Metabolomics Assessment of Human Induced Pluripotent Stem Cells: Initial Transition Patterns

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Abstract

Cell differentiation towards specific lineages remains a challenge. . In this study, non-targeted metabolomic analysis techniques were used to analyze extracellular metabolites present in samples as small as 1 microliter. HiPSCs were differentiated by initiating culture under basal medium E6 in combination with chemical inhibitors previously reported to promote differentiation toward the ectodermal lineage. B. Wnt/ -catenin and TGF- -kinase/ activin receptor, alone or in combination with inhibition of bFGF and glycogen kinase 3 (GSK-3). This is commonly used to redirect hiPSCs to the mesodermal lineage. At 0 and 48 hours, 117 metabolites were identified, including biologically relevant metabolites such as lactate, pyruvate and amino acids. By measuring the expression of the pluripotency markers OCT3/4, we were able to correlate the differentiation state of the cells with the shifted metabolites. A group of cells that underwent ectodermal differentiation showed a significant decrease in OCT3/4 expression. In addition, metabolites such as pyruvate and kynurenine showed dramatic changes under ectodermal differentiation conditions, with pyruvate consumption increased 1–2-fold and kynurenine secretion decreased 2-fold. Further metabolite analysis reveals a group of metabolites specifically associated with the ectodermal lineage and the potential of our results for characterizing hiPSCs during cell differentiation, especially under ectodermal lineage conditions.

Keywords: Non-targeted metabolomics • Extracellular metabolites• Metabolic assessment

Introduction

Human pluripotent stem cells (hPSCs) are divided into human embryonic stem cells (hESCs) generated from the inner cell mass of the blastocyst of pre-implantation embryos and human induced pluripotent stem cells generated directly from the blastocyst [1]. (hiPSC). Four transcription factors (Oct3/4, Sox2, Klf4, Myc) are introduced into somatic cells hPSCs can self-renew or transform into three germ layers: Under certain conditions, ectoderm, mesoderm and endoderm form functional cells such as hepatocytes [2]. Therefore, hPSCs represent a valuable source for the generation of functional human cells for a variety of applications, including cell-based therapy and in vitro model building.

Early differentiation of hPSCs to specific cell lineages was determined by real-time quantitative polymerase chain reaction (PCR) for gene expression, gene expression and protein expression of three lineage markers (ectoderm, mesoderm and endoderm) by western blotting. can be determined by evaluating , immunofluorescence staining, or ELISA for protein detection. Although these methods provide useful information, they are invasive and require destruction of cells to access intercellular nucleic acids and proteins [3]. Additionally, these methods can be time consuming and expensive as they require multiple steps, such as the addition of different antibodies and labeling agents. For large-scale production of hPSC-derived functional cells, a non-invasive, simple, non-invasive method for predicting and monitoring the lineage of hPSCs at early stages of differentiation to minimize wastage of time and material during cell production. A rapid and comprehensive analytical method is desirable [4].

53

Stem. Cell. Res. Reg. Med. (2023) 6(3), 53–56

The metabolome provides relevant information about cell properties. One metabolomics technique, liquid chromatography-mass spectrometry (LC-MS)-based non-targeted metabolomics, can detect hundreds of small amounts of metabolites through a relatively simple sample preparation process. Previous studies have used this technique to extract cell culture media (CCM) of hepatocytes (HepG2) and corneal epithelial cells (HCE-T) grown in microfluidic devices by collecting only small amounts at different time points.) were successfully measured. . Currently, there is no established method to assess lineage variation of hPSCs during differentiation by profiling extracellular metabolites. Therefore, implementation of an LC-MS-based non-targeted metabolomics approach represents a non-invasive solution for predicting the differentiation state of hPSCs.

In this study, we examined early differentiation of hiPSCs using chemical inhibitors previously reported to induce differentiation towards ectodermal and mesoderm lineages. CCM was collected at 0 and 48 hours and the abundance of extracellular metabolites was determined by LC-MS-based untargeted metabolomics measurements. The resulting metabolite changes were then evaluated in relation to hiPSC pluripotency status [5].

Experimental Design

Culturing human induced pluripotent stem cells

Cell culture dishes were coated with Matrigel and incubated at 37 ℃ for 30 min before initiating differentiation. (Thermo Fisher Scientific, Inc., Waltham, MA, USA) for 5 minutes at 37°C, followed by mTeSR™ Plus medium (mTeSR Plus) (STEMCELL Technologies, Cambridge). , Massachusetts, USA) and transfer the cell suspension to a 15 mL tube. Cells were centrifuged at $200 \times g$ for 3 min, supernatant removed, resuspended in mTeSR Plus medium supplemented with 10 M Y27632 (Wako, Osaka, Japan) and plated in 96-well plates at a density of 1. 1 $\times$ 10 cells were added per well to Matrigel (Thermo Fisher Scientific, Inc., Waltham, MA)-coated culture dishes and incubated for 24 hours in a humidified incubator at 37°C, 5% CO_2. Culture medium was replaced with mTeSR Plus basal medium every day except

weekends according to the manufacturer's instructions [6].

Data visualization

Independent biological triplicates were used in the study. Statistical analysis was performed using Dunnett's test, paired t-test and Tukey comparison test via GraphPad Prism 8. PCA and VIP analyzes included all samples collected at 0 and 48 hours without exclusion, including cells treated with E6 alone and in combination with chemical inhibitors, and cells cultured under mTeSR Plus conditions [7]. Venn diagrams were created using Orange 3 software developed by the Bioinformatics Laboratory, Faculty of Computer and Information Sciences, University of Ljubljana, Slovenia. Data visualization was done using GraphPad Prism 8 with R Studio and the ggplot library. A graphical overview was created at BioRender.com [8].

Measurement of extracellular metabolites

We were able to annotate 117 metabolites at annotation level 1 of the Metabolomics Standard Initiative. Peak areas were used for semi-quantitation of metabolites. The CVs of the two tISs in the QC samples were <18% and <14% in negative and positive ionization modes, respectively. In the study sample, it was <17% and <12% in negative and positive ionization modes, respectively (HEPES was not used as a tIS because it was present at high concentrations in the cell culture medium and saturated the signal). Differences between AM and RT for annotated metabolites were <15 ppm and <0.9 min, respectively [9]. Multiple metabolites were detected in single or few samples, resulting in lower QC sample concentrations and lower QC mean-to-blank ratios (<3). In such cases, only metabolites with D ratio < are extracted. 30 were reserved. Detailed information on each metabolite identification (retention time, ionization mode, m/z value), raw peak area, CV, QC mean versus blank, D ratio is provided. Principal component analysis (PCA) results were obtained over time, as evidenced by systematic changes in sample clusters at 48 h and 0 h, including all samples and chemical inhibitor combinations under mTeSR Plus and E6. Showed significant changes in metabolite levels. Furthermore, hiPSC samples in mTeSR Plus were indistinguishable from those differentiated in E6 medium at both 0 and 48 hours. Partial least-squares discriminant analysis (PLS-DA) was combined with variable

Stem. Cell. Res. Reg. Med. (2023) 6(3)

54

importance in projection (VIP) to identify unique metabolites within the study sample. This analysis revealed a significant increase in lactate levels in almost all samples. In contrast, pyruvate levels decreased during the differentiation process. His PCA of samples treated with E6 alone observed marked changes in metabolites at 48 h compared to the initial time points. However, no significant differences were observed between the different treatment groups in terms of metabolite segregation [10].

Discussion

We used two types of inhibitors for Wnt/ -catenin inhibition, IWP-2 and IWR-1 endo, which have been extensively reported in many differentiation protocols, involving neuron cells and retinal and corneal epithelial cells, and two types for the inhibition of TGF- kinase/activin receptor that are commonly reported in the differentiation of the forehead and eye lineage, SB505124 and A83-01 where the inhibition potency of A83-01 is much greater than that of other TGF- /R inhibitors. For the initiation of the differentiation toward the corneal epithelium lineage, which is initially diverted from the surface ectoderm? Additionally, we used CHIR99021 as a potent inhibitor of glycogen kinase 3 (GSK-3). It is commonly used in hPSCs to redirect them to the mesodermal lineage prior to cell differentiation into cardiomyocytes or myoblasts. Additionally, we used Y-27632 as a Rho-associated coiled-coil kinase (ROCK) inhibitor, which is widely used in hPSCs to improve survival.

To confirm the differentiation process in samples using E6 alone or in combination with another treatment, we performed immunofluorescence staining for the pluripotency markers OCT3/4. Our results show that compared to cells cultured in mTeSR Plus medium, OCT3/4 expression was significantly reduced in all samples cultured with E6 alone or in combination with chemicals or the growth factor bFGF, suggesting that cell differentiation was enhanced. Indeed, the use of E6 alone has been reported to redirect cells to neural crest progenitor cells derived from the ectodermal lineage, and the combination of E6 and bFGF has also been used to generate myoblasts derived from the mesodermal lineage. I'm here. it has been. Another combination

with A83-01, IWP-2_SB505124_bFGF, IWP-2_A83-01_bFGF, and IWR-1 endo_SB505124 resulted in greater reduction of OCT3/4, indicating greater potency in initiating cell differentiation. We have previously shown that combined inhibition of Wnt/ -catenin and TGF- -kinase/activin receptors with bFGF can bypass the ectodermal lineage and redirect cells to the corneal lineage in the first weeks of differentiation.

Conclusion

This is the first study using metabolome profiling of microscale cell culture media (CCM) from early differentiating human induced pluripotent stem cells (hiPSCs). We were able to demonstrate the onset of differentiation by observing changes in key biological metabolites such as glucose, pyruvate, and lactate, as well as changes in hiPSC metabolite markers kynurenine and tryptophan. In addition, a group of metabolites found to be specific for ectodermal differentiation. B. Indole-3-lactate, oxalate, adenosine secretion and decrease in 2-oxoglutarate. Further investigation is needed to determine the importance of these discovered metabolites in the differentiation into specific lineages.

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Stem. Cell. Res. Reg. Med. (2023) 6(3)

56