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Effect of rapid increases in oxygen levels on glucose metabolism of cancer cells

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Introduction

Objective

To evaluate the effect of oxygen concentration changing on the glucose-based acid producing activity of oral squamous carcinoma cells and normal cells

Cancer metabolism

Since the 1980s, various oncogenes and tumor suppressor genes have been discovered, and such genetic studies have become a focus of cancer research. In addition, these genes have been found to regulate cell metabolism; hence, the metabolic alterations caused by mutations have attracted attention as a common feature of cancer cells. In particular, energy production-related metabolic pathways, which support the infinite and rapid growth of cancer cells, are essential components of cancer biology.

Mammalian cells mainly produce ATP via oxidative phosphorylation under normoxic conditions. However, cancer cells increase their uptake of glucose and glycolysis and subsequently produce energy via a pathway that produces lactate, even in the presence of sufficient oxygen. This phenomenon is called the “Warburg effect” and is a well known metabolic characteristic of cancer cells.

What is hypoxia?

The environment surrounding cancer tissue is unique and is known to be hypoxic. In rapidly growing cancer tissue, oxygen consumption is increased, oxygen supply is decreased due to the abnormal blood vessel structures found in such tissue, the oxygen diffusion distance is increased, and blood flow is temporarily blocked, resulting in the development of a hypoxic region. However, the oxygen concentration within cancer tissue is expected to increase rapidly again when the oxygen supply is recovered by angiogenesis or hematogenous metastasis.

Outline of our experiment

We evaluated the effects of fluctuations in the environmental oxygen concentration on the metabolism of glucose in cancer cells using a real-time cell metabolism monitoring system(1.Morishima et al., 2017). Cancer cells were cultured under normoxic (in air) or hypoxic conditions (1% oxygen), and then the metabolic activity of these normoxically or hypoxically cultured cells was evaluated under normoxic or hypoxic conditions. We also tried to examine the metabolic regulatory mechanisms in operation in such cells by measuring the production of lactate.

Materials and methods

Cell lines used in this study

Cancer cell HSC-2, HSC-3 : Human oral squamous cell carcinoma cells

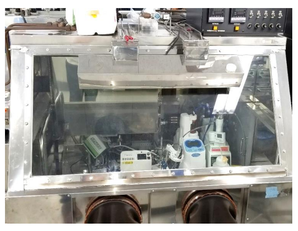
Normal cell HaCaT : Human immortalized keratinocyte cell derived from normal skin

Culture conditions

Culuture medium :E-MEM (for HSC-2, HSC-3) or D-MEM (for HaCaT) [with 2 mM L-alanyl-L-glutamine solution, 10% heat-inactivated fetal bovine serum, 100 µL/mL streptomycin, and 100 U/mL penicillin]
Conditions: 37^{O}C with humidified 21%O_2 or 1%O_2 and 5% CO_2. Sub-cultured every 3-4 days to maintain logarithmic growth and collected at 80-90% confluence.

Hypoxia experiments system

The pH-stat system used for the hypoxic experiments was placed in the hypoxic chamber, in which hypoxic conditions (oxygen concentration: 1%) were created by diluting and replacing the gas in the anaerobic glove chamber (ANB-180-P; Hirasawa, Tokyo, Japan) with nitrogen gas.



pH stat system in the anaerobic glove chamber

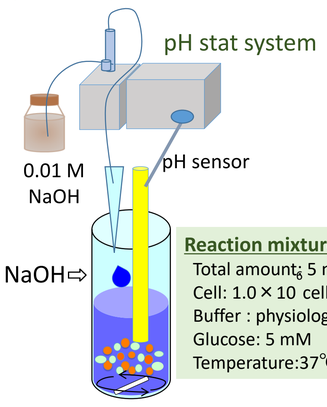
Real time monitoring of the acid-zproducing activity with pH-stat system > Figure 1

The cell suspension was mixed with 5 mM glucose as substrate at 37^{O}C and the acid production from glucose was monitored by pH-stat system (in Normoxia: AUTO pH STAT; model AUT-211S, TOA Electronics, Tokyo, Japan, In HypoxiaAT-710M pH-stat system; Kyoto Electronics Industry, Kyoto, Japan), using 0.01 M NaOH as a titrant. The metabolic activity was estimated from the amount of acid produced.

Analysis of acidic end-product levels > Figure 2

After being thawed, the stored supernatants were centrifuged again (at 1,000 g and 5℃ for 7 min), and the resultant supernatants were filtered through a polypropylene membrane (pore size: 0.20 µm; Toyo Roshi Ltd., Tokyo, Japan). The sample was analyzed by HPLC (high-performance liquid chromatography) (Shimadzu Prominence LC-20AD; Shimadzu Corporation, Kyoto, Japan) to determine the levels of acetic acid, lactic acid, formic acid, malic acid, fumaric acid, succinic acid, citric acid, α-ketoglutaric acid, oxalic acetic acid, and pyruvic acid.

[pH-stat system]



Reaction mixture
Total amount; 5 mL
Cell: 1.0×10 cells
Buffer : physiological saline
Glucose: 5 mM
Temperature:37℃

NaOH is automatically added to keep the setting pH (7.5 in this study), when the pH in reaction mixture is lowered by acid produced from substrates (glucose used in this study). The amount of NaOH added to the reaction mixture corresponds to that of acid produced by the cells.

Effects of the environmental oxygen concentration on ROS production > Figure 3

The amount of ROS produced during glucose metabolism was measured using the Cell Meter™ fluorescent intracellular ROS activity assay kit (Amplite™ ROS deep red; AAT Bioquest, Inc., Sunnyvale, CA, USA). Before the monitoring of metabolic activity, Amplite™ ROS deep red was added to the cell suspension, which was then pre-incubated at 37℃ for 20 min in normoxic or hypoxic conditions. Subsequently, glucose metabolism activity was monitored using a pH-stat system, as described above. The reaction mixture was collected before and after glucose metabolism and fluorescence intensity (excitation wavelength: 658 nm; measurement wavelength: 675 nm) was immediately measured using a fluorescence microplate reader.

Refferences:
1. Morishima H, et al. (2018). Real-time monitoring system for evaluating the acid-producing activity of oral squamous cell carcinoma cells at different environmental pH *Scientific Reports*

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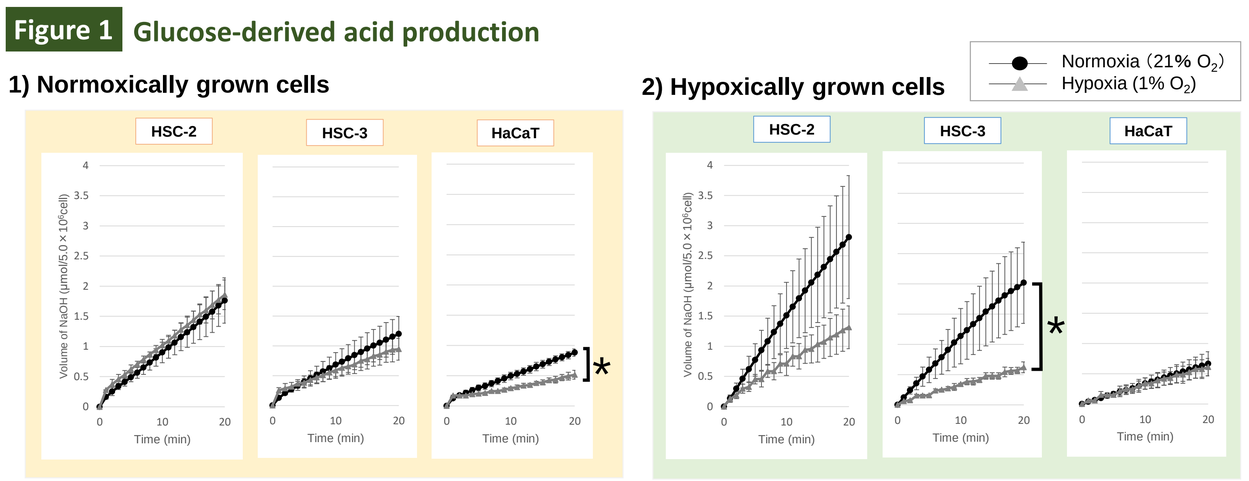
Results

Figure 1 Glucose-derived acid production

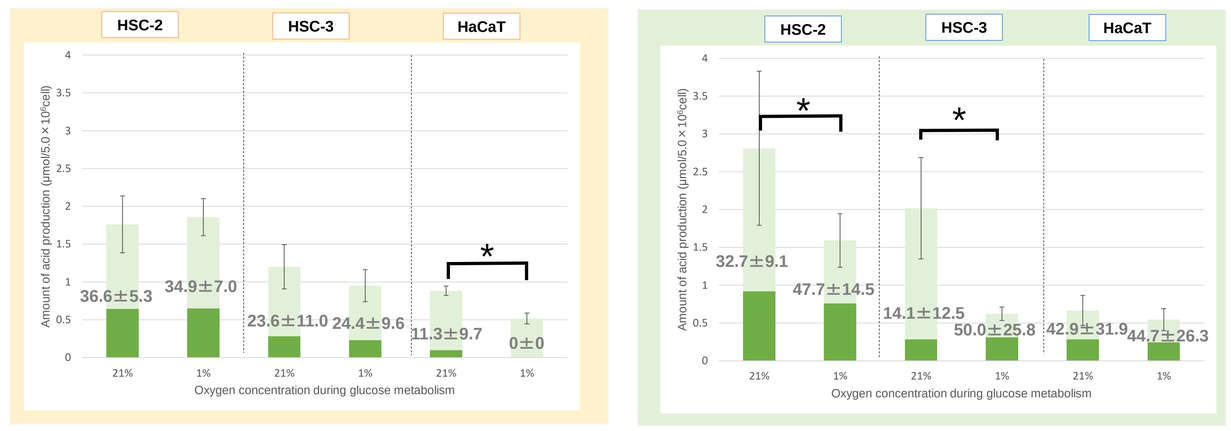


Glucose metabolism activity

1) Normoxically grown cells
HSC-2 and HSC-3 : No significant differences.
HaCaT: Significantly lower under hypoxic conditions. (0.54 to 0.63 times, p <0.05)

2) Hypoxically grown cells
HSC-2 and HSC-3: Higher metabolic activity under normoxic conditions.
(HSC-3: 2.02 to 4.79 times, p <0.05 HSC-2: 1.44 to 2.03 times, p=0.06)
HaCaT: No significant differences.

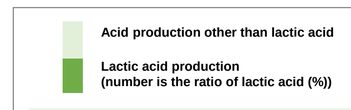
Figure 2 Amounts of lactic acid and other acids produced during glucose metabolism



The production of acidic end-products

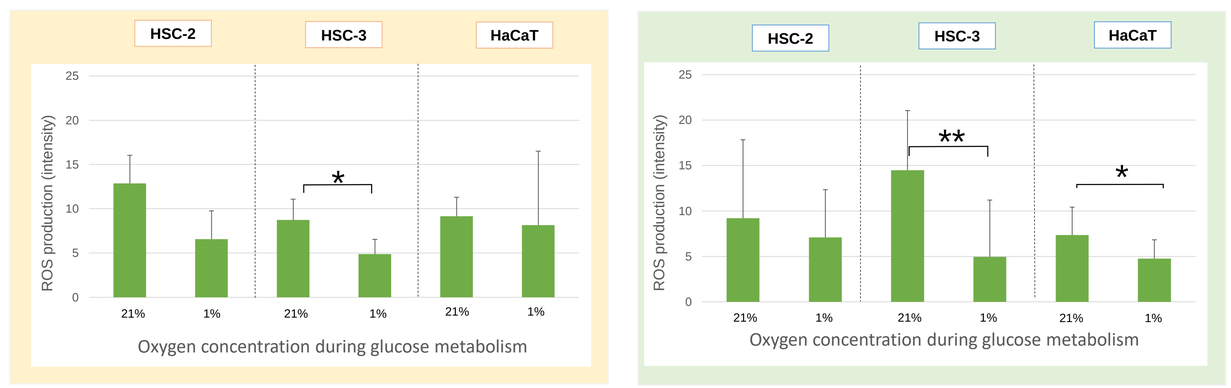
1) Normoxically grown cells
HSC-2 and HSC-3: High lactic acid ratios (23.5–36.6%)
⇒Not affected by the environmental oxygen concentration.
HaCaT: Low lactic acid ratio (11%)

2) Hypoxically grown cells
HSC-2 and HSC-3: Significant increases in their levels of acids other than lactic acid in normoxic conditions
(HSC-2: 2.31±0.89 times, p <0.05; HSC-3: 6.92±3.84 times, p <0.05).
The total acid production and lactic acid ratios of the HSC-2 and HSC-3 cells were similar to those of the normoxically cultured HSC-2 and HSC-3 cells.
HaCaT: Lactic acid ratio was about 50% both of oxygen concentrations.



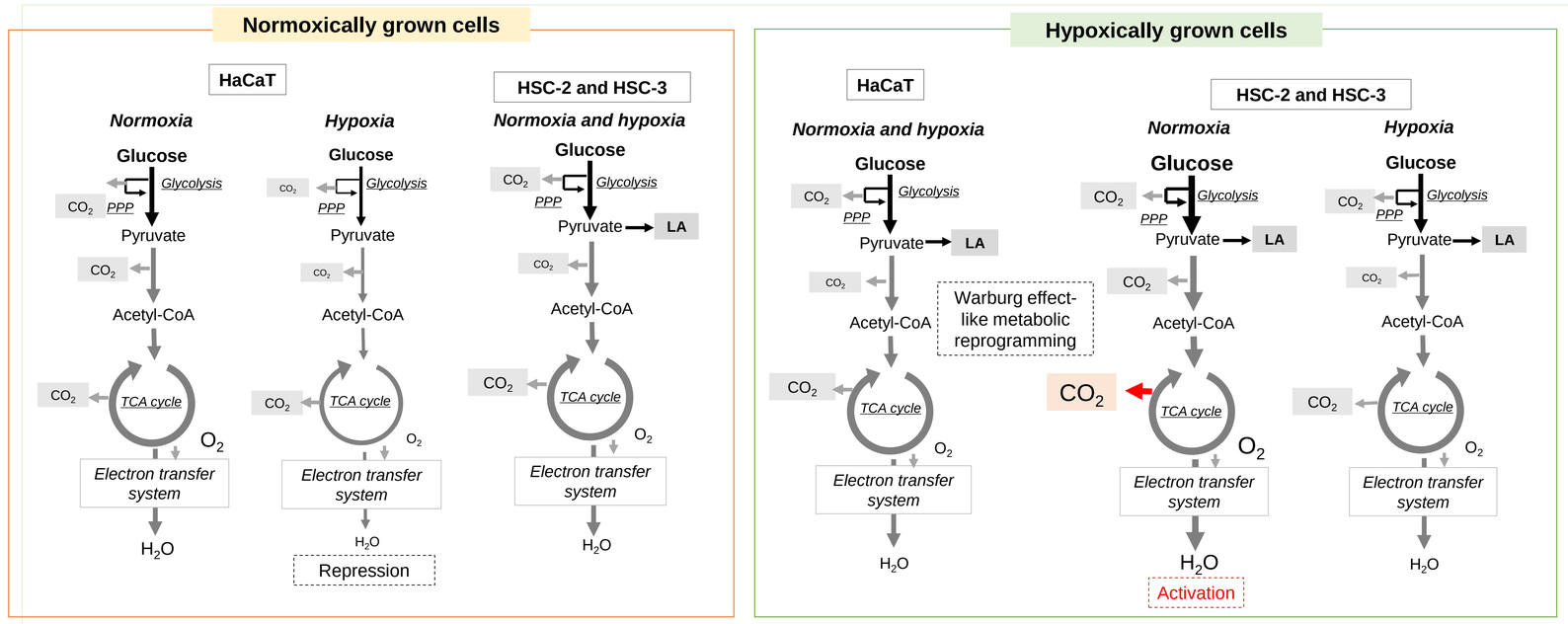
Lactic acid was the only acidic end-product detected with high-performance liquid chromatography (HPLC) analysis. The ratio of the amount of lactic acid, as measured by HPLC, to the total amount of acids, as measured by a pH-stat, was calculated (Fig. 2). Acids other than lactic acid were assumed to be carbonic acid derived from CO_2 produced by the TCA cycle.

Figure 3 Production of ROS during glucose metabolism



All the cells produced higher amounts of ROS during glucose metabolism in normoxic conditions, regardless of the culture conditions. In particular, the HSC-3 cells cultured in hypoxic conditions exhibited the greatest ROS production in normoxic conditions.

Discussion and Conclusion



LA: Lactic acid
PPP: Pentose phosphate pathway

Schematic diagram showing the metabolic effects of the environmental oxygen concentration revealed in the present study

The present study clearly demonstrated that rapid changes in the environmental oxygen concentration affect cellular glucose metabolism. Interestingly, only the hypoxically cultured cancer cells exhibited enhanced glucose metabolism, along with a metabolic shift from glycolytic to oxidative pathways in response to a rapid increase in the environmental oxygen concentration. This finding suggests that a rapid increase in the environmental oxygen concentration activates cancer cells by increasing the ATP supply through oxidative phosphorylation and signaling modifications induced by ROS production. A metabolic shift might be the first adaptive response to a rapid change in the environmental oxygen concentration and cell properties might subsequently be modified through the expression of various genes.