Determining Metabolic Switch in Induced Pluripotent Stem Cells (iPSCs)

Application Brief

Introduction

Induced pluripotent stem cells (iPSCs) have become the cell type of choice to mimic various diseases including cancer, cardiovascular and neurodegenerative diseases. The nuclear reprogramming of somatic cells, such as fibroblasts, to a pluripotent state has a dramatic effect on the metabolic requirements of the cells inducing a cancer-like (Warburg) metabolic shift from oxidative phosphorylation to glycolysis. Furthermore, it has been shown that rapidly dividing cells such as cancer cells and stem cells will preferentially switch to glycolysis-based metabolism, even in the presence of normal oxygen levels. This switch allows for a greater supply of cell growth intermediates such as amino acids, provides protection against oxidative stress, and helps to generate NADPH (Ma; *et al.*, 2013)[1].

Similar to cancer cells, it has been proposed that the metabolic shift in iPSCs has significant importance in establishing pluripotent identity. However, the factors that mitigate this metabolic shift remain unclear. Studying the mechanisms involved in cellular reprogramming could have applications not only in pluripotency induction, but also in cancer cell progression. This Application Brief describes publications that have used Agilent Seahorse XF technology to examine the metabolic shift exhibited by iPSCs.

It has been proposed that the dependence on glycolysis for ATP production is due to the cellular adaptation to their niche, a microenvironment characterized as either a hypoxic or low oxygen environment. Adaptation to this environment predominantly relies on the up-regulation of hypoxia-inducible factors (HIFs), which are oxygen-sensitive transcription factors.

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Mathieu; et al.[2] examined the metabolic switch observed in iPSCs and the roles of HIF1α and HIF2α in shifting to glycolysis from oxidative phosphorylation. The authors employed iPSCs derived from human lung fibroblasts to characterize the metabolic phenotypes of cells under various treatment conditions. As illustrated in Figure 1, it was observed that the fibroblasts exhibited increased maximal respiratory capacity compared to human embryonic stem cells (hESC) and iPSCs. These data indicate that the metabolic changes would occur early in the reprogramming process. To further examine the specific roles of HIF1α and HIF2α, they tested whether these factors are required in the metabolic shift. As shown in Figure 1B, iPSCs over expressing HIF1α resulted in reduced mitochondrial reserves as a consequence of FCCP treatment, which is similar to the metabolic profile of hESC cells.

The authors used an Agilent Seahorse XF96 Analyzer to assess the metabolic changes of iPSCs derived from human fibroblasts using the Agilent Seahorse XF Cell Mito Stress Test and Agilent Seahorse XF Glycolysis Stress Test kits. Their analyses determined that the metabolic switch occurs early in the nuclear reprogramming process, and that both HIF1α and HIF2α have sequential roles in the metabolic switch during iPSC induction.

Results and Discussion

Agilent Seahorse XF technology allows for the real-time assessment of cell metabolism, and is capable of providing measurements focused on a specific interest, without a concomitant effect on other aspects of cell metabolism.

Prigione; et al. (2014)[3] published a study focused on the HIF1α pathway contribution to metabolic reprogramming. They hypothesized that HIF1α activation would result in an early switch to glycolysis and subsequently lead to more efficient iPSC generation. The authors used an Agilent Seahorse XF24 Analyzer
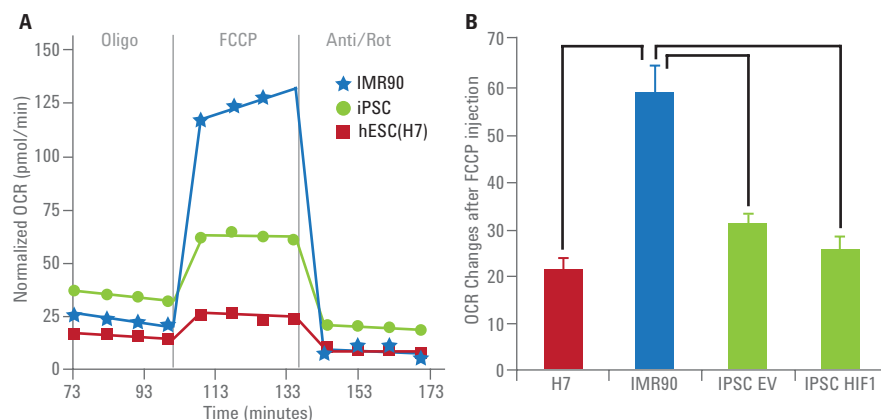

Figure 1. Metabolic switch occurs early in reprogramming, and HIFs are required for the metabolic switch in early reprogramming. A) Graphical representation of normalized OCR values following the Agilent Seahorse XF Cell Mito Stress Test protocol. The profile compares iPSCs to hESC (H7) and fibroblast cells (IMR90). B) iPSC Colonies reprogrammed using HIF1α overexpression exhibited reduced mitochondrial activity similar to the hESC (H7) cells, but distinctly different from the parental IMR90 fibroblast cells. P values were calculated using a Student's t-test, ***$p < 0.001$. Scale bars show SEM for at least three separate experiments.

to assess both oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in iPSCs generated from neonatal foreskin fibroblasts.

They observed that short-term treatment of ethyl-3,4-dihydroxybenzoate (EDHB), an inhibitor of oxygen-dependent prolyl-hydroxylases that degrades HIF1α under normal oxygen conditions, results in a decrease in oxidative phosphorylation and an increase in glycolytic metabolism. In further testing of HIF1α pathway contributions, cells that were treated with a small molecule mimicking HIF1α activation exhibited a significant OCR/ECAR decrease. Collectively, these data indicate the crucial role of HIF1α in establishing the early metabolic switch to glycolysis during the acquisition of pluripotency.

It has been demonstrated that enhancing certain factors that are normally dormant in differentiated cell populations may allow for greater efficiency in generating iPSCs and reduce the reliance on embryonic tissue for stem cell generation (Yoshida; et al., 2009)[4].

Folmes; et al., (2013)[5] examined c-Myc, a transcription factor that plays a major role in integrating cell proliferation with the regulation of energy metabolism. The authors tested whether the presence of exogenous c-Myc would have an effect on pluripotency induction. Using an Agilent Seahorse XF24 Analyzer, the authors used iPSCs-derived from mouse embryonic fibroblasts and determined the OCR. They demonstrated that the presence of c-Myc results in an increased basal glycolytic rate and glycolytic capacity; however, a significant difference was not observed in glycolytic reserve.

Based on these and other collected data, exogenous c-Myc potentiates the metabolic switch of the iPSCs to glycolysis without affecting the mitochondrial structure nor oxidative metabolism. The authors further speculate that as the glycolytic rates are closely correlated to nuclear reprogramming efficiency, the cellular utilization of c-Myc (or other similar factors) may impact the metabolic state, which, in turn, may influence the generation of any metabolism-dependent lineages.

Materials and Methods

Agilent Seahorse XF96 Cell Culture Microplates were coated with Matrigel or 0.1 % gelatin prior to cell plating, as described in a separate protocol.

iPSCs, derived from human lung fibroblasts, were cultured on γ-irradiated primary mouse embryonic fibroblasts (MEFs) in DMEM/Ham's F-1 medium, supplemented with Glutamax and 20 % knock-out serum replacer, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 50 U/mL penicillin, 50 μg/mL streptomycin, 0.1 mM β-mercaptoethanol, and 4 ng/mL basic fibroblast growth factor.

XF Analysis

Metabolic analyses of iPSCs were performed using a Agilent Seahorse XF96 Analyzer. The XF96 Analyzer enables real-time, simultaneous rate measurements of OCR and ECAR by creating a transient microchamber of a few microliters in specialized cell culture microplates.

As shown in Figure 2, iPSCs were passaged and seeded onto pre-coated XF96 Cell Culture Microplates. Cell culture media was exchanged for base medium, containing unbuffered DMEM supplemented with sodium pyruvate and either 25 mM glucose (for the XF Cell Mito Stress Test), or 2 mM glutamine (for the XF Glycolysis Stress Test) 1 hour prior and for the duration of the XF assays. Baseline OCR was calculated based on the average values obtained from time point 1–5 during the assay. Changes in OCR were calculated based on the maximal change following compound injection and compared to the previous OCR value prior to injection.
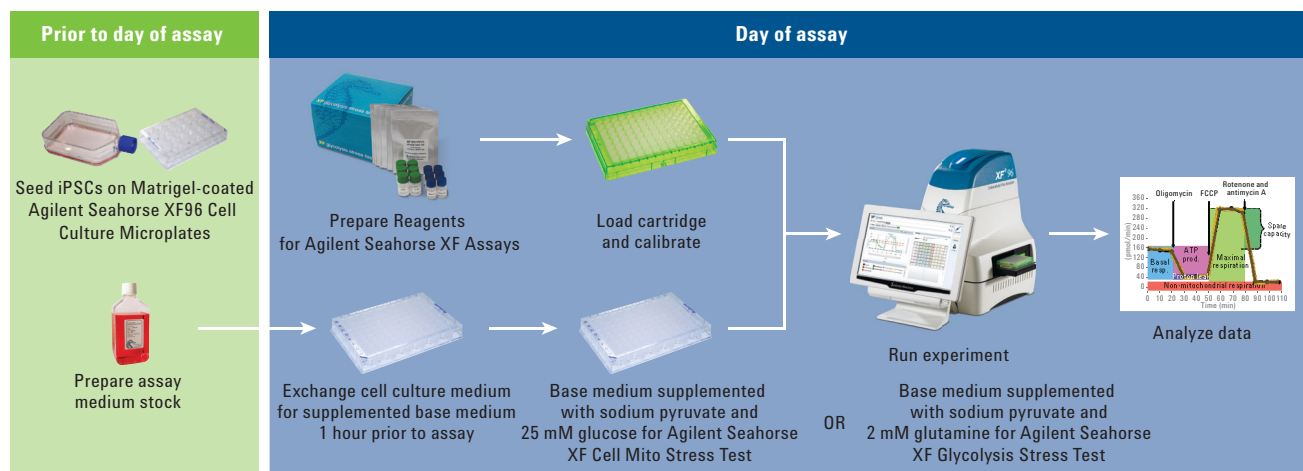

Figure 2. Flow chart of the XF assay.

References

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