Jpn. J. Cancer Res. **92**, 874–879, August 2001

SGLT Gene Expression in Primary Lung Cancers and Their Metastatic Lesions

Nobuhisa Ishikawa, Tetsuya Oguri,[1] Takeshi Isobe, Kazunori Fujitaka and Nobuoki Kohno

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

Cancer cells show increased glucose uptake and utilization in comparison with their normal counterparts. Glucose transporters play an important role in glucose uptake. We previously reported the differential gene expression of the GLUT family in primary and metastatic lesions of lung cancer. To investigate the role of Na^+/glucose cotransporter (*SGLT*) genes in cancers, we examined the levels of expression of *SGLT1* and *SGLT2* genes in primary lung cancers and their metastatic lesions. Ninety-six autopsy samples (35 primary lung cancers, 35 corresponding normal lung tissues, 10 metastatic liver lesions, and 16 metastatic lymph nodes) from 35 patients were analyzed for *SGLT1* and *SGLT2* expression by reverse transcription (RT)-polymerase chain reaction (PCR). There were no significant differences in the level of expression of either gene between the primary lung cancers and normal lung tissues. The level of *SGLT1* expression in the metastatic lesions and primary lung cancers did not differ significantly. The level of *SGLT2* expression was, however, significantly higher in the metastatic lesions of both the liver and lymph node than in the primary lung cancers. These results suggest that *SGLT2* plays a role in glucose uptake in the metastatic lesions of lung cancer.

Key words: *SGLT1* — *SGLT2* — Lung cancer

Glucose is a basic source of energy in mammalian cells. Cancer cells show increased glucose uptake and utilization relative to their normal counterparts.[1–3] Two classes of glucose transporters have been described in mammalian cells.[4, 5] One class is the facilitative glucose transporter (*GLUT*) family, which mediates the energy-independent transport of glucose. There are five functional isoforms of *GLUT* (*GLUT1*–*GLUT5*). The five transporters have different functions and distribution in human tissues. The second class is the Na^+/glucose cotransporter (*SGLT*) family, which utilizes the electrochemical sodium gradient to transport glucose against the cell's internal concentration gradient.[4, 5] Two isoforms (*SGLT1* and *SGLT2*) have been cloned in mammalian cells.[6, 7] *SGLT1* is strongly expressed in the small intestine, and is expressed at lower levels in the kidneys, liver, and lungs.[8] In contrast to *SGLT1*, the kidneys express a high level of *SGLT2*, and the small intestine does not.[9]

Previous studies have demonstrated the overexpression of *GLUT1* in various human cancers relative to normal tissues.[10] In addition, increased *GLUT3* expression has been found in several cancers.[11] Overexpression of *GLUT5* was found in breast cancers.[12] On the other hand, the expression of *SGLT* genes in cancers has not been studied, and the role of *SGLT* genes is still unclear in cancers.

Recently, we studied the expression of genes belonging to the *GLUT* family in lung cancers and their metastatic lesions.[13] Our results showed the differential expression of *GLUT* genes in primary lung cancers and their metastatic lesions. To investigate the role of the SGLT family in cancers, we examined the expression levels of *SGLT1* and *SGLT2* in primary lung cancers and their metastatic lesions.

MATERIALS AND METHODS

Patients and samples Ninety-six autopsy samples (35 primary lung cancers, 35 corresponding normal lung tissues, 10 metastatic liver lesions, 16 metastatic lymph nodes) from 35 patients with lung cancer, who had been admitted to Hiroshima University Hospital between September 1993 and October 1998, were studied. The characteristics of the patients are summarized in Table I. Eight patients had small cell lung cancer (SCLC), and 27 patients non-small cell lung cancer (NSCLC). No patient had a history of diabetes mellitus. Fresh specimens of primary lung cancers, normal lung tissues, metastatic liver lesions, and metastatic lymph nodes were obtained during autopsy after written informed consent had been obtained. It was confirmed microscopically that the tumor specimens were not contaminated by necrotic areas or normal lung tissue by hematoxylin-eosin staining. The tissues were frozen in liquid nitrogen and stored at −80°C until analysis.

RNA extraction and reverse transcription (RT)-polymerase chain reaction (PCR) Total cellular RNA was extracted using the guanidinium isothiocyanate-phenol method as described previously.[14] We confirmed that similar amounts of total cellular RNA had been extracted from

[1] To whom correspondence should be addressed.
E-mail: tetsuogu@yahoo.co.jp

Table I. Characteristics of Patients

Number of patients	35
Age (years)	
median	67
range	44–82
Sex (male/female)	27/8
Histology	
small cell lung cancer	8
non-small cell lung cancer	27
Interval between death and autopsy (h)	
median	4
range	1–16

the samples by ethidium bromide staining. cDNA was synthesized using a random hexamer (Amersham, Buckinghamshire, United Kingdom) with Superscript RNase H− reverse transcriptase (GIBCO-BRL, Bethesda, MD), as described previously.[14] To confirm the quality of the harvested RNA, the reverse-transcribed cDNA synthesized from the same amount of total RNA in each sample was subjected to PCR amplification using β-actin primers as described previously.[14] We found that the expression levels of β-actin in each sample were similar on ethidium bromide staining, even though they had been obtained from patients whose interval between death and autopsy differed.

The reverse-transcribed cDNA was subjected to PCR amplification using primers based on the *SGLT1*, *SGLT2*, and *β-actin* (internal control) gene sequences. The PCR primer sets for *SGLT1* and *SGLT2* and the procedure for PCR amplification were as described previously.[15] After predenaturation at 94°C for 5 min, the cDNA was added to 5 μl of the PCR mixture.[14] Amplification was carried out using a thermal cycler (Geneamp PCR System 2400; Perkin Elmer Applied Biosystems Division, Norwalk, CT). The PCR products of SGLT1 and SGLT2 were 354 and 240 base pairs long, respectively.

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 a ^{32}P-labeled cDNA probe. We used the SGLT1 and SGLT2 PCR products aforementioned as cDNA probes. After washing of each filter, the level of radioactivity was measured with a laser imaging analyzer (BAS-2000; Fuji Photo Film, Tokyo). The PCR products of SGLT1 and SGLT2 described above were used as the cDNA probes. In each sample, the radioactivity associated with the level of *SGLT1* or *SGLT2* expression was expressed relative to the level of *β-actin* expression in that sample.

Statistical analysis Differences between the levels of expression of each gene in the tissue samples were analyzed for significance using the paired *t* test. The statistical calculations and tests were performed using Stat View J 5.0 Software (ABACUS Co., Berkley, CA) on a Macintosh computer. The data are expressed as median and range, and differences at *P* values of less than 0.05 were considered to be significant.

RESULTS

Expression levels of *SGLT* genes Fig. 1 shows the results of RT-PCR for SGLT1, SGLT2 and β-actin in the primary lung cancers and normal lung tissues of three representative cases. There was considerable variability in the level of expression of the two *SGLT* genes among the primary lung cancers, normal lung tissues, metastatic liver lesions, and metastatic lymph nodes.

First, we compared the level of expression of the two *SGLT* genes between the primary lung cancer samples and normal lung tissue samples. The median level of *SGLT1* gene expression was 0.154 (range, 0.001–0.793) in the primary lung cancer samples, and 0.186 (0.009–0.928) in the normal lung tissue samples. The median level of *SGLT2* gene expression was 0.285 (0.010–1.183) in the primary lung cancer samples, and 0.201 (0.008–1.275) in the normal lung tissue samples. There were no significant differences in the level of expression of either gene between the primary lung cancer and normal lung tissue samples as shown in Fig. 2. We also compared the level of expression of the two *SGLT* genes between those with SCLC and those with NSCLC; no significant differences were observed in the level of expression of either gene (data not shown).

Next, we compared the level of expression of the two *SGLT* genes between the metastatic liver lesions and the

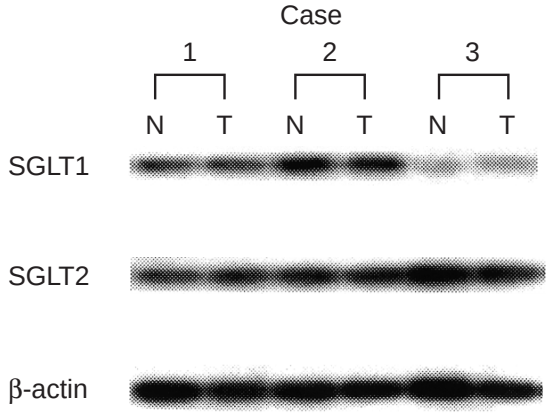

Fig. 1. Results of RT-PCR of the *SGLT1* and *SGLT2* genes in the normal lung tissues (N) and primary lung cancers (T) of three representative cases. *β-actin* was used as the control.

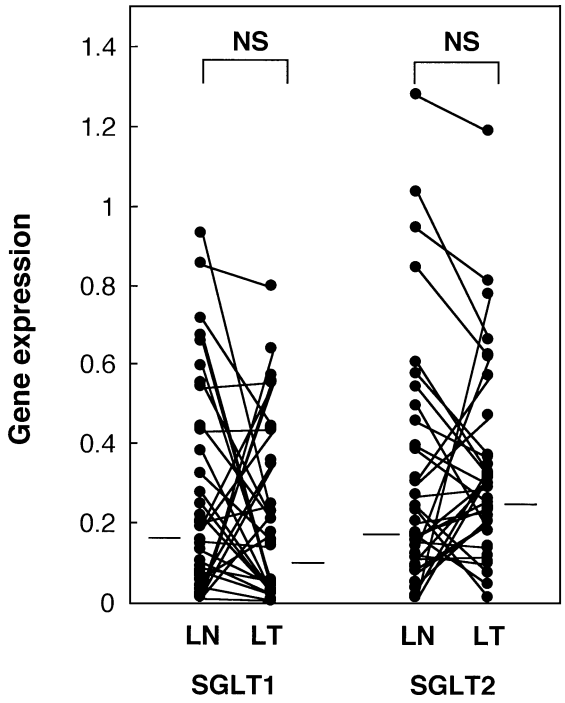

Fig. 2. Comparison of the levels of *SGLT1* and *SGLT2* expression in the primary lung cancers (LT) and normal lung tissues (LN) (n=35). RT-PCR was performed, and the level of mRNA was determined as described in "Materials and Methods." The level of *SGLT1* or *SGLT2* mRNA in a sample was expressed relative to the level of *β-actin* mRNA in that sample. Bars denote the median level of gene expression. Statistical analysis was performed using the paired t test.

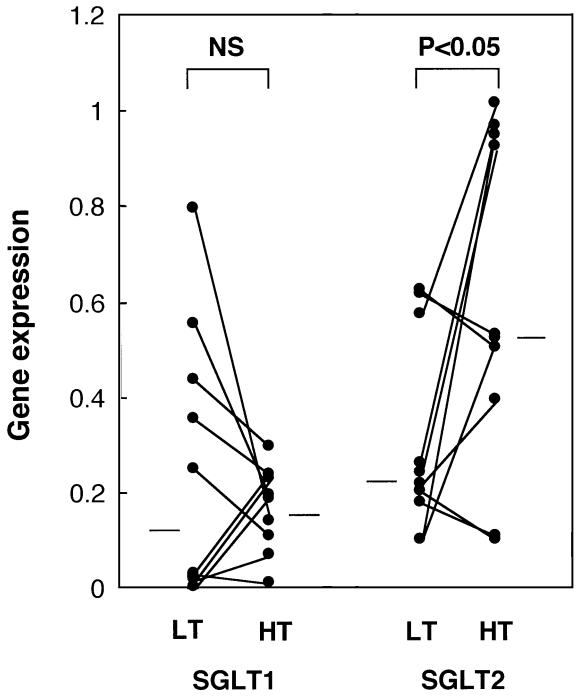

Fig. 3. Comparison of the levels of *SGLT1* and *SGLT2* expression in primary lung cancers (LT) and metastatic liver lesions (HT) (n=10). Bars denote the median level of gene expression. Statistical analysis was performed using the paired t test.

primary lung cancers, and between the metastatic lymph nodes and the primary lung cancers obtained from the respective patients. Among the 10 subjects from whom metastatic liver lesions were obtained, the median level of *SGLT1* gene expression was 0.136 (0.001–0.793) in the primary lung cancers, and 0.187 (0.008–0.293) in the metastatic liver lesions. Among the 16 subjects from whom metastatic lymph nodes were obtained, the median level of *SGLT1* gene expression was 0.097 (0.001–0.634) in the primary lung cancers, and 0.171 (0.005–1.112) in the metastatic lymph nodes. There was no significant difference in *SGLT1* gene expression between the primary lung cancers and their metastatic liver lesions, or between the primary lung cancers and their metastatic lymph nodes (liver, P=0.39, Fig. 3; lymph node, P=0.18, Fig. 4).

On the other hand, among the 10 subjects from whom metastatic liver lesions were obtained, the median level of *SGLT2* gene expression was 0.226 (0.096–0.623) in the primary lung cancers, and 0.522 (0.099–1.012) in the metastatic liver lesions. The level of *SGLT2* expression in the metastatic liver lesions was significantly higher than that in the primary lung cancers (P=0.04, Fig. 3). Among the 16 subjects from whom metastatic lymph nodes were obtained, the median level of *SGLT2* gene expression was 0.286 (0.073–1.183) in the primary lung cancers, and 0.702 (0.332–3.097) in the metastatic lymph nodes. The level of *SGLT2* expression in the metastatic lymph nodes was significantly higher than that in the primary lung cancers (P<0.01, Fig. 4).

DISCUSSION

This is the first study that provides data on the levels of *SGLT1* and *SGLT2* expression in cancer tissues. There were no significant differences in the level of *SGLT1* or *SGLT2* expression between the primary lung cancers and normal lung tissues. However, the level of *SGLT2* expression was significantly higher in the metastatic lesions than in the primary lung cancers, while the level of *SGLT1* expression in the primary lung cancers and their metastatic lesions did not significantly differ.

The *SGLT* gene family is involved in the active uptake of dietary glucose. *SGLT1* is a high-affinity, low-capacity

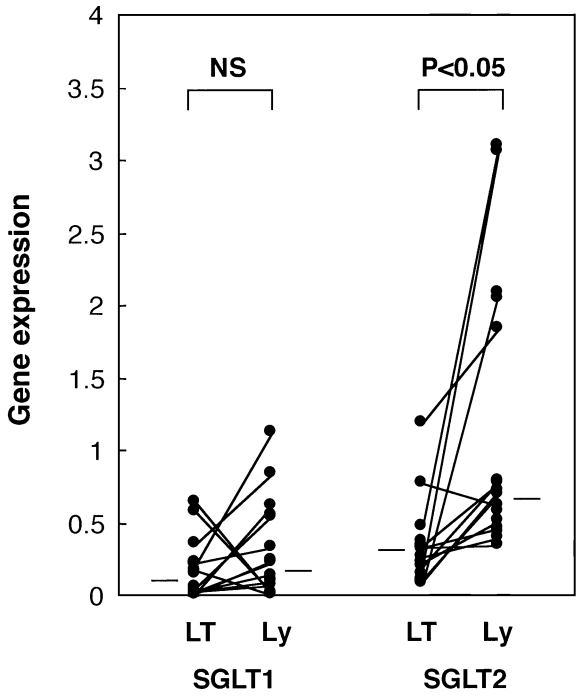

Fig. 4. Comparison of the levels of *SGLT1* and *SGLT2* expression in primary lung cancers (LT) and metastatic lymph nodes (Ly) (n=16). Bars denote the median level of gene expression. Statistical analysis was performed using the paired t test.

Na^+/glucose transporter, whereas *SGLT2* is a low-affinity, high-capacity Na^+/glucose transporter.[8, 9] The differences in the characteristics of *SGLT1* and *SGLT2* may be related to their expression in different tissues as well as their physiological properties. It was previously demonstrated that *GLUT1* expression is associated with high-glucose-consuming clones in exponentially growing cells, whereas *GLUT5* and *SGLT1* expression was associated with low-glucose-consuming clones and was absent from exponentially multiplying cells, being detectable only after confluence.[16] These results suggest that the differential consumption of glucose and cell growth rate among tumors may also contribute to the differential expression of *GLUT* and *SGLT* genes.

We previously studied the expression of *GLUT* genes in the same samples that were used in the present study. We showed that primary lung tumors expressed a significantly higher level of *GLUT1* than normal lung tissues,[13] although the expression of *GLUT3* and *GLUT5* in the primary tumors and normal lung tissues did not significantly differ. Taking these results and the findings of other reports into consideration, we concluded that *GLUT1* may play a major role in glucose uptake in primary lung cancer cells.

Although we have reported that the levels of *GLUT3* and *GLUT5* expression were significantly higher in the liver metastases of lung cancer than in the primary lung cancers,[13] we are not aware of other reports describing the differential expression of the *GLUT* or *SGLT* genes in primary lung cancers and their metastatic lesions. In this study, we found that the metastatic lesions of lung cancer expressed a significantly higher level of *SGLT2* than the primary lung cancers. These results indicate that not only *GLUT3* and *GLUT5*, but also *SGLT2* may play a role in glucose uptake in the metastatic lesions of lung cancer.

GLUT1 expression was detected in hepatomas, but not in normal liver tissues.[17] We did not find a difference in the level of *GLUT1* expression between normal liver tissues and liver metastases arising from lung cancers.[13] Different members of the *GLUT* and *SGLT* families may play a role in glucose uptake in different types of cancer. We plan to compare the level of expression of glucose transporters between primary lung cancers and their metastatic lung lesions, because these parameters may be helpful in distinguishing primary lung cancer and metastatic lung cancer.

Overexpression of *GLUT* genes has been reported to be associated with aggressive behavior in cancers and poor prognosis for survival.[18–21] These data suggest that tumors with increased glucose uptake are more metabolically active and more aggressive.

The *V-src* and *H-ras* oncogenes have been reported to regulate the levels of glucose uptake and *GLUT-1* expression, while *k-ras* is not involved in this regulation.[22, 23] However, it is not clear how *GLUT1* and *SGLT* expression are regulated. Growth factors such as platelet-derived growth factor and fibroblast growth factor mediate the levels of glucose uptake and *GLUT-1* expression.[24–26] Moreover, hypoxia induces the cellular uptake of glucose and upregulates the expression of several glucose transporters.[27, 28] The mechanism of the induction of the expression of glucose transporters in cancers is extremely complicated and remains to be elucidated.

Recently, Briasoulis *et al.*[29] reported the results of a phase I trial of glufosfamide (β-D-glucosylisophosphoramide mustard; D-19575), a new alkylating agent that targets the glucose transport system, in tumor cells. *In vitro* data suggested that a low-affinity Na^+/glucose cotransporter transports glufosfamide into tumor cells, and that the drug accumulates in the cells.[30] In that study, the dose-limiting toxicity of glufosfamide was nephrotoxicity, which may be due to the presence of Na^+/glucose cotransporters in the proximal tubule of the kidney. Further, inhibition of glucose uptake and glucose deprivation have been shown to induce apoptosis, which was blocked by *Bcl-2* expression in the cells but appeared to be independent of wild-type *p53* activity.[31, 32] It may be possible to induce apoptosis of tumor cells *in vivo* by inhibiting

glucose uptake. Thus, the *GLUT* and *SGLT* genes of tumor cells may be useful therapeutic targets for cancer treatment.

In summary, we found that primary lung cancers and their metastatic lesions express different levels of various glucose transporter isoforms. Glucose transporter inhibitors may be useful for the treatment of cancers.

(Received March 19, 2001/Revised May 10, 2001/Accepted May 23, 2001)

REFERENCES

1) Isselbacher, K. J. Sugar and amino acid transport by cells in culture—differences between normal and malignant cells. *N. Engl. J. Med.*, **286**, 929–933 (1972).
2) Holm, E., Hagmuller, E., Staedt, U., Schlickeiser, G., Gunther, H. J., Leweling, H., Tokus, M. and Kollmar, H. B. Substrate balances across colonic carcinomas in humans. *Cancer Res.*, **55**, 1317–1378 (1995).
3) Blair, S. L., Heerdt, P., Sachar, S., Abolhoda, A., Hochwald, S., Cheng, H. and Burt, M. Glutathione metabolism in patients with non-small cell lung cancers. *Cancer Res.*, **57**, 152–155 (1997).
4) Pessin, J. E. and Bell, G. I. Mammalian facilitative glucose transporter family: structure and molecular regulation. *Annu. Rev. Physiol.*, **54**, 911–930 (1992).
5) Bell, G. I., Burant, C. F., Takeda, J. and Gould, G. W. Structure and function of mammalian facilitative sugar transporters. *J. Biol. Chem.*, **268**, 19161–19164 (1993).
6) Hediger, H. A., Truk, E. and Wright, E. M. Homology of the human intestinal Na^+/glucose and *Escherichia coli* Na^+/proline cotransporters. *Proc. Natl. Acad. Sci. USA*, **86**, 5748–5752 (1989).
7) Wells, R. G., Pajor, A. M., Kanai, Y., Turk, E., Wright, E. M. and Hediger, M. A. Cloning of a human kidney cDNA with similarity to the sodium-glucose cotransporter. *Am. J. Physiol.*, **263**, F459–F465 (1992).
8) Lee, W. S., Kanai, Y., Wells, R. G. and Hediger, M. A. The high affinity Na^+/glucose cotransporter. Re-evaluation of function and distribution of expression. *J. Biol. Chem.*, **269**, 12032–12039 (1994).
9) Kanai, Y., Lee, W. S., You, G., Brown, D. and Hediger, M. A. The human kidney low affinity Na^+/glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose. *J. Clin. Invest.*, **93**, 397–404 (1994).
10) Younes, M., Lechago, L. V., Smoano, J. R., Mosharaf, M. and Lechago, J. Wide expression of the erythrocyte glucose transporter Glut1 in human cancers. *Cancer Res.*, **56**, 1164–1167 (1996).
11) Younes, M., Lechago, L. V., Smoano, J. R., Mosharaf, M. and Lechago, J. Immunohistochemical detection of Glut3 in human tumors and normal tissues. *Anticancer Res.*, **17**, 2747–2750 (1997).
12) Zamora-León, S. P., Golde, D. W., Concha, I. I., Rivas, C. I., Delgado-Lopez, F., Baselga, J., Nualart, F. and Vera, J. C. Expression of the fructose transporter GLUT5 in human breast cancer. *Proc. Natl. Acad. Sci. USA*, **93**, 1847–1852 (1996).
13) Kurata, T., Oguri, T., Isobe, T., Ishioka, S. and Yamakido, M. Differential expression of facilitative glucose transporter (*GLUT*) genes in primary lung cancers and their liver metastases. *Jpn. J. Cancer Res.*, **90**, 1238–1243 (1999).
14) Oguri, T., Fujiwara, Y., Isobe, T., Katoh, O., Watanabe, H. and Yamakido, M. Expression of γ-glutamylcysteine synthetase (γ-GCS) and multidrug resistance-associated protein (MRP), but not human canalicular multispecific organic anion transporter (cMOAT), genes correlates with exposure of human lung cancers to platinum drugs. *Br. J. Cancer*, **77**, 1089–1096 (1998).
15) Matosin-Matekalo, M., Mesonero, J. E., Delezay, O., Poiree, J. C., Ilundain, A. A. and Brot-Laroche, E. Thyroid hormone regulation of the Na^+/glucose cotransporter SGLT1 in Caco-2 cells. *Biochem. J.*, **334**, 633–640 (1998).
16) Mahraoui, L., Rodolosse, A., Barbat, A., Dussaulx, E., Zweibaum, A., Rousset, M. and Brot-Laroche, E. Presence and differential expression of SGLT1, GLUT1, GLUT2, GLUT3 and GLUT5 hexose-transporter mRNAs in Caco-2 cell clones in relation to cell growth and glucose consumption. *Biochem. J.*, **298**, 529–633 (1994).
17) Yamamoto, T., Seino, Y., Fukumoto, H., Koh, G., Yano, H., Inagaki, N., Yamada, Y., Inoue, K., Manabe, T. and Imura, H. Over-expression of facilitative glucose transporter genes in human cancer. *Biochem. Biophys. Res. Commun.*, **170**, 223–230 (1990).
18) Younes, M., Brown, R. W., Mody, D. R., Fernandez, L. and Laucirica, R. GLUT1 expression in human breast carcinoma: correlation with known prognostic markers. *Anticancer Res.*, **15**, 2895–2898 (1995).
19) Younes, M., Lechago, L. V. and Lechago, J. Overexpression of the human erythrocyte glucose transporter occurs as a late event in human colorectal carcinogenesis and is associated with an increased incidence of lymph node metastasis. *Clin. Cancer Res.*, **2**, 1151–1154 (1996).
20) Younes, M., Brown, R. W., Stephenson, M., Gondo, M. and Cagle, P. T. Overexpression of Glut1 and Glut3 in Stage I nonsmall cell lung carcinoma is associated with poor survival. *Cancer*, **80**, 1046–1051 (1997).
21) Ogawa, J., Inoue, H. and Koide, S. Glucose-transporter-type-I-gene amplification correlates with sialyl-Lewis-X synthesis and proliferation in lung cancer. *Int. J. Cancer*, **74**, 189–192 (1997).
22) Flier, J. S., Mueckler, M. M., Usher, P. and Lodish, H. F. Elevated levels of glucose transport and transporter messenger RNA are induced by ras or src oncogenes. *Science*, **235**, 1492–1495 (1987).
23) Noguchi, Y., Okamoto, T., Marat, D., Yoshikawa, T., Saitoh, A., Doi, C., Fukuzawa, K., Tsuburaya, A., Satoh, S.

and Ito T. Expression of facilitative glucose transporter 1 mRNA in colon cancer was not regulated by k-ras. *Cancer Lett.*, **154**, 137–142 (2000).
24) Hiraki, Y., Rosen, O. M. and Birnbaum, M. J. Growth factors rapidly induce expression of the glucose transporter gene. *J. Biol. Chem.*, **263**, 13655–13662 (1988).
25) Kitagawa, T., Tanaka, M. and Akamatsu, Y. Regulation of glucose transport activity and expression of glucose transporter mRNA by serum, growth factors and phorbol ester in quiescent mouse fibroblasts. *Biochim. Biophys. Acta*, **980**, 100–108 (1989).
26) Rollins, B. J., Morrison, E. D., Usher, P. and Flier, J. S. Platelet-derived growth factor regulates glucose transporter expression. *J. Biol. Chem.*, **263**, 16523–16526 (1988).
27) Iliopoulos, O., Levy, A. P., Jiang, C., Kaelin, W. G. and Goldberg, M. A. Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. *Proc. Natl. Acad. Sci. USA*, **93**, 10595–10599 (1996).
28) Leadle, J. M., O'Rourke, J. F., Bartlett, S. M., Poulton, J. and Ratcliffe, P. J. Isoenzyme-specific regulation of genes involved in energy metabolism by hypoxia: similarities with the regulation of erythropoietin. *Biochem. J.*, **313**, 809–814 (1996).
29) Briasoulis, E., Judson, I., Pavlidis, N., Beale, P., Wanders, J., Groot, Y., Veerman, G., Schuessler, M., Niebch, G., Siamopoulos, K., Tzamakou, E., Rammou, D., Wolf, L., Walker, R. and Hanauske, A. Phase I trial of 6-hour infusion of glufosfamide, a new alkylating agent with potentially enhanced selectivity for tumors that overexpress transmembrane glucose transporters: a study of the European Organization for Research and Treatment of Cancer Early Clinical Studies Group. *J. Clin. Oncol.*, **18**, 3535–3544 (2000).
30) Veyhl, M., Wagner, K., Volk, C., Gorboulev, V., Baumgarten, K., Weber, W. M., Schaper, M., Bertram, B., Wiessler, M. and Koepsell, H. Transport of the new chemotherapeutic agent beta-D-glucosylisophosphoramide mustard (D-19575) into tumor cells is mediated by the Na^+-D-glucose cotransporter SAAT1. *Proc. Natl. Acad. Sci. USA*, **95**, 2914–2919 (1998).
31) Shim, H., Chun, Y. S., Lewis, B. C. and Dang, C. V. A unique glucose-dependent apoptotic pathway induced by c-Myc. *Proc. Natl. Acad. Sci. USA*, **95**, 1511–1516 (1998).
32) Kan, O., Baldwin, S. A. and Whetton, A. D. Apoptosis is regulated by the rate of glucose transport in an interleukin 3 dependent cell line. *J. Exp. Med.*, **180**, 917–923 (1994).