selected. All-important transcription factors for the tissue type were then selected. Amazonia! database was used to learn about the expression of our selected transcription factors of interest. Transcription factors showing high expression in the selected cell type were then noted down. NCBI Gene Database was used to collect more information about the transcription factors. To study the signaling pathways (biochemical pathways) that each of our selected transcription factors are involved in Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database were used. Here, we used online bioinformatics databases to select transcription factors to directly reprogram one cell type into another, desired (or target) cell type.

3. RESULTS AND DISCUSSION

Several transcription factors that are found in liver were searched and then the liver-specific transcription factors were studied. The liver-enriched transcription factors bind to multiple promoter/enhancer sites and synergistically interact with each other to stimulate hepatocyte-specific gene transcription. Liver development requires retention of numerous proliferation specific transcription factors that required proliferation, migration, and survival of hepatic progenitor cells. Around 20 transcription factors were analyzed and out of them only those that are highly expressed in liver cells were selected for further analysis **Table 1**.

HNF1

Hepatocyte nuclear factor 1 (HNF1) is a transcription factor involved in the regulation of a large set of hepatic genes, including albumin, β-fibrinogen, and α1-antitrypsin. HNF1 is expressed in the liver, digestive tract, pancreas, and kidney. Mice lacking HNF1 has been reported to exhibit hepatic, pancreatic, and renal dysfunctions [2]. HNF1 is a major regulator of glucose homeostasis and plays an important role in controlling the postnatal functions of the liver, pancreas, and kidney. HNF1 deficient mice fail to express the hepatic phenylalanine hydroxylase gene, giving rise to hyperphenylalaninemia. The phenotype of HNF12/2 mice has revealed that this transcription factor is crucial for the transcriptional activation of genes that play key roles in phenylalanine catabolism, pancreatic β-cell glucose sensing, and renal proximal tubular reabsorption of glucose and several other metabolites. For these reasons, HNF1 can be considered a transcription factor at the crossroads of the regulation of glucose homeostasis [3]. HNF1 is highly expressed in the liver and the average liver signal 116.5. It is associated with liver specific genes. Defects can cause diabetes and liver tumors.

HNF3A

The HNF3 proteins were originally identified by their ability to interact with an important promoter element of the trans-thyretin and cxl-antitrypsin genes [4], and were subsequently shown to participate in the expression of several other liver-specific genes. HNF3A with an average liver signal of 86.4 and is involved in regulation of metabolism and in the differentiation of metabolic tissues such as the pancreas and liver; bind to *cis*-regulatory elements in hundreds of genes encoding gluconeogenic and glycolytic enzymes, serum proteins and hormones. HNF3A is also expressed in breast, prostate gland, trachea, oesophagus, etc.

HNF3B

HNF3 is a family of liver-enriched transcription factors. The binding sites of HNF3 on *cis* elements have been identified in the regulatory sequences of several hepatocyte-specific genes including albumin, α-fetoprotein, α-1- antitrypsin, trans-thyretin tyrosine aminotransferase, etc. Three members of the HNF3 family (HNF3α, HNF3β, and HNF3γ) have been so far identified and cloned [5]. HNF3B shows an average liver signal of 300.725 and is also expressed in thyroid gland, oocyte, kidney and heart but in low amounts. It serves as a transcriptional activator for liver specific genes, involved in embryonic development. This has been linked to sporadic cases of maturity onset diabetes of the young.

TABLE 1: This table contains information on transcription factors that are important for Liver

TARGET CELL TYPE	IMPORTANT TRANSCRIPTION FACTOR	EXPRESSED IN OTHER CELL TYPES	HIGHLY EXPRESSED IN OTHER CELL TYPES	KNOWN FUNCTIONS
Liver	**HNF1**	Kidney, oocytes, small intestine, skin	YES	Associated with liver specific genes. Defects can cause diabetes and liver tumors. Involved in diabetes and insulin signaling pathways.
Liver	**CEBP**	Adipose tissue ,skin	YES	Transcriptional mis-regulation in cancer, tuberculosis, pathways in cancer , acute myeloid leukemia, non alcoholic fatty liver disease.
Liver	**HNF4**	Kidney , skin, pancreatic beta cells	YES	AMPK signaling pathway, maturity onset diabetes of the young, liver development.
Liver	**HNF3A**	Breast, trachea, bronchus, prostate gland, lymph nodes, oesohagus, stomach	YES	Regulation of metabolism and in the differentiation of metabolic tissues such as the pancreas and liver; bind to *cis*-regulatory elements in hundreds of genes encoding gluconeogenic and glycolytic enzymes, serum proteins and hormones.
Liver	**HNF3B**	Thyroid gland, kidney, heart	YES	Transcriptional activator for liver specific gene, involved in embryonic development. Pathway-Maturity onset diabetes of the young.
Liver	**NR1H2**	Lung, skin, heart	YES	Key regulators of macrophage function, controlling transcriptional programs involved in lipid homeostasis and inflammation. Pathway- Insulin resistance.
Liver	**NR1H3**	Thyroid, kidney, placenta, skin, heart	YES	Key regulators of macrophage function, controlling transcriptional programs involved in lipid homeostasis and inflammation. Pathway- Insulin resistance, PPAR Signaling pathway, Hepatitis C, NAFLD

HNF4

HNF4 was first identified by its interaction with a *cis*-regulatory sequence within the trans-thyretin and cd-antitrypsin promoters. The predicted amino acid sequence of HNF4 revealed that it is a member of the nuclear receptor superfamily. Like other members of the family, HNF4 contains a zinc finger region and binds DNA as a dimer. HNF4 possesses a proline-rich region at the carboxy- terminus and three serine-threonine regions located throughout the molecule that very likely are implicated in transcription activation. HNF4 is also phosphorylated at tyrosine, serine, and to a lesser extent at threonine residues, and various inhibitors and stimulators of protein kinase pathways modify the HNF4-mediated transcriptional activation [6]. The average liver signal for HNF4 was found to be 95.0. It is mostly expressed in liver, kidney, skin, pancreatic beta cells and is critical for liver development. It is involved in AMPK signaling pathway, maturity onset diabetes of the young.

NR1H2 & NR1H3

Nuclear receptors are ligand-activated transcription factors that coordinate gene expression in response to hormonal and environmental signals. Members of the superfamily that work as heterodimers with the retinoid X receptor (RXR) serve as sensors of dietary components, orchestrating the physiological response to nutrients. LXR-α and LXR-β (also called NR1H3 and NR1H2, respectively) are RXR partners that recognize oxidized cholesterol and control gene expression linked to cholesterol and fatty acid metabolism. Activation of LXRs results in decreased atherosclerosis in rodents[9]. LXR ligands have anti-diabetic effects as well, decreasing liver glucose output and increasing peripheral glucose disposal.Treatment of rodents with synthetic LXR ligands results in decreased hepatic gluconeogenesis and increased lipogenesis, indicating that LXR serves as a transcription factor that integrates liver carbohydrate and lipid metabolism [8, 9, 10]. The average liver signals for NR1H2 and NR1H3 were found to be 130.875 and 130.15, respectively. They are also expressed in lung, skin, thyroid, kidney, placenta and heart. These factors are also involved in peroxisome proliferator-activated receptors (PPARs) signaling pathway and insulin resistance pathway.

CEBP

CCAAT/enhancer-binding protein (CEBP), a member of the basic leucine zipper transcription factor family, was cloned as a DNA-binding protein from rat liver [11, 12]. It is involved in transcriptional misregulation in cancer, tuberculosis, pathways in cancer, acute myeloid leukemia, non-alcoholic fatty liver disease. CEBPa transcripts are present in a variety of cells, but the protein is detected only in differentiated hepatocytes, adipocytes, intestinal epithelial cells, pregranulocyte, and myelonoblastic cell lines. CEBP shows an average liver signal of 1572.95 and is also expressed in skin, adipose tissue, brain, lungs.

4. CONCLUSION

Out of all the transcription factors that were selected, the best ones to use in the direct reprogramming efforts to make cells of our target cell type are HNF1, HNF3A, HNF3B and HNF4. In the above studied transcription factors, CEBP was one of the transcription factors that was found to be involved in transcriptional mis-regulation in cancer, tuberculosis, pathways in cancer, acute myeloid leukemia, non-alcoholic fatty liver disease. Hence, CEBP transcription factor cannot be used in direct reprogramming because it might lead to the formation of unwanted products that may be harmful to the human body. Most importantly, this analysis gives insight into the fact that with the help of transcription factors it is possible to directly reprogram one cell type into another cell type. Additional analysis and validation of the transcription factors will be required to determine the clinical value.

5. ACKNOWLEDGEMENT

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