Cell Cycle Dependency of [18F]FDG Uptake by Cancer Cells in Vivo

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Introduction

2-Deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) has been shown to be a useful radiotracer for tumor detection by positron emission tomography (PET). Our recent in vivo autoradiographic study[1] has demonstrated higher accumulation of [^{18}F]FDG in the macrophages and young granulation tissues than the tumor cells. Quantitative microautoradiographic studies revealed that the intratumoral neoplastic and non-neoplastic cellular components have different glucose uptake patterns in the same tumor tissue[2]. In this study, we demonstrate the cell cycle-dependency of [^{18}F]FDG uptake by cancer cells in vivo by double tracer microautoradiography.

Materials and Methods

The study protocol with animals was approved by the laboratory animal care and use committee of Tohoku University.

Ten week-old C3H/He female mice were injected subcutaneously with a 0.1-ml suspension of 10^7 syngeneic FM3A mammary carcinoma cells on their left thighs and 10^6 syngeneic MH134 hepatoma cells on their right thighs. Tracer experiments were performed 10 days after tumor transplantation.

Seven C3H mice bearing both FM3A and MH134 tumors were intravenously injected with a mixture of 1 mCi (37 MBq) of [^{18}F]FDG and 30 μCi (1.11 MBq) of [6-^{3}H]thymidine ([^{3}H]Thd) in 0.2 ml and killed 1 hr later. The tumors were quickly dissected and prepared for frozen sectioning. Several 3.5 μm- thick sections were processed for the microautoradiography using NTB2 nuclear emulsion[1] just after the tracer experiment for [^{18}F]FDG. Three days after, following the [^{18}F]FDG microautoradiography, the second autoradiography was performed using ET2F stripping film[3]. Under a safety light, the slides with the tissue sections and the first autoradiogram were covered with ET2F stripping film and the film coated slides were then exposed for 3 weeks. After the exposure, the sections

were developed, fixed, and stained with hematoxyline and eosin. The cells in the DNA synthesis phase (S phase) of cell cycle are labeled with [^{3}H]Thd. The [^{3}H]Thd labeled cells were identified by focusing a transmitted light brightfield microscope on the upper emulsion layer of ET2F film. The tumor cells were divided into S phase and non-S phase cells by the [^{3}H]Thd labeling. Grain counts of [^{18}F]FDG autoradiogram were obtained by focusing the microscope on the lower emulsion layer (NTB2) using a micrometer.

Results

Figure 1 shows a pair of double-tracer micro-ARG of cell proliferating area in MH134 tumor 1 hr after injection of a mixture of [^{18}F]FDG and [^{3}H]Thd. It is possible to compare the distribution of S and non-S phase cells visually and the grain levels of [^{18}F]FDG in the same area. S phase cells seem to have less grain density than the non-S phase cells. Table 1 represents the cellular [^{18}F]FDG uptake in the cells in S phase, mitosis (late metaphase-anaphase), and non-S/non-M phase selected by [^{3}H]Thd labeling and mitosis. [^{18}F]FDG uptake was the highest ($p<0.001$) in non-S/non-M phase cells. The uptake was the lowest ($p<0.001$) in mitotic cells.

Discussion

In the 1950s and 60s, the principal process providing energy for mitosis was thought to be primarily by glucose oxidation. The rate of glucose oxidation was related to an activation of the enzymes of the hexose monophosphate shunt[4,5]. These studies also proposed the concepts of the cell cycle dependency of the rate of glucose oxidation, which increased in the period between divisions and decreased during mitoses. Recent flow cytometric analysis enabled the precise investigations of various events depending on each cell cycle phase not only mitosis. Stimulation of glucose transport is one of the early events that occur prior to the reinitiation of DNA synthesis in quiescent cells[6,7]. In proliferative thymocytes, concomitant increases were observed in the rates of DNA synthesis and glycolysis. However, the maximal increment in the glycolytic rate preceded that of DNA synthesis[8]. Insulin binding sites were the greatest in G_2+M phases followed by G_0/G_1, and the lowest in S[9]. These studies support our result that higher [^{18}F]FDG uptake was observed in the cells in non-S/non-M (late metaphase to anaphase) phases; therefore, G_0/G_1 and G_2/M-partial. The cell cycle is the set of events that is responsible for the duplication of the cell[10]. Cycling cells may produce the energy for S phase during the G_0/G_1 and the energy for M phase during the G_2 phase of cell cycle.

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Table 1. Cell cycle dependency of [^{18}F]FDG uptake and cell density of each tumor.

	Number of grains / cell			Cell density
	S phase	M phase	non-S/non-M phase	(cells/10^4 μm^2)
FM3A	3.37±0.71	1.84±0.80	5.48±1.12	48.9± 5.4
MH134	5.97±2.10	1.53±1.33	8.70±1.79	57.4±11.0

Mean±S.D. Grain numbers per cell.

S phase: [^{3}H]Thd labeled cells.

M phase: histologically confirmed mitotic cells in late metaphase to anaphase.

non-S/non-M phase: cells except S and M phase.

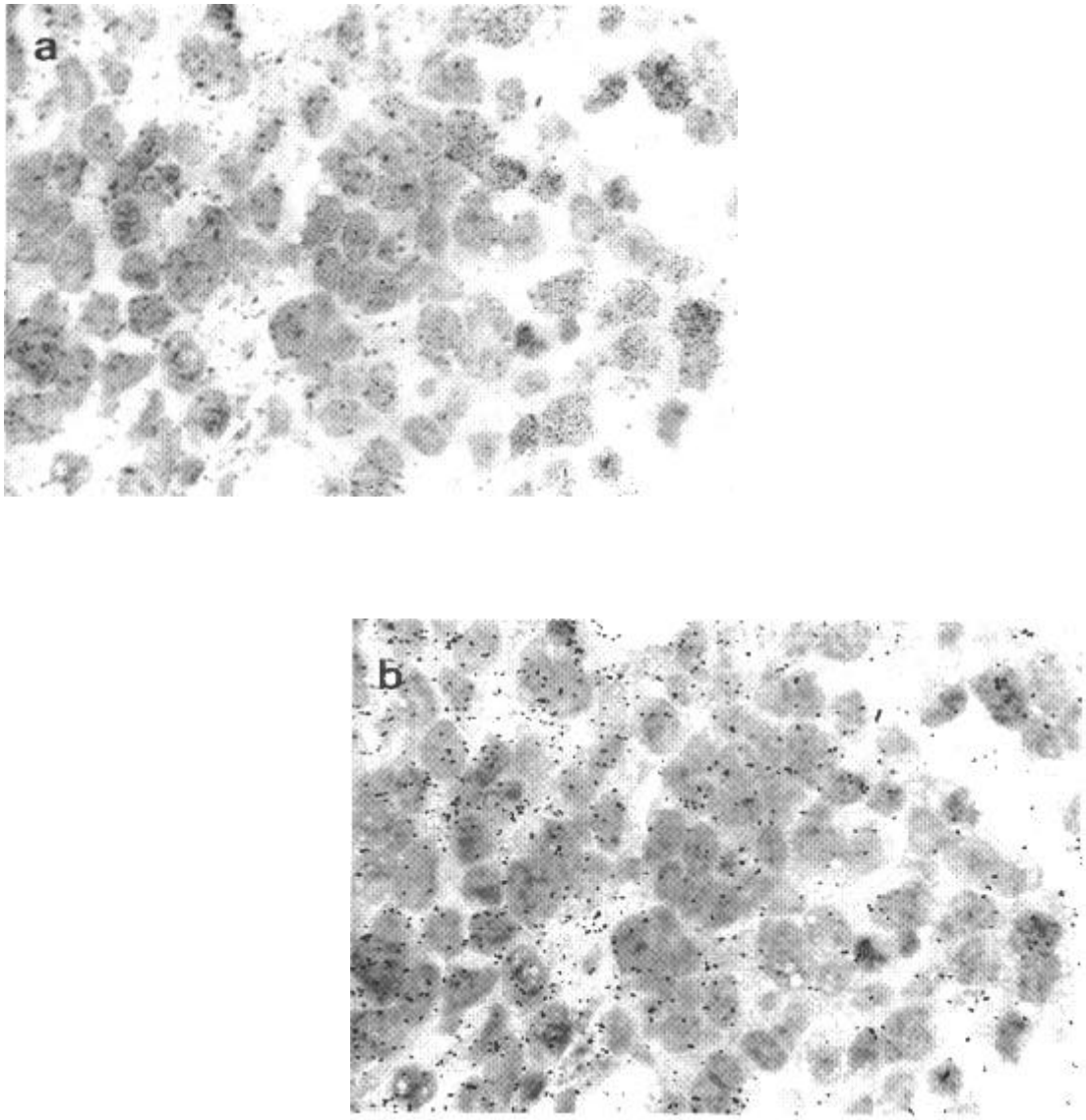

Fig. 1. A pair of double-tracer microautoradiograms with [^{18}F]FDG and [^{3}H]Thd. a: [^{18}F]FDG and b: [^{3}H]Thd in MH134. [^{18}F]FDG autoradiogram was focused on the lower emulsion (NTB2) layer. [^{3}H]Thd autoradiogram was focused on the upper emulsion (ET2F stripping film) layer of the same area.