Visualization of metabolic profile of immune cells using metabolic inhibitors

Introduction

There is a diversity of metabolic profiles in cancer cells, and these profiles can differ widely even within the same cancer.[1] Dependence on aerobic glycolysis, one of the various metabolic profiles, is also known to be common in immune cells.[2] This dependence generates competition for energy sources between cancer cells and immune cells in the tumor microenvironment, and has been implicated in immune cell dysfunction.[3] Cells also alter their metabolism in response to the environment. Analysis of cell metabolism is therefore a key factor in understanding cell function. Methods for evaluating cell metabolism by analyzing culture media components are available, but conventional methods based on at most a few samples per day are inadequate for observing changes in cell metabolism over time. Given this background, PHC engineered a Live cell metabolic analyzer with electrochemical In-Line sensors and established a method for continuously measuring glucose and lactate concentrations in culture medium. In this study, we attempted to visualize cell metabolic profiles by continuously measuring changes in cell metabolism using a glycolysis inhibitor and a mitochondrial metabolism inhibitor.

Method

This study used THP-1 cells from an acute monocytic leukemia cell line (obtained from JCRB Cell Bank: JCRB0112.1), and NB-4 cells from an acute promyelocytic leukemia cell line (obtained from CLS - Cell Lines Service GmbH: 300299). THP-1 and NB-4 cell lines are known to be dependent on fatty acid metabolism and glycolysis, respectively.[4] Both cell lines were cultured for 72 hours in RPMI 1640 Medium supplemented with 10% FBS, during which the concentrations of glucose and lactate were measured continuously using the Live cell metabolic analyzer.

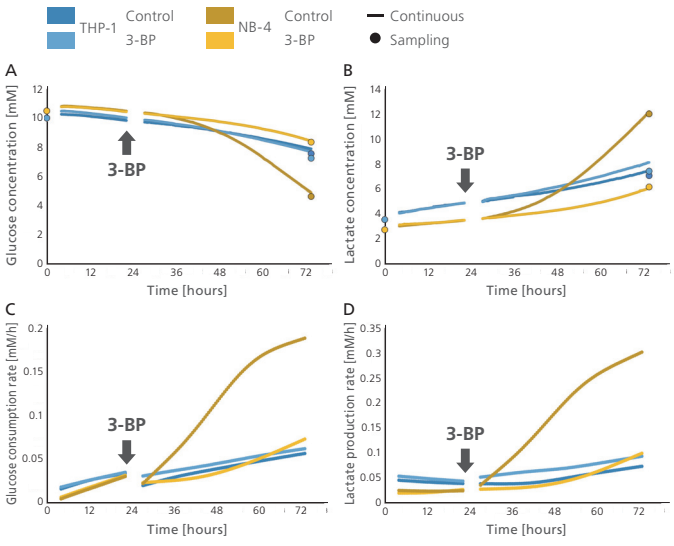
In Experiment 1, the hexokinase inhibitor 3-bromopyruvate (3-BP) was added to a final concentration of 12.5 µM, 24 hours after cell seeding. This allowed us to examine the kind of metabolic changes that occur when glycolysis is inhibited in cell lines with different metabolic properties.

In Experiment 2, the ATP synthase inhibitor oligomycin (Oligo) was added to a final concentration of 0.25 µM (0.1% DMSO), 24 hours after cell seeding, allowing us to examine the kind of metabolic changes that occur in both cell lines when mitochondrial metabolism is inhibited.

In Experiments 1 and 2, the concentrations of glucose and lactate in culture medium at the end of continuous measurements were compared based on the results from the Live cell metabolic analyzer and from colorimetric analysis.

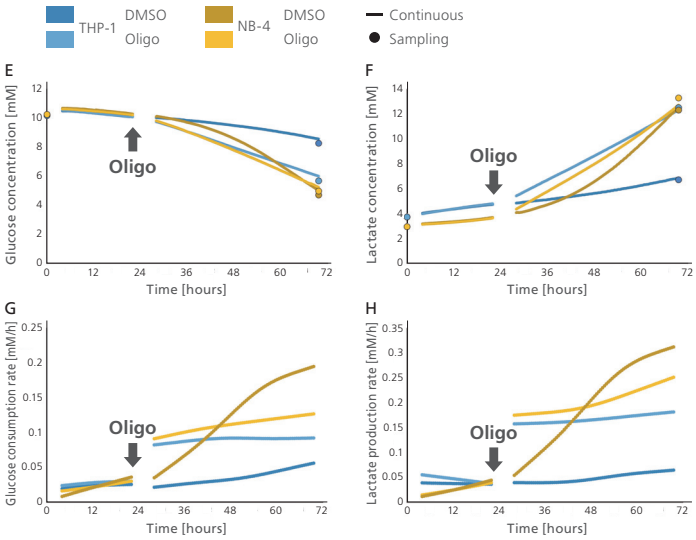
Result 1

The accuracy of results from continuous measurements was initially assessed. When the results from the Live cell metabolic analyzer were compared with those from colorimetric analysis for concentrations of glucose and lactate in culture medium at the end of continuous measurements, the maximum discrepancy was 9%. It was therefore concluded that measurements were sufficiently accurate (A and B). Next, the glucose consumption rate and lactate production rate were calculated from changes in glucose and lactate concentrations determined by continuous measurements (C and D). Comparison of these values showed that the metabolic rate of both glucose and lactate in NB-4 cells increased considerably, but the increase tapered off 60 hours after cell seeding. Furthermore, the metabolic rate in NB-4 cells was inhibited immediately after adding 3-BP, and this inhibition continued throughout the remainder of the experiment. In contrast, THP-1 cells showed only a small change in metabolic rate, and no change in response to 3-BP.



Result 2

When the results from the Live cell metabolic analyzer were compared with those from colorimetric analysis for concentrations of glucose and lactate in culture medium at the end of continuous measurements, the maximum discrepancy was 6% (E and F). When the glucose consumption rate and lactate production rate were calculated from glucose and lactate concentrations determined by continuous measurements, the metabolic rates of both cell lines showed a dramatic increase in response to Oligo (G and H). However, although the metabolic rate in NB-4 cells increased as a result of Oligo treatment, it was higher in the DMSO group 45 hours after cell seeding.



Discussion

Inhibition of glycolysis by 3-BP treatment only affected NB-4 cells, and continuous measurements demonstrated that NB-4 cells have glycolysis-dependent properties. Furthermore, a change in metabolic rate occurred 60 hours after seeding, suggesting that there was also a change in the state of the cells at this time.

A metabolic shift may have occurred because Oligo treatment promoted glycolysis in THP-1 cells and mitochondrial ATP production was inhibited. Continuous measurements were thus able to show that THP-1 cells have mitochondrial metabolism-dependent properties. The change in metabolic rate after Oligo treatment differed between THP-1 cells and NB-4 cells, suggesting that responsiveness to metabolic inhibitors changes over time depending on the cell type.

Conclusion

We developed a method for continuously measuring glucose and lactate concentrations using a Live cell metabolic analyzer with In-Line sensors. We also confirmed that the continuous measurements were sufficiently accurate regardless of whether an inhibitor was used.

Continuous measurements of the culture medium made it possible to visualize changes in cell metabolism in response to the environment, rather than just providing a snapshot of cell metabolism. Evaluation of the differing responsiveness of each cell type to metabolic inhibitors over time using continuous measurements was also useful for elucidating the metabolic properties of cells.

Understanding metabolism in immune cells is important for elucidating the properties of those cells.[5] It is also well known that immune cells dynamically change their own metabolism during the phases of activation, proliferation, and exhaustion.[6] The close relationship between cell function and metabolism has been the focus of research on immune cell therapies, particularly those targeting cancer. We expect that the use of the Live cell metabolic analyzer with In-Line monitoring will contribute to new discoveries in research on the metabolic properties of immune cells.

Reference

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