PHCbi

LiCellMo

Application Note

Metabolic rate monitoring of undifferentiated iPS cells and expression analysis of differentiation markers

Introduction

Reprogramming somatic cells to induced pluripotent stem cells (iPSCs) requires a shift in the main metabolic pathway from oxidative phosphorylation (OXPHOS) to the glycolysis.[1) 2)] This metabolic shift takes place at an early stage of reprograming, before self-renewal and expression of pluripotency genes, and undifferentiated iPSCs show significantly greater glycolytic metabolism than somatic cells.[3)] Therefore, a close relationship between pluripotency and the glycolysis is surmised.

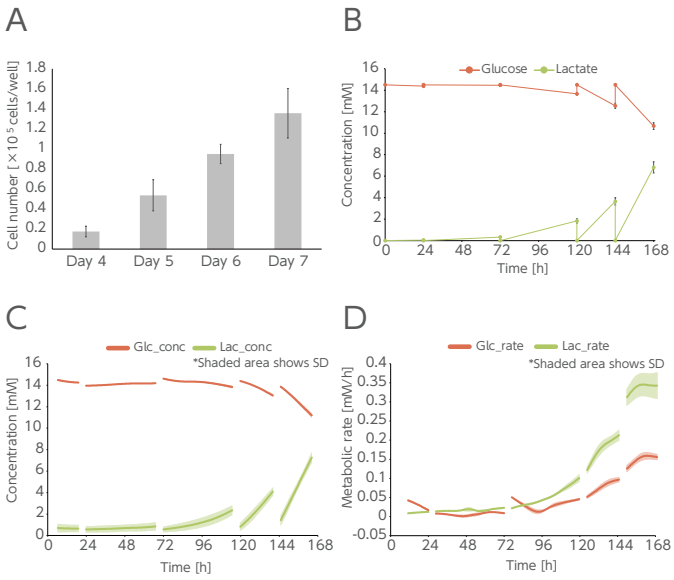
Since the state of undifferentiated iPSCs is a major factor in the quality and efficiency of subsequent processes such as freezing stock and differentiation induction, it is necessary to understand the constantly changing state of the cells to carry out culture operations at the best possible time. In general cell culture, qualitative judgements are often based on the experience of the practitioner, which is an issue for ensuring uniform quality. One method for quantitative evaluation of the state of cells is to evaluate cell metabolism through analysis of the culture medium,[4)] but it is not possible to gain an accurate understanding of the state of cells by sampling several times a day. Though there are evaluation methods using automated sampling, this is difficult for cultures in multi-well plates that are used in laboratories. Therefore, PHCbi developed the Live cell metabolic analyzer, which can continuously measure glucose and lactate concentrations in the culture medium in common, commercially-available, 24-well plates through in-line monitoring. This allows changes in cell state over time to be quantified.

Method

1231A3 iPSCs (HPS0381 was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan.) were cultured for 7 days (168 h) in 24-well plates according to the standard protocol,[5)] and glucose and lactate concentrations were continuously measured using the Live cell metabolic analyzer. The culture medium was StemFit® AK02N (Ajinomoto Healthy Supply). Y-27632 was added at 24 h after passage culture, and the culture medium was replaced at 24, 72, 120, and 144 h. The medium was collected each time it was replaced, and glucose and lactate concentrations were measured by colorimetry. Cell state was evaluated using qRT-PCR to measure expression levels of undifferentiated markers (NANOG, OCT3/4) and differentiated markers (PAX6 in the ectoderm, Brachyury in the mesoderm, GATA4 in the endoderm).

Result 1

The cells continued to increase throughout the 7 days of culture (Fig. A). The colorimetric measurements of the medium that was collected when it was replaced showed that, over time, glucose concentration decreased and lactate concentration increased (Fig. B). Continuous measurements taken using the Live cell metabolic analyzer allowed visualization of the continuous changes in glucose and lactate concentrations in a way that would not be possible with measurements taken when the medium was replaced (Fig. C). In addition, comparison of the concentration measurements obtained from the electrochemical sensor of the Live cell metabolic analyzer and the colorimetric concentration measurements of the medium that was collected when it was replaced showed that glucose concentration measurements in the range of 1–27 mM diverged by -2 – +4%, and lactate concentration measurements in the range of 1–15 mM diverged by +11 – +18%, from which it was concluded that the accuracy was sufficient. The glucose consumption rate and the lactate production rate were calculated from the continuous concentration measurement data, and the results indicated that there was a point at which the increase turned to a decrease at 130 h, following replacement of the medium at 120 h, and at 155 h, following replacement of the medium at 144 h (Fig. D).



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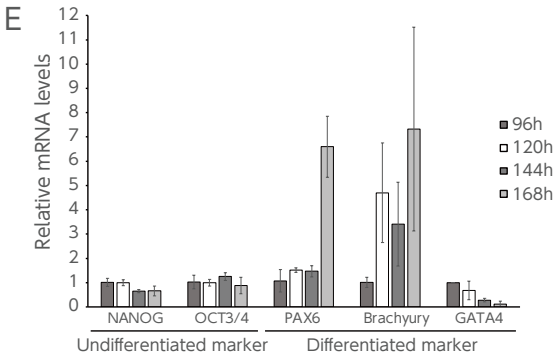
Result 2

Gene expression analysis was carried out at 96 h, 120 h, 144 h, and 168 h to evaluate the cells during the second half of culture, when the glucose consumption rate and lactate production rate are reduced (Fig. E). No change was seen in expression of NANOG or OCT3/4, which are markers of undifferentiated iPSCs, whereas there were increased expressions of PAX6 and Brachyury, which are early differentiation markers. Expression of GATA4 decreased.



Discussion

In this experiment, iPSCs were cultured using the standard culture protocol for 7 days. As the cells continued to proliferate over the 7 days, the rate of glucose consumption and lactate production decreased may be restated as a decrease in the per-cell rate of glucose consumption and lactate production. This result suggests that the glycolysis is suppressed at 130 h and 155 h after the start of culture. The glycolysis is more active in iPSCs than in somatic cells, but it has been reported that the glycolysis is suppressed in iPSCs in which differentiation is induced by retinoic acid.[6] Furthermore, it has been reported that iPSCs cultured in medium with no bFGF and induced to differentiate spontaneously show no decrease in undifferentiated marker expression and an increase in early differentiation marker expression.[7] Thus, since there is a close relationship between the pluripotency of iPSCs and the glycolysis, there may be a correlation between the tendency toward suppression of the glycolysis and increased expression of early differentiation markers in the latter stage of culture.

Conclusion

The Live cell metabolic analyzer allows continuous measurement of glucose concentration and lactate concentration during culture in a multi-well plate. With this device, it was possible to visualize changes in the glycolysis of iPSCs between medium replacements that could not be seen with the conventional method of sampling several times a day. In addition, the results of continuous concentration measurement were shown to have equivalent accuracy to the results of measurement by the usual method of colorimetry.

We expect that changes in the glucose consumption rate and the lactate production rate as quantitative markers of the changes in state taking place within cells will allow for objective judgements, which will also ensure the production of high-quality cells.

Reference

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