사람 암세포와 단핵세포에서 고포도당 농도에 의한 FDG 섭취 저하의 서로 다른 기전

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Decreased glucose uptake by hyperglycemia is regulated by different mechanisms in human cancer cells and monocytes

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Abstract

To clarify the difference in glucose uptake between human cancer cells and monocytes, we studied [^{18}F] fluorodeoxyglucose (FDG) uptake in three human colon cancer cell lines (SNU-C2A, SNU-C4, SNU-C5), one human lung cancer cell line (NCI-H522), and human peripheral blood monocytes. The FDG uptake of both cancer cells and monocytes was increased in glucose-free medium, but decreased in the medium containing 16.7 mM glucose (hyperglycemic). The level of Glut1 mRNA decreased in human colon cancer cells and NCI-H522 under hyperglycemic condition. Glut1 protein expression was also decreased in the four human cancer cell lines under hyperglycemic condition, whereas it was consistently undetectable in monocytes. SNU-C2A, SNU-C4 and NCI-H522 showed a similar level of hexokinase activity (7.5 - 10.8 mU/mg), while SNU-C5 and monocytes showed lower range of hexokinase activity (4.3 - 6.5 mU/mg). These data suggest that glucose uptake is regulated by different mechanisms in human cancer cells and monocytes. **(Korean J Nucl Med 2002;36:110-120)**

key words : Inflammatory cells, cancer, glucose, F-18-fluorodeoxyglucose, hyperglycemia, glucose transporter

Introduction

Glucose uptake and glycolysis are generally increased in cancer cells.[1-4] Positron emission tomography (PET) study using F-18-fluorodeoxyglucose (FDG), an analogue of 2-deoxy-D-glucose, has been used for the detection of primary cancer and its metastasis. This increased glucose uptake, however, has also been found in active inflammation and injured tissue[1,5] due to activated macrophages.[6-8] These findings sometimes cause considerable

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confusion in PET tumor interpretation using FDG.

A variety of mechanisms have been proposed to explain the increased glucose use seen in cancer cells and inflammatory cells.[9-11] Enhanced rates of glucose uptake, increased hexokinase concentration and increased glucose transporter expression have been reported.[2,3,6,12] Enhanced FDG uptake is closely associated with increased expression of the facilitative glucose transporter (Glut).[10,13-15] Glut is a cellular membrane protein and shows a tissue-specific distribution consisting of Glut1 (erythrocyte), Glut2 (liver), Glut3 (brain), Glut4 (muscle-fat) and Glut5 (small intestine). Increased expression of Glut isoforms was reported in a variety of cancers. Increased levels have been reported for Glut1 and Glut3 in head and neck tumors.[16] Glut1, Glut2 and Glut4 in human breast cancer[10], Glut1 and Glut3 in central nerve system (CNS) tumors and glioblastoma multiforms[17], and Glut1, Glut2 and Glut3 in gastrointestinal cancer and pancreatic cancer. Increased Glut1 and decreased Glut2 levels in hepatoma were also reported.[18,19] Glut expressions in inflammatory cells were also changed. Expressions of Glut1, Glut3, and Glut5 were reported in macrophages.[5,7] During the differentiation from human monocytes into macrophages, a progressive increase of Glut1, rapid decrease of Glut3, and delayed increase of Glut5 were detected.[20] However, it has been accepted that *Glut1 is the most important glucose transporter in* cancer cells and macrophages.[7,12,21]

Glucose metabolism in many cells changes according to the glucose concentration. FDG uptake in tumor cells is decreased in hyperglycemia[4,22], but little is known concerning the effect of hyperglycemia on inflammatory cells. In this study, we tried to define the difference between tumor cells and inflammatory cells in terms of glucose uptake and their respective mechanisms. We determined the effect of glucose concentration on FDG uptake, Glut1 expression and hexokinase activity in both human cancer cells and monocytes.

MATERIALS AND METHODS

Reagents

RPMI 1640 medium and fetal calf serum (FCS) were purchased from Gibco (Grand Island, NY). Rabbit anti-Glut1 antibody was purchased from East Acres Biologicals (Southbridge, MA) and peroxidase-linked anti-rabbit antibody was purchased from Amersham (Buckinghamshire, England). All other chemicals were purchased from Sigma (St. Louis, MO) unless otherwise stated.

Cancer cell culture

Human colon carcinoma cells, SNU-C2A, SNU-C4 and SNU-C5 (24), as well as human lung adenocarcinoma NCI-H522, were obtained from the Korean Cell Line Bank. Cells were grown in RPMI 1640 medium containing 10% FCS, 100 U/ml penicillin and 100 (g/ml streptomycin at 37℃ in a 5% CO_2 incubator.

Preparation of human peripheral blood monocytes

Human peripheral blood monocytes (hMP) were isolated from the blood of healthy donors, kindly provided by a blood bank at Seoul National University Hospital (Korea). The blood was layered on the top of Hitopaque 1077 and Hitopaque 1119 gradient, and centrifuged at 700 × g for 30 min at room temperature. The mononuclear layer was transferred to a new tube, washed three times in 10 ml of phosphate buffered saline (PBS), and centrifuged at 200 × g for 10 min. Red blood cells were lysed with Tris-buffered ammonium chloride (pH 7.2) and washed in 10 ml PBS. Monocytes were incubated for 4 h in RPMI 1640 medium at 37℃ in a 5% CO_2 incubator. After adherence, supernatants were discarded and the adherent cells were used for the experiments. Stimulation of monocytes was performed by the addition of a final 10 μg/ml of lipopolysaccharide (LPS; Difco, Detroit, MI).

Measurement of FDG uptake

Glucose uptake was measured as reported previously.[12] Briefly, cancer cells and monocytes were suspended in RPMI 1640 containing various concentrations of glucose (0 mM, 5 mM, and 16.7 mM), placed 48 well plates for either 4 h or 24 h, and washed with glucose free HEPES-buffered saline (HBS; 140 mM NaCl, 1 mM $CaCl_2$, 5 mM KCl, 2.5 mM $MgSO_4$ and 20 mM HEPES, pH 7.4). After incubation, wells were filled with 500 μl of HBS containing 1 μCi/ml of F-18-FDG and further incubated for 1 h at 37℃. FDG uptake was determined after washing three times with HBS. The cells were solublized in 500 μl of 1% sodium dodecyl sulfate (SDS), and the radioactivity incorporated into the cells was measured using a Packard gamma counter (Cobra II, Downers Grove, IL). Cellular protein was measured by a modified Lowry method using bovine serum albumin as a standard.

Northern blot analysis of Glut1

Total RNA was isolated from cells cultured in various glucose concentrations using an Ultraspec-II RNA kit (Biotecx Laboratory, Houston, TX). Twenty micrograms of total RNA were denatured with formaldehyde and electrophoresed on 1.2% formaldehyde agarose gel. The gel was blotted onto a nylon membrane filter (Amersham, Arlington Heights, IL) for 20 h and washed with 6 × standard saline citrate (SCC) for 5 min. The blot was hybridized to 864 base pair fragment (2×10^6 cpm/ml ^{32}P-labeled Glut1 probe; ATCC59630) in hybridization solution (10 × Denhardt solution, 5 × saline-sodium phosphate-EDTA buffer (SSPE), 1% SDS and 100 μg/ml salmon sperm deoxyribonucleic acid) at 42℃ for 20 h. The blot was washed with 2 × SSPE containing 0.1% SDS for 10 min at room temperature and washed two more times with 1 × SSPE containing 0.1% SDS for 15 min at 65℃. The filter was autoradiographed using AGFA Scofix MFC (AGFA, Belgium) at -70℃. The fragment of Glut1 cDNA used as a probe was that which encoded the portion of the transporter beginning at the large cytoplasmic loop and ending at the C-terminus of the protein. Reverse transcription-polymerase chain reaction (RT-PCR) was performed according to the manufacturer's instruction (Promega; Madison, WI) with Glut1-specific primers (sense, TACCCTGGATGTCCTATCTG; antisense, CACAC AGTTGCTCCACATAC), corresponding to nucleotides 1261 - 1281 and 1449 - 1469 of the human Glut1 cDNA sequence.

Western blot analysis of Glut1

Cancer cells and monocytes were suspended in RPMI 1640 containing 10% FCS and incubated for 4 h at 37℃ in 5% CO_2 incubator. The cells were washed with PBS and medium containing various concentrations of glucose was added. After incubation for either 4 h or 24 h at 37℃ in 5% CO_2 incubator, the cells were lysed with lysis buffer (20 mM Tris, pH 8.0, 137 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM Na_3VO_4, 2 mM EDTA, 1 mM PMSF, 20 μM leupeptin, and 0.15 units/ml aprotinin). Cell lysates were centrifuged for 10 min at 400 × g to remove nuclei and unbroken cells. The supernatant was clarified by centrifuging for 10 min at 2500 × g, and the supernatant product from this centrifugation was further centrifuged for 10 min at 28500 × g. The resultant centrifugation pellet was suspended in PBS. Next, samples were heated at 95℃ for 5 min with 0.2 volume of 5 x Laemmli sample buffer and then 20 μg of the cell lysates was resolved on 10% SDS-PAGE. The gels were transferred onto nitrocellulose membrane (Schleicher & Schuell, Keene, NH) and maintained overnight. Blots were incubated with rabbit anti-Glut1 antibody (1:2500 dilution; Charles River Pharmservices, MA) for 1 h and washed. Antibodies were detected with peroxidase-linked anti-rabbit antibody (Amersham), and developed with the ECL method (Amersham) according to the manufacturer's instructions.

Hexokinase activity

Increased FDG uptake is generally related to increased levels of hexokinase in vitro, although there is no convincing evidence in vivo.[2,23] Hexokinase activity was measured as reported previously.[12,24] Briefly, cells (1×10^8 cell/ml) were homogenized in 50 mM Tris-HCl (pH 7.4) containing 0.15 M KCl, 5 mM β-mercaptoethanol and 1 mM EDTA, and centrifuged at 100,000 $\times$ g for 30 min. Fifty micro liters of supernatant was incubated for 10 min at 37℃ in 20 mM Tris buffer (pH 7.6) containing 220 mM D-glucose, 0.75 mM adenosine triphosphate, 8 mM $MgCl_2$, 1.12 mM β-nicotinamide adenine dinucleotide phosphate, and 20 μg of glucose-6-phosphate dehydrogenase (125 units/ml) in a total volume of 2.5 ml. The increase in absorbance at 340 nm was measured using a spectrophotometer. One unit of activity catalyzed the formation of 1 μmol glucose-6-phosphate per minute. The controls which contained the aforementioned medium, except either glucose or the cell lysates, were corrected for any reduction of oxidized nicotinamide adenine dinucleotide phosphate not caused by hexokinase. Bovine serum albumin was used as a standard for protein assay measured by a modified Lowry method.

Statistical analysis

The statistical significance of differences between experimental groups was analyzed using Student's t-test, and data were expressed as the mean $\pm$ SD. Figures were plotted using Sigmaplot 4.0 (Sigma, MO).

RESULTS

Decreased FDG uptake under hyperglycemic condition

The glucose uptake of human cancer cells and peripheral blood monocytes was determined at glucose free (0 mM), normal (5.5 mM) and high (16.7 mM) glucose concentrations. Among the tested cell lines, SNU-C5 showed the highest FDG uptake (Fig. 1). Increased glucose concentration

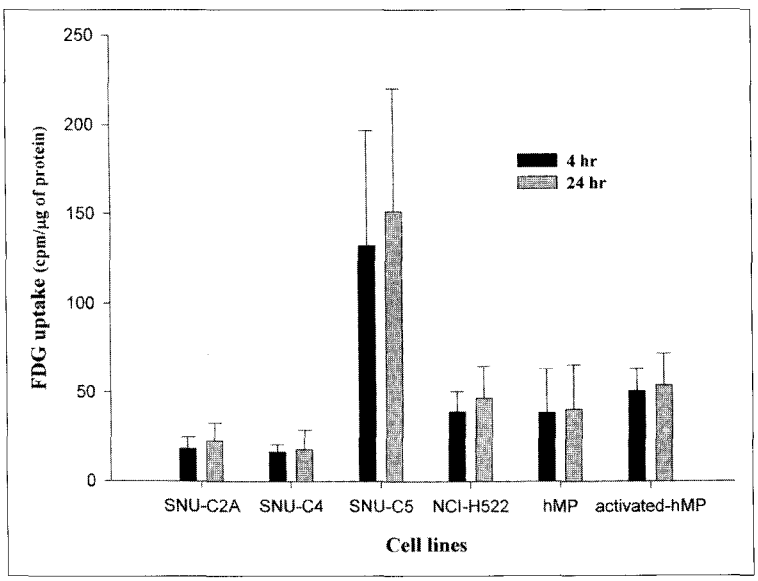

Fig. 1. FDG uptake in human cancer cells and peripheral blood monocytes (hMP) after 4 h and 24 h incubation in media containing 5.5 mM glucose. Each assay was performed four times and the result represents the mean±SD of the four separate experiments, except NCI-H522 (n=2).

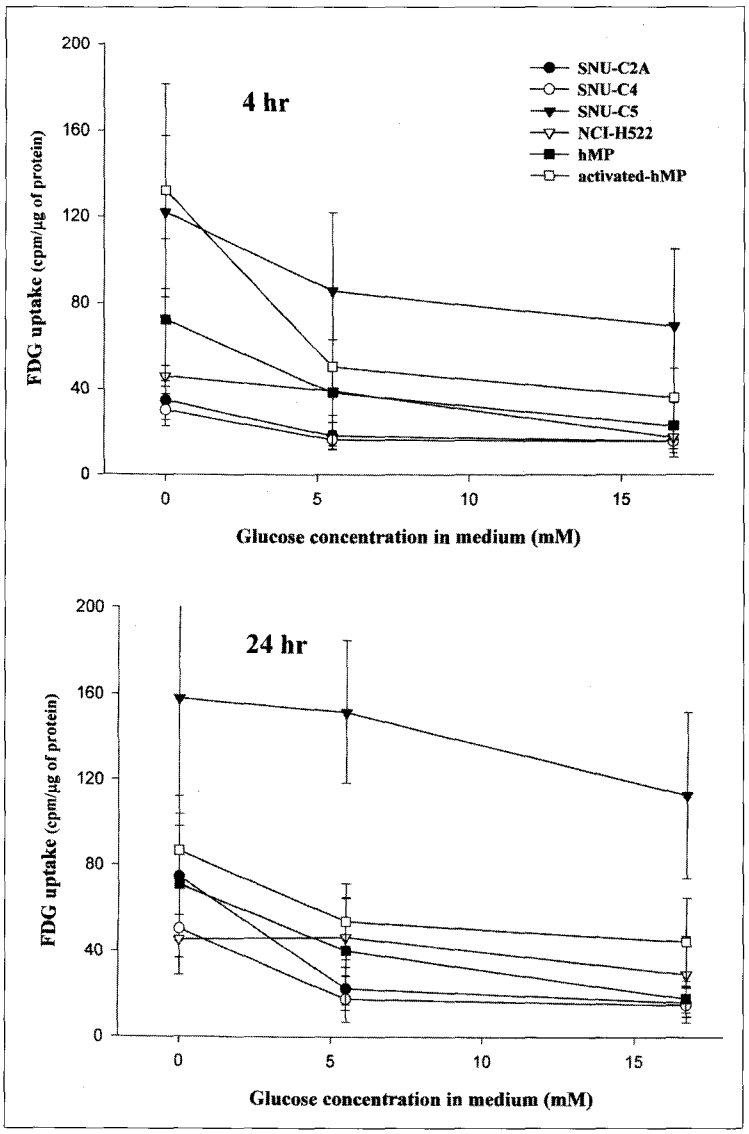

Fig. 2. FDG uptake in human cancer cells and peripheral blood monocytes (hMP), after 4 h (upper panel) and 24 h (lower panel) incubation, in media containing various concentrations of glucose (n=5).

reduced FDG uptake in both human cancer cells and monocytes after 4 h and 24 h incubation (Fig. 2).

FDG uptake was higher in glucose free media at 4 h and 24 h in all the cancer cell lines except NCI-H522. However, increased FDG uptake by SNU-C2A and SNU-C4 was not significant as the glucose concentrations changed from 5.5 mM to 16.7 mM. FDG uptake at 16.7 mM glucose was 72.6 ± 12.1% (SNU-C2A), 86.2 ± 4.1% (SNU-C4), 67.0 ± 6.7% (SNU-C5), 49.0 ± 22.2% (NCI-H522), 49.8 ± 10.4% (hMP) and 67.9 ± 13.5% (activated-hMP) of the uptake in glucose free medium at 24h (Fig. 3). Although under hyperglycemic condition the decrease was sustained for 24h incubation, the longer incubation did not significantly alter FDG uptake.

Expression of Glut1 mRNA

Northern blot analysis with Glut1 cDNA probe

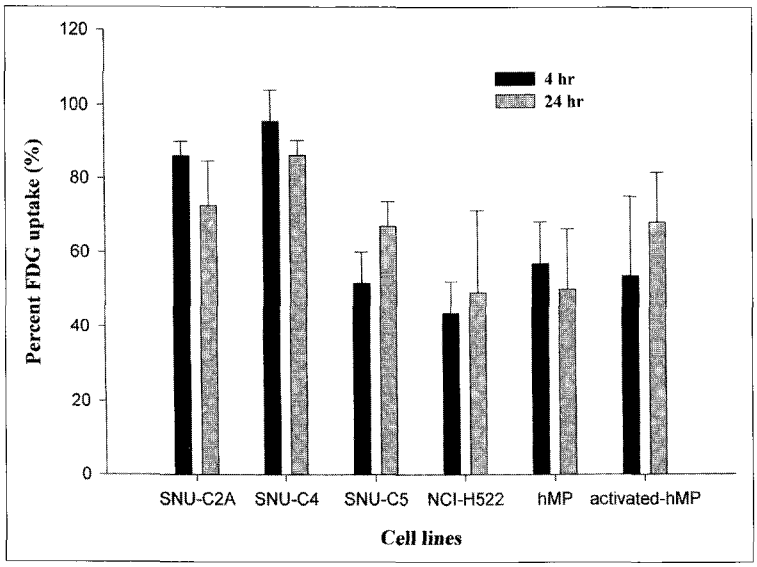

Fig. 3. Percent FDG uptake in human cancer cells and peripheral blood monocytes (hMP) after 4 h and 24 h incubation under hyperglycemic condition (16.7 mM).

revealed that Glut1 mRNA was expressed in human colon cancer cells and NCI-H522, while it was negligibly expressed in monocytes under normoglycemic condition. The Glut1 mRNA level in all four cancer cell lines was decreased in hyperglycemia, whereas it remained negligible in monocytes (Fig. 4). Northern blot data were consistent with the result from RT-PCR analysis (data not shown).

Effect of hyperglycemia on Glut1 expression

In normoglycemia, SNU-C5 exhibited the highest Glut1 expression among the human colon cancer cells (SNU-C5 > SNU-C2A ≅ SNU-C4), monocytes

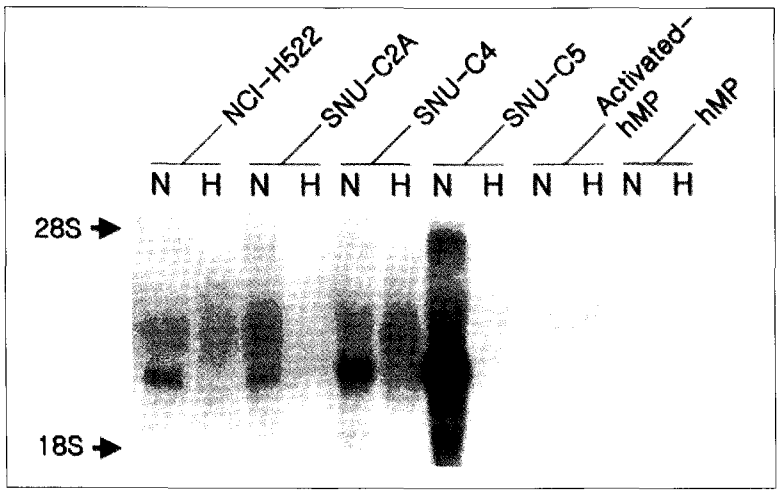

Fig. 4. Expression of Glut1 mRNA. After cells were incubated for 24 h in RPMI 1640 containing 5.5 mM and 16.7 mM glucose, 20 ㎍ of total RNA was electrophoresed on 1.2% formaldehyde agarose gel, blotted to nylon membrane, and probed with human Glut1 cDNAs. N represents normoglycemia (5.5 mM) and H represents hyperglycemia (16.7 mM).

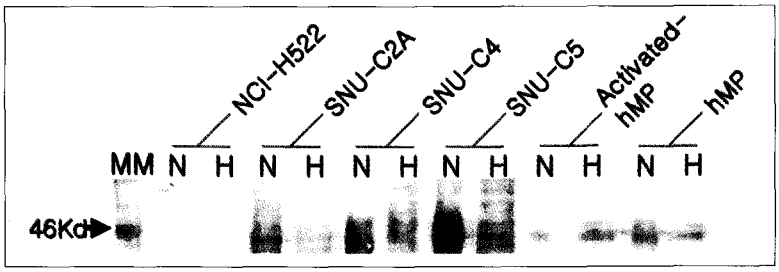

Fig. 5. Effect of glucose concentration on Glut1 expression. After cells were incubated for 24 h in RPMI 1640 containing 5.5 mM and 16.7 mM glucose, 20 ㎍ of cell membrane fractions were subjected to 10% SDS-PAGE and immunoblotted using rabbit anti-Glut1 antibody. N represents normoglycemia (5.5 mM) and H represents hyperglycemia (16.7 mM).

showed modest Glut1 expression, while NCI-H522 hardly express Glut1 even at a rabbit anti-Glut1 antibody concentration increased twenty times (1:100 dilution). The expression of Glut1 in human colon cancer cells was decreased at 16.7 mM glucose concentration, whereas it was consistently undetectable in monocytes (Fig. 5).

Hexokinase activity

Hexokinase activities of human cancer cells and monocytes are summarized in Table 1. SNU-C2A, SNU-C4 and NCI-H522 showed similar levels of hexokinase activity (7.5 – 10.8 mU/mg), whereas SNU-C5 and monocytes showed lower hexokinase activity (4.3 – 6.5 mU/mg) ($p<0.05$). The hexokinase activity under normoglycemic and hyperglycemic conditions was similar. The hexokinase activity of activated monocytes was significantly higher than that of unactivated monocytes under hyperglycemia ($p<0.005$).

DISCUSSION

Positron emission tomography using FDG is a

Table 1. Hexokinase activity in human cancer cells and monocytes (hMP)

Cell lines	Glucose conc. (mM)	Hexokinase activity (mU/mg)
SNU-C2A	5.5	8.7 ± 1.7*
	16.7	8.8 ± 3.3
SNU-C4	5.5	10.8 ± 4.4
	16.7	9.4 ± 3.5
SNU-C5	5.5	5.1 ± 2.7
	16.7	5.6 ± 2.0
NCI-H522	5.5	7.5 ± 2.8
	16.7	8.3 ± 2.3
hMP	5.5	5.7 ± 2.6
	16.7	4.3 ± 2.0
Activated-hMP	5.5	5.4 ± 1.8
	16.7	6.5 ± 1.5

* Mean ±SD, n=6-8

useful tool for the detection and evaluation of cancer, because most cancer cells show increased glucose uptake. However, several inflammatory lesions, such as active tuberculosis and bacterial infection, also show increased glucose uptake.[7,21,25] Following burn injury and infection, monocytes augment their cellular glucose uptake.[7] Glucose is the primary metabolic substrate of monocytes, which are critical components of host response to injury and infection. This characteristic causes considerable errors in the interpretation of FDG-PET scans.

In this study, we used human colon cancer cell lines (SNU-C2, SNU-C4, SNU-C5) and one lung cancer cell line (NCI-H522) to compare the glucose uptake with that of human monocytes. We had previously measured the FDG uptake in human colon cancer cells and colon cancers xenografted in nude mice, and observed that the FDG uptake in SNU-C5 was very high compared to that in SNU-C4 and SNU-C2.[12] As a medium for these cells, we selected NCI-H522, in which, as is evident in Fig. 1, FDG uptake was between that of SNU-C5 and other human colon cancer cell lines.

It is generally known that glucose concentration regulates the uptake of glucose into the cell.[23,26] In the current study, we compared FDG uptake in cancer cells and monocytes at different glucose concentrations, in order to characterize the relationship between glucose concentration and glucose uptake in cancer cells and inflammatory cells. FDG uptake was increased in glucose free media, and a high concentration of glucose reduced FDG uptake in both human cancer cells and monocytes.

Although the expression of Glut in cancer cells is variable depending on cancer type, most cancer tissues showed increased expression of Glut1.[2,9,10] In the present study, Glut1 expression was much higher in SNU-C5 than in either SNU-C4 or SNU-C2A, in terms of both mRNA and protein level. Glut1 expression in SNU-C5, SNU-C2A and SNU-C4 cells was decreased under hyperglycemic condition, and was correlated with FDG uptake data. Glut1 mRNA expression in NCI-H522 was also decreased under hyperglycemic condition, while its protein expression was very low in both normoglycemia and hyperglycemia. The expression of Glut1 mRNA and its corresponding protein was not correlated with FDG uptake data, and the expression of Glut1 mRNA was not correlated with its corresponding protein in NCI-H522 cells.

Glucose is also delivered via Glut1 in the phagocytic process of macrophages. Camelli et al. reported that macrophages augment their cellular glucose uptake following burn injury and infection, an augmentation which is facilitated by an increased Glut1 mRNA and protein level.[7] Macrophages co-cultured with LPS in vitro showed a similar response.[21] The consumption and uptake of glucose by macrophages in culture were accelerated two to three-fold by LPS stimulation accompanying an increased expression of Glut1 and its mRNA. We also found that increased uptake of FDG in human monocytes activated with LPS. However, expression of Glut1 mRNA and protein in normoglycemia was smaller in monocytes than in human colon cancer cells, and those expression levels were not changed in hyperglycemia. FDG uptake and Glut1 expression in activated monocytes were increased compared to those in monocytes. Activation of macrophages induced Glut1 redistribution from the intracellular compartments toward the cell surface.[21] A similar phenomenon was reported by Haspel et al., who found augmented Glut1 protein expression, but not Glut1 mRNA, in murine fibroblasts after oxygen and nutrient deprivation.[27] We speculated that relocalization of Glut1 present in cytoplasm to cell membrane might be the cause of this phenomenon in monocytes.

Hexokinase is variably distributed in mammalian

tissues and its activity is increased in many cancer cells.[1,28] However, hexokinase contributes to glucose uptake to varying degrees depending on cell type. In the present study, SNU-C5 and monocytes showed slightly lower hexokinase activity than did the other cancer cells, and increased glucose concentration had no effect on hexokinase activity in either cancer cells or monocytes. The hexokinase activity of activated monocytes was significantly higher than that of unactivated monocytes under hyperglycemia ($p<0.005$). These findings suggest that glucose concentration does not play a significant role in hexokinase activity, especially in cancer cells.

In summary, glucose uptake was decreased as glucose concentration increased in both human cancer cells and monocytes. The decreased glucose uptakes at high glucose concentration in human cancer cells and monocytes appear to be regulated by different mechanisms. Human colon cancer cells regulate it by reducing Glut1 expression, whereas the human monocytes use other mechanisms.

Acknowledgements

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요 약

목적: FDG PET은 악성종양의 진단에 유용하게 쓰이고 있으나, 염증에도 섭취되어 진단에 어려움이 있다. 염증에서 F-18-FDG 섭취는 단핵세포에서 포도당대사가 항진되어 나타난다. 이 연구에서는 사람의 암세포와 단핵세포간에 포도당대사에 차이가 있는지 알아보고자 하였다. **대상 및 방법**: 사람의 대장암 세포주(SNU-C2A, SNU-C4, SNU-C5)와 폐암 세포주(NCI-H522), 단핵세포를 포도당 농도가 다른 배지에서 각각 배양시키고, FDG 섭취와 포도당운반체 1(Glut1)의 발현, hexokinase 활성도의 변화를 비교 분석하였다. **결과**: 포도당이 없는 배지에서는 암세포와 단핵세포 모두에서 FDG 섭취가 증가되나 포도당 고농도(16.7 mM)에서는 섭취가 감소하였다. 이 고농도에서 Glut1 mRNA의 발현은 대장암 세포주, 폐암 세포주에서 감소하였다. 고농도의 포도당 배지에서 Glut1 단백질의 발현도 4종류의 암세포에서 모두 감소하였으나, 단핵세포에서는 변화가 없었다. SNU-C2A, SNU-C4, NCI-H522 세포에서 hexokinase의 활성도는 비슷하였고, 단핵세포와 SNU-C5에서는 약간 증가하였다. **결론**: 포도당 섭취에 있어서 사람의 암 세포주와 단핵세포는 서로 다른 기전을 보이고 있다. 대장암 세포는 포도당 농도에 의한 포도당 섭취 변화가 Glut1에 의하여 조절되나, 단핵세포는 다른 기전을 가지고 있다.

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