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

Takayasu Kurata, Tetsuya Oguri, Takeshi Isobe,1 Shin-ichi Ishioka and Michio Yamakido

Second Department of Internal Medicine, Hiroshima University Faculty of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551

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 Rosai 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 guanidium 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...
of PCR buffer (100 mM Tris-HCl [pH 9.0], 500 mM KCl), 1 µl of 15 mM MgCl$_2$, 2 µl of distilled water, 0.2 µl of 20 mM 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 and sequenced them: GLUT1, forward 5'-TCTATGGGCTTGAATTTCAG-3', reverse 5'-TCACCTGGGAATCAGCCCG-3'; GLUT3, forward 5'-'AAAGTGAGCTGAGGACCTGCTGACGG-3', reverse 5'-AAAGATCCAACAAACCGCAGCCTTG-3'; GLUT5, forward 5'-AGCTGGTGATGTATGGCATC-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. 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 $\chi^2$ 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 4 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

| Characteristic | Number |
|---------------|--------|
| Male/female   | 27/8   |
| Age (median)  | 67 (44–82) |
| Histology     |        |
| non-small     | 26     |
| small         | 9      |
| Smoking status| 31/4   |
| Interval to autopsy (median) | 4 h (1–16) |
| Liver metastasis (+)| 10     |
| non-small     | 6      |
| small         | 4      |

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**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.
The 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 liver tissues \((P=0.0012; \quad \text{GLUT5}, \quad P<0.0001)\), 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 \((P=0.1040; \quad \text{Fig. 3})\). However, GLUT3 and GLUT5 expression levels in metastatic liver lesions were significantly higher than in primary tumors \((\text{GLUT3}, \quad P=0.0102; \quad \text{GLUT5}, \quad P=0.0019; \quad \text{Fig. 3})\).

**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
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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 sLex synthesis and proliferation, and sLex 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 lymph nodes 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.19) 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.

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. Statistical analysis was done using the Mann-Whitney U-test.

Fig. 3. Expressions of the (A) GLUT1, (B) GLUT3, and (C) GLUT5 genes in primary lung tumors (LT), metastatic liver tumors (HT).

![Graphs showing GLUT1, GLUT3, and GLUT5 expressions](image-url)
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, 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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