論文審査の要旨及び担当者

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（論文審査の要旨）

論文題名：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）

This study examined correlation between energy metabolism and cell-cycle progression in a human colon cancer cell line HCT116. A cell cycle phase-dependent fluorescent marker Fucci was introduced into HCT116 cells to distinguish between cells in G_1 phase and those in S + G_2-M phases. Results suggested that as the cell cycle progressed from G_2-M to G_1 phases, the dependence of energy production on glycolysis in the cancer cells was increased, whereas the mitochondrial energy production was reciprocally decreased.

In the defense of the thesis, duration of each cell-cycle phases and the time points for collecting cell samples at specific cell-cycle phases were inquired. The applicant answered that results of analyses for DNA contents in the cancer cells undergoing double-thymidine block treatment suggested that S phase continued for three to four hours, G_2-M phase lasted about one hour, and G_1 phase lasted more than ten hours in HCT116 cells. According to these data, collected cell samples at the time points of three, seven, and 12 hours after releasing procedure of the thymidine blockade to ensure that at least greater than 80% of cells exhibited the same phase in cell-cycle progression as judged by Fucci fluorescence *in vitro*. Secondly, the question raised was why the model of splenic xenograft transplants in NOG mice was used in this study and how cancer cells and blood vessels were differentiated in foci microscopically. The applicant answered as follows: the model chosen for the current study is known to display hepatic metastasis through portal vein with high reproducibility. Another important beneficial point for the choice of the experimental model is a feasibility to examine metabolic interactions between the metastatic cancer and host tissues such as liver, where carbohydrates and/or amino acids are enriched. Regarding the latter question, the applicant answered that Fucci-HCT116 cancer cells can be distinguished from the host-derived cells including hepatocytes and blood vessels through high-magnification intravital microscopy or by immunohistochemistry. A question was raised regarding fundamental differences of metabolic systems between cancer cells and normal cells. The applicant answered that similar to cancer cells, proliferating normal cells are also known to possess higher activity of glycolysis in G_1 phase than in other phases, and that the current results collected by imaging mass spectrometry suggested that concentrations of ATP, UTP, and UDP-GlcNAc were extremely higher in cancer cells irrespective of cell-cycle phase than in hepatocytes. The applicant showed unpublished data indicating that HCT116 cells under G_1 phase utilizes varied free amino acids to synthesize reduced and oxidized glutathione and to thereby recover from oxidative stress that appears to be dominant in G_2-M phase.

Since the main data in this work were collected from HCT116 wild-type and p53-null cells, it was inquired by a referee whether application of the notion to other types of cancer cells was feasible. The applicant answered to this question by showing that efflux of lactate indicated a significant peak in G_1 phase in HeLa cells *in vitro*, whereas imaging of lactate in tumors *in vivo* has not been examined yet. Results of extracellular acidification rates or lactate in this work were also explained. The data showing an increase in effluxes of lactate after ^{13}C-glucose challenge support a notion that G_1- cancer cells were supported mainly by glycolysis. Subsequently, it was discussed as to what molecules might regulate metabolic changes in cell-cycle progression. Although there are many candidates such as Wnt signal do exsit, many studies suggested that p53 plays a key role in regulation of both energy metabolism and cell-cycle progression. The current study actually showed that p53 was important for suppression of glycolysis, especially in G_2-M phase. The referees pointed out that determination of amounts and activities of p53 in specific cell-cycle phases should be necessary for future study. Finally, according to the referees' suggestions, the conflict between low ATP production in mitochondria (mtATP) and high mitochondrial inner membrane potential (ψ_{mt}) in G_1 phase were discussed. The applicant speculated that most of pyruvate was converted to lactate, while only a small fraction entered TCA cycle in G_1 phase. Furthermore, NADH could be generated through up-regulated glutaminolysis to maintain a high ψ_{mt} in G_1 phase.

As described above, although some improvements are necessary for future studies, mechanism of cell-cycle dependent changes in energy metabolism should be clarified further. All referees agreed that results of the current study *in vivo* and *in vitro* are important to further understand cell-cycle specific energy metabolism, being helpful for developing cancer therapies in which conventional anti-cancer reagents that make cancer cells stop in G_2-M phase may be used in combination with those enhancing oxidative stress.