Mechanisms Related to [^{18}F]Fluorodeoxyglucose Uptake of Human Colon Cancers Transplanted in Nude Mice

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[^{18}F]Fluorodeoxyglucose ([^{18}F]FDG), a glucose analogue, has been widely used for tumor imaging. To investigate the mechanisms related to [^{18}F]FDG uptake by tumors, an experiment involving nude mice was performed. **Methods:** Human colon cancer cell lines SNU-C2A, SNU-C4 and SNU-C5 were transplanted to nude mice. Using immunohistochemical staining and Western blot, the expression of glucose transporter (Glut) isoforms (Glut-1~5) in xenografted tumors was analyzed. For the analysis of messenger ribonucleic acid (mRNA) expression, reverse-transcription polymerase chain reaction and Northern blot were used and the enzyme activity of hexokinase in cancer tissues was measured by continuous spectrophotometric rate determination. **Results:** [^{18}F]FDG uptake in SNU-C4 and SNU-C5 cells was higher than in normal colon cells. Among these cells and xenografted tumors, SNU-C5 showed the highest level of [^{18}F]FDG uptake, followed by SNU-C4 and SNU-C2A. An immunostaining experiment showed intense staining of Glut-1 in SNU-C5 tumors but somewhat faint staining in SNU-C4. SNU-C5 tumors also showed positive staining with Glut-3, although this was not the case with SNU-C2A and SNU-C4. Western blot analysis showed the expression of Glut-1 and Glut-3 in all tumors. Experiments involving Northern blot analysis and reverse-transcription polymerase chain reaction confirmed the overexpression of Glut-1 mRNA in all tumors, with the highest level in SNU-C5. The level of Glut-3 mRNA was also elevated in SNU-C5 tumors but not in SNU-C2A and SNU-C4. The enzyme activity of hexokinase did not vary among different tumors. **Conclusion:** Gluts, especially Glut-1, are responsible for [^{18}F]FDG uptake in a nude mouse model of colon cancer rather than hexokinase activity. Increased numbers of glucose transporters at the plasma membrane of cancer cells is attributed to an increased level of transcripts of glucose transporter genes and may be a cause of increased [^{18}F]FDG uptake, at least in colon cancer tumors.

Key Words: colon cancer; [^{18}F]fluorodeoxyglucose; glucose transporter; glucose transporter messenger ribonucleic acid; hexokinase

J Nucl Med 1999; 40:339–346

Cancer cells are known to show increased rates of glycolysis metabolism (*1*), and on the basis of this a PET study using [^{18}F]fluorodeoxyglucose ([^{18}F]FDG), a glucose analogue, has been used for the detection of primary and metastatic tumors (*2*).

To account for these increases, a variety of mechanisms has been proposed; these include an enhanced rate of glucose uptake and changes in the level and/or activity of glycolytic enzymes, especially hexokinase and glucose-6-phosphatase, in cancer cells (*3–5*). Recent studies have shown that glucose influx across the cell membrane is mediated by a family of structurally related proteins known as glucose transporters (*6*). Activated glucose transporters (Gluts), an increased concentration of hexokinase and a decreased concentration of glucose-6-phosphatase are considered to be the characteristics of cancer cells (*7,8*).

Gluts have several isoforms and Glut-1 and/or Glut-3 have been reported to show increased expression in various tumors (*9–12*). Brown et al. (*12*) reported a significant correlation between Glut-1 expression and [^{18}F]FDG accumulation in rat breast cancer, whereas Higashi et al. (*3*) showed a close correlation between Glut-1 expression and standardized [^{18}F]FDG PET uptake values in human pancreatic carcinoma. However, there is controversy over the basic mechanism of increased expression of Gluts at the protein level. Some investigators have reported the overexpression of Glut-1 messenger ribonucleic acid (mRNA) in cancers (*13–15*), although Haspel et al. (*16*) found that in murine fibroblast, oxygen and nutrient deprivation augumented Glut-1 expression but not Glut-1 mRNA. This finding suggested the translocation of cytoplasmic Glut-1 into the cell membrane rather than an increased level of mRNA transcription. However, whether there is correlation between the enhanced level of Glut and its corresponding mRNA has not been fully evaluated in vivo.

It is generally believed that in vitro [^{18}F]FDG uptake in cancer cells is related to increased levels of hexokinase (*14,17*), although in human cancer tissue in vivo, there is as yet no convincing evidence for this concept (*4*).

To investigate the mechanisms related to [^{18}F]FDG uptake

Received Feb. 25, 1998; revision accepted May 27, 1998.

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in tumors, we performed an animal experiment. After measuring the [^{18}F]FDG uptake of human colon cancers xenografted in nude mice, immunohistochemical staining and Western blot, reverse-transcription polymerase chain reaction (RT-PCR) and Northern blot and continuous spectrophotometric rate determination were used, respectively, to evaluate the expression of various Gluts, their mRNA expression and their hexokinase activity.

MATERIALS AND METHODS

Cell Culture

Human colon carcinoma cells SNU-C2A, SNU-C4 and SNU-C5 (*18*) were obtained from the Korean Cell Line Bank and normal colon cell FHC was purchased from American Type Culture Collection (Manassas, VA). Cells were grown in RPMI1640 media (GIBCO, Grand Island, NY) with 100 U/mL penicillin, 100 μg/mL streptomycin and 10% fetal calf serum (GIBCO) at 37°C in 5% CO_2 incubator.

In Vitro Uptake of [^{18}F]FDG by Cells

Adherent cancer cells and normal colon cells were plated for 4 and 24 h, respectively, in RPMI1640 media and were washed with glucose-free *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)–buffered saline (HBS) ([in mmol/L]: NaCl, 140; $CaCl_2$, 1; KCl, 5; $MgSO_4$, 2.5; and HEPES, 20; pH 7.4). The wells were filled with 500 μL HBS containing 1 μCi/mL [^{18}F]FDG and were incubated for 1 h at 37°C. Uptake of [^{18}F]FDG was determined by three washes with HBS. The cells were incubated in 500 μL 1% sodium dodecyl sulfate (SDS) and incorporated radioactivity in 0.5 mL of solubilized cells was measured by a Packard gamma counter (Cobra II; Packard, Downers Grove, IL). Cellular protein was measured by a modified Lowry method using bovine serum albumin as a standard.

In Vivo Uptake of [^{18}F]FDG by Transplanted Colon Cancer Tumors

Seven male nude mice were xenografted with SNU-C2A, SNU-C4 and SNU-C5 cell lines; three cell lines (1×10^7 cells) were implanted subcutaneously in the flanks and thighs of each mouse. After 2–4 wk, tumors weighing about 1 g were excised and used for this study; they grew most rapidly in SNU-C5 xenograft followed by SNU-C4 and SNU-2A xenografts. The tail vein of nude mice was injected with 37 MBq (1 mCi) [^{18}F]FDG, and 1 h after injection the mice were killed. Normal organs and tumors were excised and weighed and radioactivity was measured using a Packard gamma counter. Biodistribution data were expressed as percentages of injected dose per gram of tissue.

Expression of Glucose Transporter

Immunohistochemistry. Eight-micrometer frozen sections were obtained from tumor tissues and were fixed with ethanol. The sections were incubated with each of the anti-Glut antibodies for 2 h at 25°C. Rabbit polyclonal antibodies against Glut-1, Glut-2, Glut-3, Glut-4 and Glut-5 were purchased from Charles River Pharmservices (Southbridge, MA) and were diluted to 1:500 or 1:100 as recommended by the manufacturer. Each section was stained for Glut isoforms using the avidin/biotin conjugate immunoperoxidase procedure (*19*). The sections were incubated with the second antibody for 30 min at 25°C and washed with phosphate-buffered saline for 15 min. Then they were incubated with streptavidin peroxidase and washed with phosphate-buffered saline. 3,3'-Diaminobenzidine tetrahydrochloride was used as a substitute substrate-chromogen solution, and sections were counterstained with Meyer's hematoxylin. Adjacent sections incubated with excess rabbit immunoglobulin G were used as a negative control.

Western Blot Analysis. Colon cancer tumors were lysed with Tris-buffered saline (pH 7.5) and the lysate was centrifuged for 10 min at 2000 rpm to remove nuclei and unbroken cells. The supernatant was centrifuged for 10 min at 5000 rpm and the supernatant from above was centrifuged for 10 min at 17,000 rpm. The pellet was resuspended in a small amount of phosphate-buffered saline.

After electrophoresis of 20-μg membrane protein on 10% SDS-polyacrylamide gels, these were transferred to nitrocellulose filters and were stored overnight. The blots were incubated with rabbit anti-Glut antibody (1:2500 dilution; Charles River Pharmservices) as a primary antibody for 1 h at room temperature and were washed three more times. The antibody was detected with peroxidase-linked anti-rabbit antibody (Amersham, Buckinghamshire, England) as a secondary antibody. After washing, the antibody binding was detected with chemiluminescence reagent (ECL; Amersham). All procedures were performed as recommended by the manufacturers.

Expression of Glut mRNA

RNA Extraction. Total cytoplasmic RNA was prepared by the acid guanidium thiocyanate-phenol-chloroform extraction method (*20*). After surgical procedures, normal human colon tissue was obtained; poly $(A)^+$ mRNA was purified from total cytoplasmic RNA using the poly A Tract mRNA isolation system (Promega, Madison, WI).

RT-PCR. Reverse-transcription reaction was performed according to the manufacturer's instructions for the reverse-transcription system (Promega). All oligonucleotides were supplied by the Central Laboratory of the College of Pharmacy, Ewha Womans University, Seoul, Korea. Primers for the amplification of Glut isoforms and β-actin as an internal control were as follows: Glut-1–specific primers, 5'-ATGGAGCCCAGCAGCAAG-3' (forward) and 5'-CACTTGGGAATCAGCCCC-3' (reverse), corresponding to nucleotides 180–197 and 1638–1655 of the human Glut-1 complementary DNA (cDNA) sequence (*21*); Glut-2–specific primers, 5'-ATGACAGAAGATAAGGTCACT-3' (forward) and 5'-CACAGTCTCTGTAGCTCCTAG-3' (reverse), corresponding to nucleotides 39–59 and 1590–1610 of the human Glut-2 cDNA sequence (*22*); Glut-3–specific primers, 5'-ATGGGGACACAGAAGGTCACC-3' (forward) and 5'-GACATTGGTGGTGGTCTCCTT-3' (reverse), corresponding to the nucleotides 243–263 and 1710–1730 of the human Glut-3 cDNA sequence (*23*); Glut-4–specific primers, 5'-ATGCCGTCGGGCTTCCAACAG-3' (forward) and 5'-GTCGTTCTCATCTGGCCCTAA-3' (reverse), corresponding to the nucleotides 146–166 and 1651–1672 of the human Glut-4 cDNA sequence (*24*); β-actin primers, 5'-TACAATGAGCTGCGTGTGGC-3' (forward) and 5'-ATGTCACGCACGATTTCCC-3' (reverse), corresponding to nucleotides 193–212 and 539–557 of the mouse β-actin cDNA sequence (*25*).

Northern Blot Analysis. Two and four micrograms of mRNA were denatured with formaldehyde and transferred to a nylon membrane. The filters were hybridized to 864–base pair fragment for the detection of Glut-1 mRNA, 858–base pair fragment for Glut-2, 870–base pair fragment for Glut-3 and 867–base pair fragment for Glut-4. To produce cDNA fragments, the forward primer common to all isoforms (5'-GGATTC9*Eco*RI)TGTCCCGAGAGCCC-3') and the isoform-specific reverse primers as described earlier were used for polymerase chain reaction. The fragment of Glut cDNA

used as a probe encoded the portion of the transporter beginning at the large cytoplasmic loop and ending at the C terminus of the protein.

The probe was hybridized in hybridization solution (10× Denhardt solution, 5× saline-sodium phosphate-EDTA buffer, 1% SDS and 100 μg/mL salmon sperm deoxyribonucleic acid [DNA]) at 45°C overnight. After being washed twice in 2× standard saline citrate and 0.1% SDS at room temperature and four times in 0.5× standard saline citrate at 55°C, the ^{32}P-labeled blots were detected by exposing them to x-ray film for 5 d.

Quantitative evaluation of Glut and β-actin expression was performed using densitometry. The mRNA levels of Glut were expressed as a ratio of the optical density units for Glut to β-actin.

Hexokinase Activity

Hexokinase was assayed by measuring the oxidized nicotinamide adenine dinucleotide phosphate reduction rate with glucose-6-phosphate dehydrogenase (*26*). Briefly, tumors were homogenized in 50 mmol/L Tris-HCl (pH 7.4) containing 0.15 mol/L KCl, 5 mmol/L β-mercaptoethanol and 1 mmol/L ethylenediaminetetraacetic acid and were centrifuged at 100,000*g* for 30 min. Fifty microliters of supernatant were incubated in 20 mmol/L Tris buffer (pH 7.6) containing 220 mmol/L D-glucose, 0.75 mmol/L adenosine triphosphate, 8 mmol/L $MgCl_2$, 1.12 mmol/L β-nicotinamide adenine dinucleotide phosphate and 20 μL glucose-6-phosphate dehydrogenase (125 U/mL) in a total volume of 2.5 mL for 10 min at 25°C. The increase in absorbance at 340 nm was recorded on a spectrophotometer. Control assays contained the aforementioned medium except either glucose or the extract; these controls were corrected for any reduction of oxidized nicotinamide adenine dinucleotide phosphate not caused by hexokinase. One unit of activity catalyzed the formation of 1 μmol glucose-6-phosphate/min under the assay conditions described earlier. Crystalline bovine serum albumin was used as a standard.

Statistical Methods

Values representing the data were expressed as means ± SD. The statistical significance of differences among colon cancer tumors was analyzed using Student *t* test. A *P* value of <0.05 was considered significant.

RESULTS

[^{18}F]FDG Uptake by Cancer Cells and Tumors

As shown in Table 1, [^{18}F]FDG uptake in SNU-C4 and SNU-C5 colon cancer cells was higher than in normal FHC colon cells. Uptake by SNU-C5 cancer cells was highest followed by SNU-C4 cells. The percentage of [^{18}F]FDG uptake relative to injected dose per gram of tumor is shown in Table 2. SNU-C5 tumors showed the highest uptake, followed by SNU-C4 and SNU-C2A successively.

TABLE 1
[^{18}F]FDG Uptake in Human Cell Lines

Cell line	FDG uptake (cpm/mg of protein)
Normal colon cell (FHC)	33,192 ± 2,692
Colon cancer cells	
SNU-C2A	24,916 ± 2,474
SNU-C4	77,192 ± 10,941
SNU-C5	226,986 ± 115,855

Each value is expressed as mean ± SD of three experiments.

TABLE 2
[^{18}F]FDG Uptake in Colon Cancer Tumor Xenografted in Nude Mice

Tumor	FDG uptake (%ID/g)
SNU-C2A	3.68 ± 0.71*
SNU-C4	4.03 ± 1.06
SNU-C5	4.97 ± 0.92*

*Statistically significant according to Student *t* test, $P < 0.01$.
%ID/g = percentage injected dose per gram.
Each value is expressed as mean ± SD for seven subjects.

Expression of Glut Isoforms

In immunohistochemistry (Fig. 1), intense staining of Glut-1 was observed in SNU-C5 tumors. SNU-C4 tumors were also positive for Glut-1 staining, although its intensity was somewhat faint and heterogeneous in tumor tissues. Glut expression was prominent in the central area compared with the peripheral area of tumors. SNU-C2A tumors showed no definite staining. SNU-C5 tumors showed positive staining with Glut-3, although SNU-C2A and SNU-C4 did not. Positive staining of Glut was in the cell membranes but not in the cytoplasmic granules (*12*). All tumors were negative for the expression of Glut-2, Glut-4 and Glut-5, but on Western blot analysis, all were positive for Glut-1 and Glut-3 (Fig. 2). However, there was no expression of other Glut isoforms in these tumors.

Expression of Glut mRNA

For RT-PCR assay, isoform-specific polymerase chain reaction primers for the amplification of Glut-1, Glut-2, Glut-3 and Glut-4 cDNA were designed to produce an approximately 1.4-kilobase (kb)-long product. When the total RNAs from normal tissue and colon cancer tumors were used for an RT-PCR, a product of approximately the correct size was found. We found the following: (a) There was much more Glut-1 message present in SNU-C5 tumors than in SNU-C4 and SNU-C2A tumors (Fig. 3, lanes 2–5). (b) Glut-2–specific primers resulted in the production of no visible polymerase chain reaction product (Fig. 3, lanes 6–9). (c) There was much more Glut-3 message present in SNU-C5 tumors than in normal tissue and SNU-C2A tumors; however, there was no Glut-3 message in SNU-C4 tumors (Fig. 3, lanes 10–13). (d) With Glut-4–specific primers, SNU-C2A and SNU-C5 tissues showed slight polymerase chain reaction products; in contrast, no such product was found in normal colon and SNU-C4 tumors (Fig. 3, lanes 14–17). (e) The same amount of polymerase chain reaction product using β-actin–specific primers as an internal control was observed in all lanes, suggesting that the same amount of RNA template was used (Fig. 3, lanes 18–21).

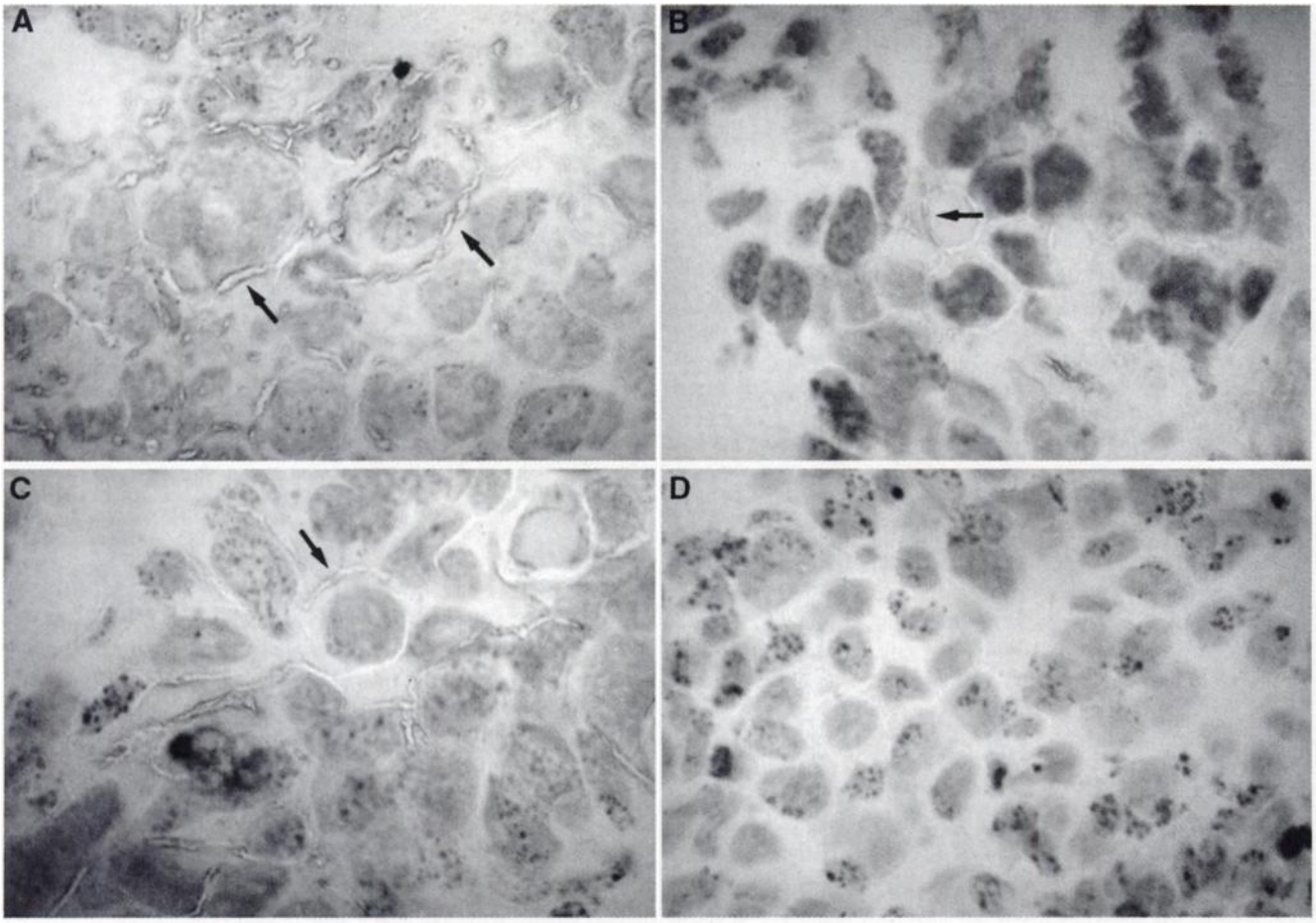

FIGURE 1. Immunohistochemical staining findings of human colon cancer tumors xenografted in nude mice (×1000). SNU-C5 tumors show (A) intense and homogeneous Glut-1 staining and (B) faint Glut-3 staining in cell membranes. (C) SNU-C4 tumors also show somewhat faint and heterogeneous Glut-1 staining, although (D) SNU-C2A tumors show no definite Glut-1 staining.

Northern blot analysis with Glut-1 cDNA probe showed that Glut-1 mRNA was expressed in SNU-C2A, SNU-C4 and SNU-C5 tumors (Fig. 4). The *Glut-1* gene was expressed at a higher level in SNU-C5 tumors. The Glut-1 to β-actin transcript count ratio of SNU-C2A, SNU-C4 and SNU-C5 tumors was 0.19, 0.31 and 2.03, respectively. The count ratio of normal colon was 0.22. The level of Glut-3 mRNA was also elevated in SNU-C5 tumors. There was no expression of Glut-2 and Glut-4 in colon cancer tumors.

Hexokinase Activity

Hexokinase activity, which was not significantly different among various colon tumors, is summarized in Table 3.

DISCUSSION

In this study, we investigated the relationship between [^{18}F]FDG uptake and the expression of Gluts and their mRNA and to hexokinase activity. The primary purpose of this study was to identify which isoforms of Gluts are expressed, to relate the expression of Gluts at the level of protein and its transcript and to clarify whether there is an increase of hexokinase activity in human colon tumors obtained from nude mice. We used human colon cancer cell lines SNU-C2A, SNU-C4 and SNU-C5, all of which were established from undifferentiated adenocarcinomas and showed different growth rates (*18*). Their features therefore appeared to provide an interesting basis for study of the correlation between [^{18}F]FDG uptake, the activities of Gluts and hexokinase activity according to growth rate, regardless of cell type and pathology.

In this study, we found the difference of [^{18}F]FDG uptake of colon cancer cells between in vitro and in vivo results. The difference of [^{18}F]FDG uptake in the in vitro model was larger than that in the in vivo model. This phenomenon is commonly found in a biodistribution study of several radiotracers (*27*). Several additional factors influence the [^{18}F]FDG uptake of an in vivo tumor. Vascular factors such as tumor blood flow, tumor blood volume and other biological factors, such as [^{18}F]FDG amount injected and hypoxic area in tumor, all would affect [^{18}F]FDG uptake in nude mouse tumor model.

Table 4 summarizes the results of expression of Gluts and their mRNAs in human colon cancer tumors xenografted in nude mice. The results of RT-PCR with Glut-1– and Glut-2–specific primers agreed with those of Northern blot

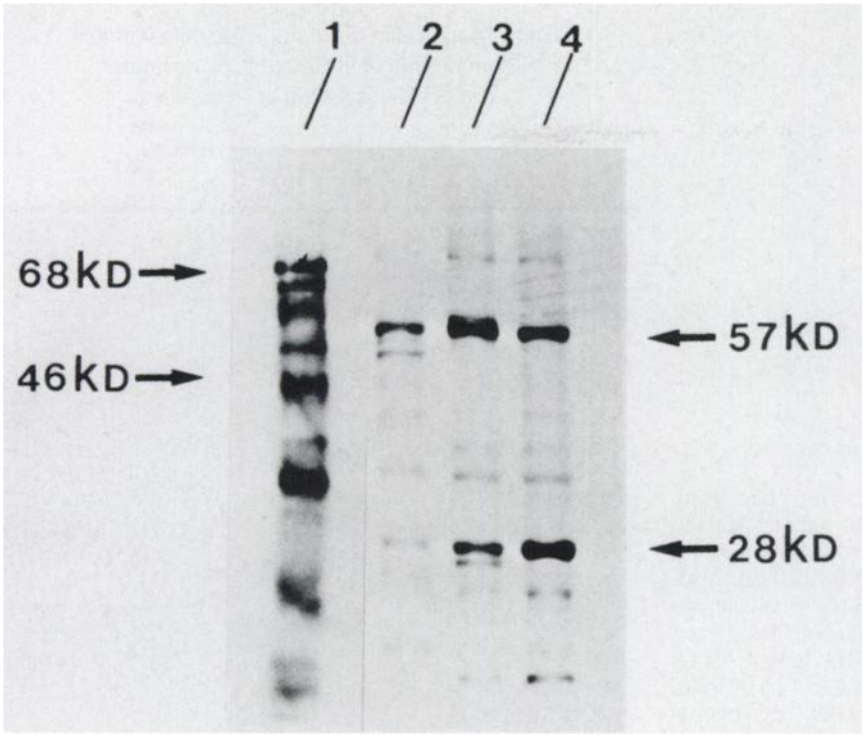

FIGURE 2. Western blot analysis of Glut-1 using lysates from colon cancer tumors. As a major band, a dimer at approximately 57 kD and a minor band at 28 kD were detected. Lane 1 = molecular marker; lane 2 = SNU-C2A; lane 3 = SNU-C4; lane 4 = SNU-C5.

analysis, although there were discrepancies between the data from immunohistochemistry, Western blot, RT-PCR and Northern blot. RT-PCR detected the presence of Glut-3 and Glut-4 messages in SNU-C2A tumors, but these were not detected by Northern blot analysis. This result might be because of the high sensitivity of the RT-PCR reaction. These data, however, indicate that as in the case of breast cancer, Glut-1 and Glut-3 are overexpressed in SNU-C5 tumors, which grew more rapidly than SNU-C2A and SNU-C4.

The ability to transport glucose, required for oxidative and nonoxidative adenosine triphosphate production, across the cell membrane is a common feature of all eukaryotic cells that survive. Anaerobic reactions are known to produce various sugar-containing macromolecules (*6*); cancer cells tend to use more glucose compared with normal cells, and the amount used is greatest in more rapidly growing tumors (*1*). The high glycolytic rate is inevitable for rapidly proliferating cancers, not only to supply a major energy source but also to provide such cells with precursors for nucleotide and lipid biosynthesis (*4*). Several explanations have been proposed for this finding, such as the possibility of increased activity of the Glut itself, an increased number of Gluts at the plasma membrane, an increased expression of

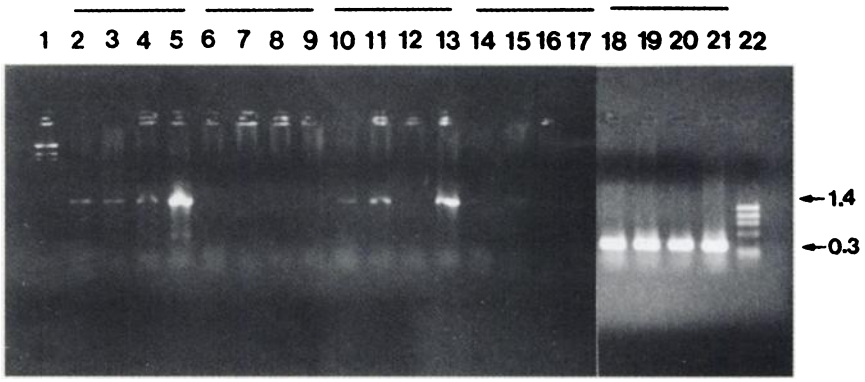

FIGURE 3. Polymerase chain reaction product of human Glut-1 amplified from total RNAs of SNU-C2A, SNU-C4 and SNU-C5 tumors. The 1.4-kb RT-PCR products of Glut-1 were confirmed (arrow). Although quantities of Glut-1 mRNA from SNU-C2A, SNU-C4 and SNU-C5 by RT-PCR cannot be calculated exactly, it appears that greatest quantity was obtained from SNU-C5 and the least from SNU-C2A. Lanes 2–5 = polymerase chain reaction products of Glut-1; lanes 6–9 = Glut-2; lanes 10–13 = Glut-3; lanes 14–17 = Glut-4; lanes 18–21 = β-actin; lane 1 = λ -*Hind*III marker; lane 22 = ϕ × 174-*Hae*III marker. Lanes were in order of normal tissue (lanes 2, 6, 10, 14 and 18), SNU-C2A (lanes 3, 7, 11, 15 and 19), SNU-C4 (lanes 4, 8, 12, 16 and 20) and SNU-C5 (lanes 5, 9, 13, 17 and 21).

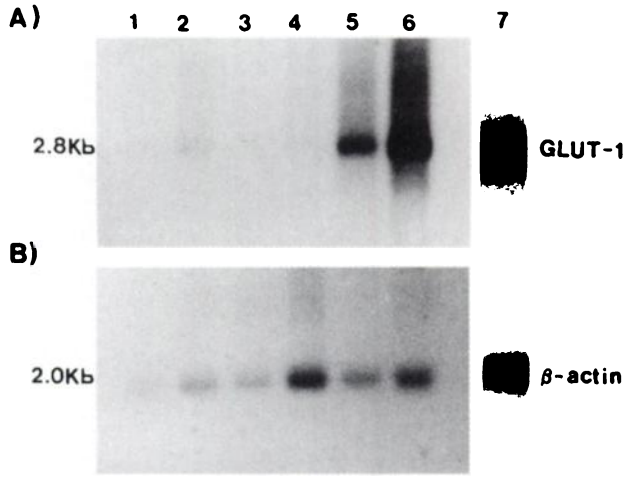

FIGURE 4. (A) Glut-1 and (B) β-actin Northern blot using mRNAs of SNU-C2A, SNU-C4 and SNU-C5 tumors. Human Glut-1 or β-actin cDNAs were hybridized to mRNAs from colon cancer tumors (SNU-C2A, SNU-C4 and SNU-C5) and these were blotted to nylon membranes after 1.2% formaldehyde agarose gel electrophoresis of 2 and 4 g of each mRNA. Lanes were in order of SNU-C2A (lane 1 = 2 μg mRNA; lane 2 = 4 μg mRNA), SNU-C4 (lane 3 = 2 μg mRNA; lane 4 = 4 μg mRNA), SNU-C5 (lane 5 = 2 μg mRNA; lane 6 = 4 μg mRNA) and normal colon tissue (lane 7 = 2 μg mRNA).

their transcripts (*7–15*) and changes in the level and/or activity of glycolytic enzymes, specifically hexokinase and glucose-6-phosphatase, compared with normal cells (*1,4,7,17*).

Six mammalian Glut isoforms have been identified so far. The human genes encoding these proteins are named Glut-1~5 and Glut-7. Glut-6 is a pseudogene that is not expressed at the protein level (*28*). These mammalian Glut genes belong to a large supergene whose protein products are involved in the transport of a variety of hexose and other carbon compounds (*29*). Overexpression of the high-affinity transporters Glut-1 and Glut-3 has been reported in a variety of cancers (*5*) and, in particular, Glut-1 overexpression has been reported frequently (*7–13*).

There are several ways to enhance the expression of Glut at the cell membrane (*30*). After short-term stimulation, such as viral infection or heat shock, enhancement of the number of Gluts at the cell membrane is rapid and not associated with any changes in Glut-1 protein or mRNA levels.

TABLE 3
Hexokinase Activities in Colon Cancer Tumor Xenografted in Nude Mice

Tumor	Hexokinase activity (mU/mg)
SNU-C2A	10.88 ± 1.03*
SNU-C4	11.84 ± 1.23*
SNU-C5	9.56 ± 3.18*

*Statistically insignificant according to Student *t* test, $P > 0.05$. Each value is expressed as mean ± SD for seven subjects.

TABLE 4
Expression Profile of Glut and Glut Messenger Ribonucleic Acid in Human Colon Cancer Tumors Xenografted in Nude Mice

	Tumors		
	SNU-C2A	SNU-C4	SNU-C5
Immunohistochemistry			
Glut-1	−	+	++
Glut-2	−	−	−
Glut-3	−	−	+
Glut-4	−	−	−
Glut-5	−	−	−
Western blot			
Glut-1	+	+	+
Glut-2	−	−	−
Glut-3	+	+	+
Glut-4	−	−	−
Glut-5	−	−	−
RT-PCR			
Glut-1	+	+	+++
Glut-2	−	−	−
Glut-3	+	−	++
Glut-4	+	−	+
Northern blot			
Glut-1	+	+	++
Glut-2	−	−	−
Glut-3	−	−	+
Glut-4	−	−	−

Glut = glucose transporter; RT-PCR = reverse-transcription polymerase chain reaction.

Pasternak et al. (*31*) showed that Glut-1 of BHK cells was translocated from intracellular sites to the cell membrane by immunofluorescence microscopy. Higashi et al. (*3*) observed the cytoplasmic granular staining pattern of Glut-1 by immunohistochemical analysis, suggesting that Glut-1 was located in the trans-Golgi network and intracellular vesicles.

Interestingly, Brown et al. (*12*) noted increased expression of Glut-1 in hypoxic tumor areas. They hypothesized that hypoxic cells may adapt to an increased dependency on glycolysis as their main energy source and that glucose influx is regulated by the translocation of Glut-1. However, some investigators found that after long-term glucose deprivation, enhancement of Glut-1 activity is observed and appears to result from a decreased rate of transporter inactivation (*16*). This might be one reason for the different [^{18}F]FDG uptake at the cellular level and at the tissue level.

On the other hand, many growth factors and cytokines intensely stimulate Glut-1 by means of increased Glut-1 mRNA levels. Similar findings have been reported for rodent fibroblasts transfected with *src* or *ras* oncogenes (*32*) or transformed by the Fujinami sarcoma virus (*33*). It was noticed that increased mRNA levels result from increases in the rate of transporter gene transcription and are followed by the increased synthesis of Glut-1 protein.

The translocation of Gluts is effective for increased glucose influx in early phase but is not a major factor in

delayed and continuous increased influx. The major factor for excessive Gluts expression in malignant tumors is supposed to be delayed and continuous response. This kind of oncogene-mediated late-phase response seems to be performed by intracellular trafficking pathways, including trans-Golgi network. We found increased [^{18}F]FDG influx caused by increased Glut mRNA expression as well as increased Glut expression.

In this study, we found increased levels of Glut-1 and Glut-3 mRNA and overexpression of Glut-1 and Glut-3 in animal tumor models of SNU-C5 colon carcinoma, a finding that agrees with those of previous reports (*13–15*). Reske et al. (*13*) reported the selective overexpression of Glut-1 mRNA in pancreatic cancer and Haberkorn et al. (*14*) showed overexpression of Glut-1 mRNA in animal mammalian carcinoma. In addition, we have shown that the expression of Glut-1 and its mRNA was highly elevated in SNU-C5 tumors, which showed the highest uptake of [^{18}F]FDG. This finding suggests a direct corelationship between [^{18}F]FDG uptake and the expression of Glut-1 and its mRNA. We also found overexpression of Glut-3 in SNU-C5 tumors. Similarly, Yamamoto et al. (*15*) reported overexpression of Glut-1 and Glut-3 mRNA in gastrointestinal carcinomas and therefore this might be one of the mechanisms causing increased [^{18}F]FDG uptake in SNU-C5 tumors. Interestingly, SNU-C5 tumors grew most rapidly in this study.

It is generally believed that [^{18}F]FDG uptake in tumors is related to hexokinase activity in cancer tissue (*14*). Besides Glut, hexokinase is also responsible for [^{18}F]FDG uptake in the brain tissue (*34*). Some investigators have reported increased activity of hexokinase together with increased Glut-1 expression in cancer cell lines (*7,8*). Our results, however, showed no clear discrepancy of hexokinase activity among colon cancer tumors SNU-C2A, SNU-C4 and SNU-C5. This similarity among tumors with different growth and [^{18}F]FDG uptake rates suggests that hexokinase probably has no role in [^{18}F]FDG uptake. We suppose that glucose consumption and its basic mechanism could be different at tissue and cellular levels. To determine [^{18}F]FDG uptake in a variety of tumor types, the role of hexokinase should be studied further.

CONCLUSION

The findings of this study suggest that in an animal model of human colon carcinoma, there is a close correlation between [^{18}F]FDG uptake and increased Glut-1 and Glut-3 expression. The presence of increased Gluts in cancer cells is attributed mainly to overexpression of Glut genes, although this phenomenon is not yet fully understood. In this study, it was found that hexokinase activity and [^{18}F]FDG uptake were not related.

ACKNOWLEDGMENTS

We thank Mee Kyung Hong for excellent technical assistance and Eun Ju Nam and Jung Hee Choi for administrative assistance. This work was supported in part by grant KOSEF SRC-56-CRC-97K3-1601–02–02–3 from the Korea Scientific and Engineering Foundation for the Cancer Research Center, Seoul National University.

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