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Differential Expression of Facilitative Glucose Transporter (*GLUT*) Genes in Primary Lung Cancers and Their Liver Metastases

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Glucose uptake is mediated by members of the facilitative glucose transporter (GLUT) family. Malignant cells take up more glucose than their normal counterparts. The aim of this study was to investigate the gene expression levels of the GLUT family, especially *GLUT1*, *GLUT3*, and *GLUT5* in primary lung cancer, metastatic liver tumors, and normal lung tissues, and to compare the expression levels of primary and metastatic tumors with those of normal tissues. We analyzed 105 autopsy samples (35 primary lung tumors, 35 corresponding normal lung tissues, 25 normal liver tissues, and 10 metastatic liver tumors) from 35 patients using the quantitative reverse transcription polymerase chain reaction. The *GLUT1* gene expression levels in primary lung tumors were significantly higher than those in normal lung tissues. In liver metastatic lesions, the *GLUT3* and *GLUT5* gene expression levels were significantly higher than those in primary lung tumors, but there were no differences in *GLUT1* expression levels between primary and metastatic liver tumors. Our results show that the gene expression pattern of the GLUT family is different between primary and metastatic liver tumors and suggest that the energy transporters in metastatic tumors may be different from those in primary tumors.

Key words: *GLUT1* — *GLUT3* — *GLUT5* — Lung cancer — Metastatic lesion

Malignant cells take up and use more glucose than normal cells.[1–3] The facilitative glucose transporters (GLUTs) mediate the transport of glucose into cells. Five GLUTs, GLUT1–GLUT5, have been isolated so far in humans,[4–9] and have different distribution and physiological properties.[10, 11] GLUT1 is widely expressed in normal tissues for basal glucose transport and increased glucose supply for growing or dividing cells.[11, 12] GLUT3 is a low-*Km* isoform responsible for glucose uptake, abundant in brain.[11] Unlike GLUT1–4, GLUT5 is a major fructose transporter and is expressed at relatively high levels in the small intestine and sperm cells.[8, 11]

Recently, increased expression of *GLUT1* and *GLUT3* was found in various human cancers,[13–19] and overexpression of *GLUT5* was found in breast cancers.[20] The appearance of GLUT1 and GLUT3 is correlated with aggressive biological behavior.[21] On the other hand, GLUT2 and GLUT4 seemed inappropriate for glucose uptake by lung cancer tumors.[22] However, the roles of the GLUT isoforms in human cancers are still uncertain. Moreover, these previous results were based on comparison of *GLUT* expression between malignant primary cells and their normal counterparts.

We examined the gene expression levels of the *GLUT* family, especially *GLUT1*, *GLUT3*, and *GLUT5*, in primary lung cancer, metastatic tumors, and normal lung tissues, and compared them between primary and metastatic tumors.

MATERIALS AND METHODS

Patients and samples We studied 105 autopsy samples (35 primary lung tumors, 35 corresponding normal lung tissues, 25 normal liver tissues, and 10 metastatic liver tumors) from 35 patients with lung cancer admitted to Hiroshima University Hospital and Chugoku Rousai General Hospital between September 1993 and September 1997. No patients had a history of diabetes mellitus. Fresh specimens of primary lung tumors, normal lung tissues, and metastatic liver tumors were obtained during autopsy after written informed consent had been obtained. We discarded necrotic parts and normal tissues. The tissues were frozen in liquid nitrogen and stored at −80°C until analysis.

Reverse transcription polymerase chain reaction (RT-PCR) Total cellular RNA was extracted by the guanidinium isothiocyanate-phenol method, and cDNA was synthesized by using random hexamers (Amersham, Buckinghamshire, UK) with Superscript RNase H− reverse transcriptase (GIBCO-BRL, Bethesda, MD).[23]

The reverse-transcribed cDNA from each sample was PCR-amplified with primers based on the *GLUT1*, *GLUT3*, *GLUT5*, and *β-actin* (internal control) gene sequences. After pre-denaturation at 94°C for 5 min, the cDNA was added to 5 μl of PCR mixture, comprising 1 μl

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of 10× PCR buffer (100 m*M* Tris·HCl [pH 9.0], 500 m*M* KCl), 1 μl of 15 m*M* $MgCl_2$, 2 μl of distilled water, 0.2 μl of 20 m*M* dNTPs (Takara, Tokyo), 0.2 μl of 50 μM forward primer, 0.2 μl of 50 μM backward primer, and 0.4 μl of (0.2 U) *Taq* polymerase (Promega, Madison, WI). We synthesized the GLUT1, GLUT3, and GLUT5 primers[4, 6, 8, 18)] and sequenced them: GLUT1, forward 5′-TCATCGTGGCTGAACTCTTCAG-3′, reverse 5′-TCA-CACTTGGGAATCAGCCCC-3′; GLUT3, forward 5′-AAAGTCCCTGAGACCCGTGGCAGG-3′, reverse 5′-AAGATCCAACAAACCGCAGCCTTG-3′; GLUT5, forward 5′-AGCTGCTGTCCATCATCGTC-3′, reverse 5′-CGATGCTGATGTATGGCATC-3′. All PCR products were subcloned and sequenced; their sequences were identical to the corresponding partial cDNA sequences. Amplification was done in a thermal cycler (Geneamp PCR System 2400; Perkin Elmer Applied Biosystems Division, Norwalk, CT) under the following conditions: denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min, followed by a final incubation at 72°C for 7 min. The lengths of the PCR products were 314 bp (GLUT1), 314 bp (GLUT3), and 303 bp (GLUT5). To determine the optimal number of amplification cycles, we tested the accuracy of the quantitative PCR procedure in a titration experiment.[21)] The optimal numbers were 24 for GLUT1, 20 for GLUT3, and 28 for GLUT5. We used the *β-actin* gene as an internal control. The sequences of its primers were: forward 5′-AAGAGAGGCATCCTCACCCT-3′ and reverse 5′-TACATGGCTGGGGTGTTGAA-3′. The PCR conditions were as follows: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, followed by a final incubation at 72°C for 7 min. Twenty amplification cycles with these primers were carried out, and the PCR products were 218 bp long, corresponding to *β-actin* cDNA.

Quantification of mRNA expression The PCR products were electrophoresed on 2% (w/v) agarose gels, transferred to nylon membranes (Hybond N+; Amersham), and subjected to hybridization analysis with ^{32}P-labeled cDNA probes. After each filter was washed, the radioactivity was measured with a laser imaging analyzer (BAS-2000; Fuji Photo Film, Tokyo). The PCR products of GLUTs 1, 3, and 5 were used as cDNA probes. The gene expression in each sample was expressed as the yield of the target gene relative to that of the *β-actin* gene (Fig. 1).

Statistical analysis Contingency table analyses based on χ^2 statistics were used to determine the significance of associations between categorical variables. Differences between the expression levels of each gene in tissue samples were analyzed with the Mann-Whitney *U*-test. The statistics were done with StatView J4.11 (Abacus Co., CA) for Macintosh. All statistical tests were two-sided; the data were expressed as medians and ranges; and differences for which $P<0.05$ were considered to be significant.

RESULTS

Patient characteristics Table I presents patients' characteristics. There were 27 men and 8 women, ranging in age from 44 to 82 years (median 67 years). Nine had small cell lung carcinoma (SCLC) and 26 had non-small cell lung carcinoma (NSCLC). Almost all (31 of 35) had been smokers. The interval between death and autopsy ranged from 1 to 16 h (median 4 h).

Expression levels of *GLUT* genes The expression levels of *GLUT* genes varied considerably among lung tumors, normal lung tissues, and metastatic lesions (Table II). There were no significant differences in age, sex, smoking history, histology, treatment history, or interval from death to autopsy between groups (data not shown).

First, we compared the expression levels of the *GLUT* genes between tumors and normal tissues. The expression

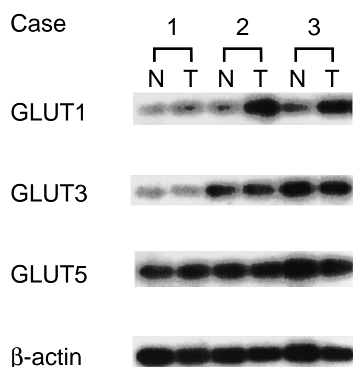

Fig. 1. Expressions of the *GLUT1*, *GLUT3*, and *GLUT5* genes in normal lung tissues (N) and primary lung tumors (T) are shown relative to *β-actin* expression using RT-PCR. The results of three representative cases are shown.

Table I. Patients' Characteristics

Number	35
Male/female	27/8
Age median (range)	67 (44–82)
Histology	
non-small	26
small	9
Smoking status yes/no	31/4
Interval to autopsy median (range)	4 h (1–16)
Liver metastasis (+)	10
non-small	6
small	4

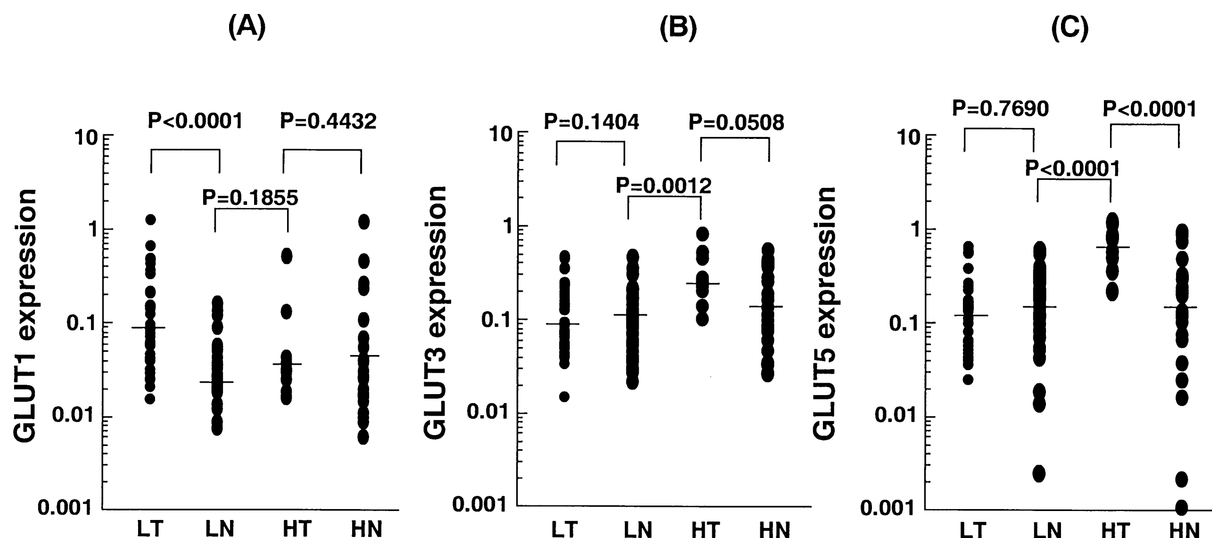

Fig. 2. Expressions of the (A) *GLUT1*, (B) *GLUT3*, and (C) *GLUT5* genes in primary lung tumors (LT), normal lung tissues (LN), metastatic liver tumors (HT), and normal hepatic tissues (HN). Statistical analysis was done using the Mann-Whitney *U*-test.

Table II. Expression Levels of *GLUT* Genes

Tissue	*GLUT1*	*GLUT3*	*GLUT5*
Normal lung	0.022 (0.006–0.137)	0.083 (0.022–0.458)	0.152 (0.003–0.418)
Lung tumor	0.076 (0.016–1.055)	0.122 (0.015–0.457)	0.150 (0.027–0.706)
Normal liver[a]	0.028 (0.006–1.306)	0.103 (0.023–0.511)	0.105 (0.001–0.732)
Liver metastasis[b]	0.036 (0.018–0.614)	0.208 (0.078–0.738)	0.755 (0.253–1.024)

The data are expressed as the median (range) of 35 patients.
a) Twenty-five samples of normal liver tissues were available.
b) Ten samples of metastatic liver tumors were available.

level of *GLUT1* in lung tumors was significantly higher than in normal lung tissues (P<0.0001), but those of *GLUT3* and *GLUT5* were not (Fig. 2). In contrast, in metastatic liver tumors, *GLUT3* and *GLUT5* expression levels were significantly higher than those in normal lung tissues (*GLUT3*, P=0.0012; *GLUT5*, P<0.0001), but that of *GLUT1* was not (Fig. 2). Furthermore, *GLUT5* levels in metastatic liver tumors were significantly higher than those in normal liver tissues (P<0.0001), and *GLUT3* tended to be higher (P=0.0508). On the other hand, for *GLUT1*, there was no significant difference between metastatic liver tumors and normal liver tissues (Fig. 2).

Next, we compared the expression levels of *GLUT* genes between primary and metastatic liver lesions of 10 patients. There was no significant difference in *GLUT1* expression levels between primary tumors and metastatic liver lesions (P=0.1040; Fig. 3). However, *GLUT3* and *GLUT5* expression levels in metastatic liver lesions were significantly higher than in primary tumors (*GLUT3*, P=0.0102; *GLUT5*, P=0.0019; Fig. 3).

DISCUSSION

In the present study, we examined the gene expression pattern of the GLUT family in primary lung cancer, metastatic tumors, and normal lung tissues. *GLUT1* was overexpressed in primary lesions compared with normal tissues, whereas *GLUT3* and *GLUT5* were overexpressed in metastatic lesions compared with primary lesions.

Recent immunohistochemical studies found *GLUT1* overexpression in primary lung tumors relative to normal tissues.[16, 22] Our result, based on molecular biological methods, is consistent with these studies. On the other hand, only a few primary lung tumors showed *GLUT3*-positive staining,[16, 21] and *GLUT5* overexpression in lung cancer has not been reported. In this study, we detected no

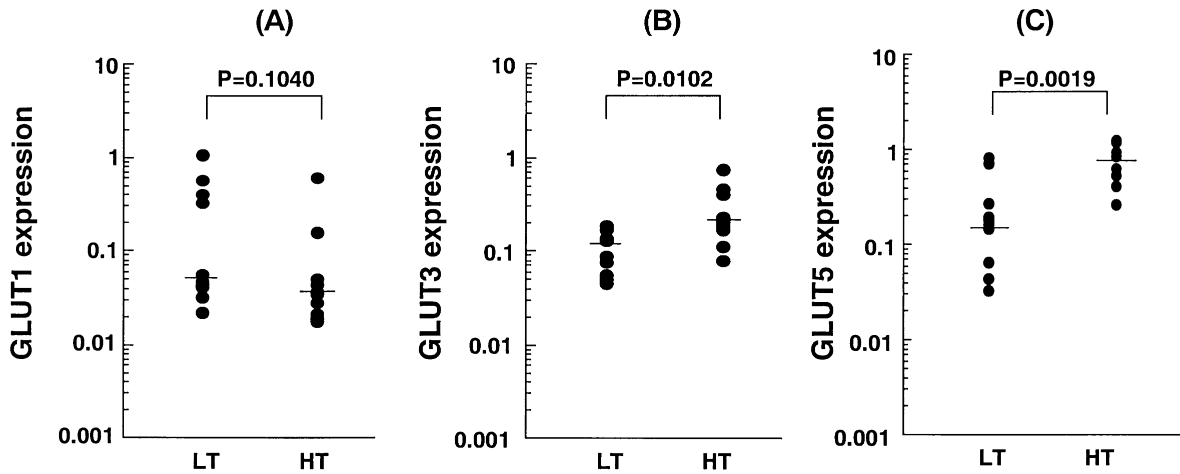

Fig. 3. Expressions of the (A) *GLUT1*, (B) *GLUT3*, and (C) *GLUT5* genes in primary lung tumors (LT), metastatic liver tumors (HT). Statistical analysis was done using the Mann-Whitney *U*-test.

significant differences in *GLUT3* and *GLUT5* overexpression between primary tumors and normal tissues. Based on these results, we suggest that *GLUT1* plays a role in glucose uptake mainly in primary lung cancer and that *GLUT1* overexpression could be a diagnostic marker for primary lung cancer. Younes *et al.* indicated that overexpression of *GLUT1* in stage I NSCLC was associated with poor survival.[21] Ogawa *et al.* suggested that *GLUT1* expression might promote metastasis, because amplification of *GLUT1* occurred in association with sLe^x synthesis and proliferation, and sLe^x plays an important role in the metastatic potential of tumors.[18] Based on the present results, we can not comment on any relationship of *GLUT* gene expression with tumor metastasis, because we could not obtain samples of metastases from all cases. However, the results suggest that amplification or overexpression of *GLUT1* in primary lung tumors is associated with aggressive tumor behavior or poor prognosis.

So far as we know, primary lung cancers and their metastatic tumors have not been compared. This study is the first that shows different expression patterns between primary and metastatic liver tumors of lung cancer. We also investigated the expression pattern of small samples of metastatic lymph nodes and found that the expression levels of *GLUT3* there tended to be higher (data not shown). Previously, Yamamoto *et al.* showed by northern blotting analysis that levels of *GLUT3* mRNA in colon cancer were higher than those in liver metastasis of colon cancer, whereas the levels of *GLUT1* mRNA were almost the same and the levels of *GLUT5* were lower in the primary tumor.[13] These results are in contrast to ours and suggest that different kinds of tumors show different expression patterns of GLUT isoforms between primary and metastatic tumors. Interestingly, we found *GLUT5* overexpression in metastatic liver tumors compared with normal liver tissues. This indicates that metastatic liver tumors of lung cancer may have a unique capacity to transport fructose for energy. Taken together, these results suggest that, in lung cancer, the mechanisms of glucose or fructose entry and use may be different between primary and metastatic tumors.

Recently, Kan *et al.* suggested that inhibition of glucose transport induced apoptosis in an interleukin-3-dependent cell line and indicated that growth-factor-mediated or oncogene-mediated increases in glucose uptake may represent an important regulatory point in the suppression of apoptosis.[24] Similarly, Shim *et al.* suggested that glucose deprivation induced extensive apoptosis of lung carcinoma that overexpressed c-*myc*.[25] Further, Martell *et al.* suggested that the expression level and rate of increase of GLUT paralleled increased vincristine resistance, active vincristine efflux, and decreased vincristine accumulation in murine erythroleukemia cell lines, and that GLUT inhibitors bound to multidrug-resistance-associated protein or to GLUT proteins directly or indirectly overcame drug resistance mediated by multidrug-resistance-associated protein.[26] Similarly, Vera *et al.* suggested that GLUT plays an important role in the modulation of multidrug resistance.[27] Based on these reports, we consider that GLUT inhibitors will play an important role in cancer therapy in the future, because they may directly induce apoptosis or indirectly produce therapeutic benefits in addition to conventional chemotherapy agents by overcoming drug resistance. Because we found different expression patterns of GLUT isoforms between primary and metastatic tumors, it will be necessary to choose appropriate GLUT inhibitors for the target tumors.

Empirically, it is sometimes difficult to distinguish primary lung cancer from metastatic lung tumor. The expression pattern of GLUT isoforms in metastatic liver tumors of colon cancer was found to be different from those in hepatoma,[13] which suggests that GLUT isoforms may be different between primary and metastatic tumors of lung cancer. Therefore, we plan to study whether it is possible to distinguish primary lung cancer from metastatic lung cancer by using GLUT isoforms in bronchoscopic biopsy samples.

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