主 論 文 要 旨

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Energy management by enhanced glycolysis in G_1 phase in human colon cancer cells *in vivo* and *in vitro*

(*In vivo*および*in vitro*におけるヒト大腸がん細胞のG_1期解糖系亢進によるエネルギー維持)

(内容の要旨)

Activation of aerobic glycolysis in cancer cells is well-known as Warburg effect, although its relation to cell cycle progression remains unknown. In this study, human colon cancer HCT116 cells were labeled with a cell cycle phase-dependent fluorescent marker Fucci to distinguish between cells in G1 phase and those in S + G2-M phases. In order to investigate coordination between energy metabolism and cell cycle progression of cancer cells *in vivo*, I have utilized the Fucci-labeled HCT116 cells to provide a liver metastasis model in super-immunodeficient NOG mice. The combination of the Fucci-system, the mouse model for metastasis, and newly developed atrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) with high spatial resolution enabled us to reveal spatial distributions and local concentrations of various metabolites in the cancer cells in different cell cycle phases *in vivo*. Measurement points corresponding to tumor cells with Fucci-green fluorescence (S + G2-M phases) and those with Fucci-red fluorescence (G1 phase) were assigned precisely using built-in fluorescence image capturing system. Ion density images from MALDI-IMS displayed in situ distributions of 10 metabolites involved in energy and redox metabolism. I found that relative concentrations of ATP, UTP, NADH, UDP-N-acetylglucosamine (UDP-GlcNAc), and GSSG in cells in G1 phase were significantly greater than those in S + G2-M phases in the foci, suggesting that the cells exhibited cell cycle phase-dependent energy metabolism, possibly executing glycolysis more actively in G1 phase than in the other phases.

To understand cell cycle-dependent changes in energy metabolism clearly, I synchronized HCT116 cells in S, G2-M, and G1 phases by using the double thymidine block method. The detected fluorescence introduced by Fucci system was consistent with the results for the DNA contents of the cells analyzed by flow cytometry. The cell numbers, cell volumes, and protein amounts of the synchronized populations, which were derived from the same numbers of cells inoculated prior to thymidine treatment, were determined. The cell numbers unchanged from S to G2-M phase, and then increased twice in G1 phase. The volumes of individual cells reached the maximum in G2-M phase and showed a dramatic decrease in G1 phase. The total protein amounts of collected cells in different phases tended to increase gradually.

Since p53 is a key regulator of glycolysis as well as of cell-cycle progression, I also collected data by employing isogenic HCT116 cells with p53-null mutation. Both WT and p53-null cells were cultured at 3g/L glucose and at a euglycemic level (1g/L glucose). The same experimental protocol for synchronization could be applied to all sets of conditions as judged by DNA contents.

To know whether cells under conditions in culture exhibited cell cycle phase-dependent metabolic changes similar to those seen *in vivo*, I used synchronized cells in S, G2-M, and G1 phases to determine cellular concentrations of the same set of the metabolites. Results show that concentrations of ATP, GSH, and GSSG were higher in G1 phase than in the other phases significantly. In order to obtain comprehensive information on changes in energy metabolism in cell cycle progression, I also measured various metabolites in glycolysis, TCA cycle, and pentose phosphate pathway (PPP) by employing capillary electrophoresis/electrospray ionization/mass spectrometry (CE/ESI/MS). The cellular amounts of the metabolites in each of the three pathways were almost unchanged in the cell cycle progress. For analyses of metabolic flux, cells were labeled with medium containing ^{13}C6 -labeled glucose for 5min. Amounts of ^{13}C-labeled metabolites (at least one carbon atom was labeled) showed that the metabolites in glycolytic pathway were labeled more quickly than those in TCA cycle and in PPP in the cell cycle progression. Because lactate was the predominant metabolite which is released from cells as a result of glycolysis, I examined amounts of extracellular lactate and extra cellular acidification rate (ECAR) in the progress of cell cycle. Accumulation of extracellular lactate and release rates of lactate significantly increased in G1 phase in HCT116 WT cells under both 1g/L and 3g/L of glucose, suggesting that glycolysis is markedly accelerated in G1 phase to greater extents than in the other phases. The increase in accumulation of extracellular lactate in wild type cells was halted in G2-M phase. On the other hand, the extracellular lactate in p53-null cells at 3g/L glucose was being accumulated with almost linear pattern throughout the cell cycle progression. Furthermore, when the releasing rates, which were normalized by cell numbers, were compared among the three sets of the data, p53-null mutation might activate glycolysis. Extracellular acidification rate (ECAR) was also significantly increased in G1 phase in HCT116 WT cells cultured with both 1g/L and 3g/L of glucose and in HCT116 p53-null cells with 1g/L glucose.

I measured activities of phosphofucto-1-kinase (PFK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and pyruvate kinase (PK) to investigate possible causes of the changes in glycolytic activities throughout cell cycle phases. The activities of the three enzymes in S, G2-M, and G1 phases gave similar patterns to one another; for all of the three enzymes the activities in S and G1 phases were similar to each other and higher than those in G2-M phase. These results may explain in part changes in glycolytic rates and ECAR in the cell cycle progression.

I subsequently examined how mitochondrial oxidative phosphorylation activity changed in the cell cycle progression and found that cells in G2-M phase exhibited the highest dependence of energy production on mitochondrial respiration. The fraction of mitochondrial ATP production were the highest values in G2-M phase together with the greatest levels of cellular reactive oxygen species (ROS), a by-product from the electron transfer. The highest inner mitochondrial membrane potential in G1 phase, however, implied that the potential was not so efficiently consumed to produce ATP in G1 phase as in S and G2-M phases.

In the present study, several lines of evidence indicated cell cycle phase-dependent changes in energy metabolism in culture. First, the energy production in G1 phase highly depended on glycolysis with only a small fraction of mitochondrial respiration. Second, the G2-M phase exhibited the highest dependence of energy production on mitochondrial respiration. Third, the energy metabolism in S phase mostly exhibited an intermediary between G1 and G2-M phases. Thus, as the cell cycle progressed from G2-M to G1 phases, the dependency of energy production on glycolysis in the cancer cells was drastically increased while mitochondrial contribution was reciprocally decreased. These findings highlight a possibility that cell cycle-specific metabolic pathways might serve as a new target of anti-cancer treatment.