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Increases in mRNA Levels of Glucose Transporters Types 1 and 3 in Ehrlich Ascites Tumor Cells During Tumor Development

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Abstract A common feature of many tumors is an increase in glucose catabolism during tumor growth. We studied the mechanism of this phenomenon by using Ehrlich ascites tumor bearing mice as the animal model. We found that Ehrlich ascites tumor cells possess only glucose transporter 1 (GLUT1) and GLUT3 but no GLUT2, GLUT4, or GLUT5. The mRNA levels of GLUT1 and GLUT3 increased progressively in the tumour during development; however, there were no changes observable in mRNA levels of glucose transporters of all types in brain, liver, and heart of the host mice. These findings suggest that Ehrlich ascites tumor augments its glucose transport mechanism relative to other tissues in response to its unique growth needs. J. Cell. Biochem. 67:131–135, 1997.

Key words: Ehrlich ascites tumor; glucose transporter; mRNA

A frequent characteristic of many tumors is an increase in glucose utilisation [Dills, 1993]. Using mice that bear the Ehrlich ascites tumor (EAT) as our model, we found that, in developing EAT cells, the glucose uptake rate and the cytochalasin-B binding activity varied inversely with the glucose concentration in the blood and peritoneal fluid of the host [Chan et al., 1983], and it was suggested that glucose regulates its own transport in EAT cells at the gene level [Fung et al., 1986], with phosphoribosyl pyrophosphate (PRPP) acting as the catabolic repressor [Choy et al., 1988; Fung et al., 1996]. Since regulation of glucose transport might be an important antitumor strategy [Merrall et al., 1993], it is important to examine how a tumor cell adaptively changes its glucose transport system during growth.

Recent studies of mammalian cells have revealed that facilitative transport of glucose across the cell membrane is mediated by six tissue- and cell-specific types of glucose transporters (GLUT) [Gould and Holman, 1993; Mueckler, 1994]. It has been suggested that two of these isoforms, GLUT1 and GLUT3, may be responsible for malignant transformation [White and McCubrey, 1995]. Increased levels of GLUT1 and GLUT3 mRNA and/or protein have been found in extracts of human gastrointestinal tumors [Yamamoto et al., 1990], as well as in tumors of the brain [Nagamatsu et al., 1993; Boado et al., 1994], head-and-neck [Mellanen et al., 1994], bladder, breast, kidney, lung and ovary [Younes et al., 1996]. However, since the comparisons were done in cancer biopsy specimens of the patients against normal ones, it is uncertain whether the increases in GLUT mRNA are associated with stages of tumor development. In the present study, we characterized the mRNA of GLUTs of EAT cells and examined quantitatively their changes in EAT cells during tumor development in mice.

MATERIALS AND METHODS

Materials

Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Nylon membrane (Zeta Probe) for electrophoresis was purchased from BioRad (Hercules, CA). Megaprime labeling sys-

Abbreviations: EAT, Ehrlich ascites tumor; GAPD, glyceraldehyde-3-phosphate dehydrogenase; GLUT, glucose transporter; PRPP, phosphoribosyl pyrophosphate.

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tem was from Amersham (Aylesbury, UK). Preparations of plasmid DNA of human GLUT3, GLUT5, and glyceraldehyde-3-phosphate dehydrogenase (GAPD) were obtained from American Type Culture Collection (ATCC, Rockville, MD). Murine-GLUT2 probe and murine-GLUT4 probe were generously provided by Dr. G.I. Bell of the University of Chicago, U.S.A. Human-HepG2-GLUT1 probe was generously provided by Dr. B. Thorens of the University of Lausanne, Switzerland.

Ehrlich Ascites Tumor-Bearing Mice

Ehrlich ascites tumor (EAT), Ny Klein cell type, was maintained by weekly intraperitoneal implantation in albino mice (strain ICR). Adult 30- to 35-g male mice were inoculated i.p. with 10^7 EAT cells harvested from mice bearing tumors for 9 days. The cells were prepared in 0.2 ml phosphate-buffered saline (PBS), pH 7.4. Tumor-bearing mice were killed by cervical dislocation on days 1, 2, 4, 7, 9, and 11. For the fasting study, the mice were fasted for 24 h and 48 h, respectively, before inoculation of 10^7 EAT cells harvested from mice bearing tumors for 9 days. The implanted cells were collected from fasting and nonfasting mice 3 h after inoculation.

Serum Glucose Concentrations

The serum glucose concentrations were determined according to instructions for the glucose determination kit obtained from Sigma Chemical Co.

RNA Preparation

EAT cells were washed with ice-cold PBS, and the cell pellets were resuspended in 10 volumes of Solution D (4 M guanidinium isothiocyanate, 25 mM sodium citrate, 0.5% sarkosyl, 0.01% 2-mercaptoethanol, pH 4). Total RNA was isolated according to the method of Chomczynski and Sacchi [1987].

Northern Blot Hybridization

RNA samples were denatured by heating in 6% formaldehyde and 50% formamide at 60°C for 15 min and then separated on 1% agarose gel containing 2.2 M formaldehyde by electrophoresis (20 µg/lane) and subsequently transferred to a nylon membrane. The membranes were prehybridized at 65°C for 3 h in the prehybridization solution (1.0 M NaCl, 1% sodium dodecyl sulfate [SDS]) and then hybridized in the hybridization solution (10% dextran sulfate, 1.0 M NaCl, 1% SDS, 100 µg/ml denatured salmon sperm DNA) with ^{32}P-labeled cDNA probes for 16 h according to the method described by Hardy et al. [1985]. The human-HepG2-GLUT1 probe is a 2.5-kb full-length clone. The murine-GLUT2 probe and the murine-GLUT4 probe is 1.2 kb cDNA and 1.3 kb cDNA, respectively. The human-GLUT3, GLUT5, and GAPD probes were prepared from corresponding clones from ATCC. The cDNA of GAPD was used to monitor the expression of the internal control gene. The cDNAs were labelled with [^{32}P]dCTP by megaprime labelling system. Following hybridization, the membranes were washed for 1 h at 65°C in low stringency wash (2 × SSPE, 1% SDS; where 1 × SSPE = 150 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.4) or medium stringency wash (0.5 × SSPE, 1% SDS). Membranes were exposed to X-ray film at −70°C with intensifying screen. The abundance of mRNA was quantitated by densitometry of autoradiographic bands using a laser densitometer (Molecular Dynamic Densitometer, model SI).

RESULTS

Figure 1 shows the Northern blot analysis for glucose transporter genes in 9-day-old EAT cells. Probing with specific cDNA of human or murine origins, we detected two isoforms of glucose transporters in EAT cells. A strong message of 2.7-kb size was detected by human-GLUT1 cDNA probe (Fig. 1a, lane 1). For comparison, the same probe hybridizing with the mRNA from murine brain is presented in lane 2 of the same figure. As GLUT1 is the major glucose transporter gene expressed in murine brain [Nagamatsu et al., 1994] and as human-GLUT1 and murine-GLUT1 were reported to show 95% identity [Gould and Bell, 1990], we conclude that the 2.7-kb message is the GLUT1 isoform. Neither GLUT4, GLUT2, nor GLUT5 mRNA was detectable in EAT cells, even after long exposure, although they were found in mRNA extracts from murine heart (Fig. 1b, lane 2), murine kidney and liver (Fig. 1c, lanes 2 and 4), and murine intestine (Fig. 1e, lane 2), respectively. GLUT3 was putatively identified in EAT cells using a human-GLUT3 cDNA probe (Fig. 1d, lane 1). This isoform was also present in murine testis [Yano et al., 1991] (Fig. 1d, lane 2) and is 3.2 kb in size.

The mRNAs levels of GLUT1 and GLUT3 were measured in EAT cells harvested at various stages of tumor growth. The results are

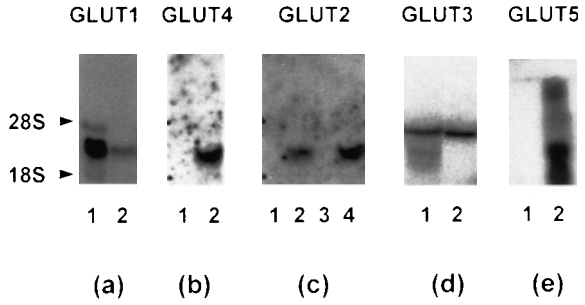

Fig. 1. Expression of mRNA levels of glucose transporters in tumor cells and organs in mice bearing EAT cells. Samples of total RNA extracted from EAT, brain, heart, kidney, liver, testis, and intestine were separated by electrophoresis on 1% agarose-formaldehyde gel and blotted onto nylon filter. Hybridization was performed with random primed human GLUT1, human GLUT3, murine GLUT2, murine GLUT4, and human GLUT5 cDNA probes as indicated above the panels. The positions of 28S and 18S rRNAs are indicated. **a: lane 1,** EAT; **lane 2,** brain. **b: lane 1,** EAT; **lane 2,** heart. **c: lane 1,** EAT; **lane 2,** kidney; **lane 3,** EAT; **lane 4,** liver. **d: lane 1,** EAT; **lane 2,** testis. **e: lane 1,** EAT; **lane 2,** intestine.

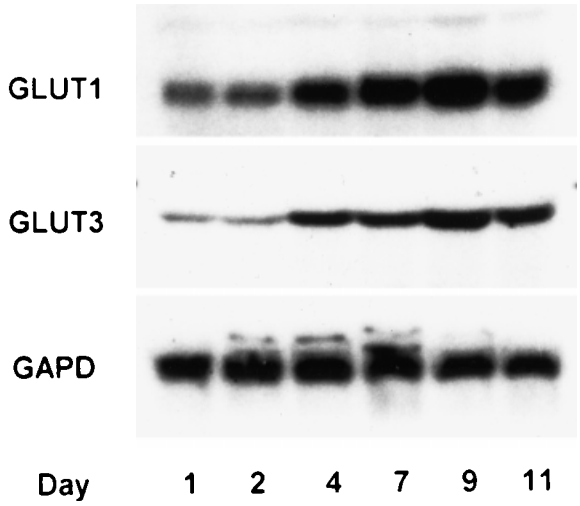

Fig. 2. Expression of mRNA of GLUT1, GLUT3, and GAPD in EAT cells during tumor growth in mice. EAT cells (10^7) were inoculated into mice on day 0. Groups of mice were killed on days 1, 2, 4, 7, 9, and 11. Tumor cells were pooled and washed. Total RNA were extracted for Northern blot analysis as described in Methods. Each group consisted of at least 10 mice.

shown in Figure 2. For internal control, the GAPD gene was used. It is already seen from Figure 2 that both GLUT isoforms increased progressively with tumor age, reaching maximum on day 9 and then declined. The increase, from day 1 to day 9, for both GLUT1 and GLUT3, was about 4.5-fold.

The increase in glucose transporter gene expression during tumor development appears to be confined to tumor cells. Other normal cells of

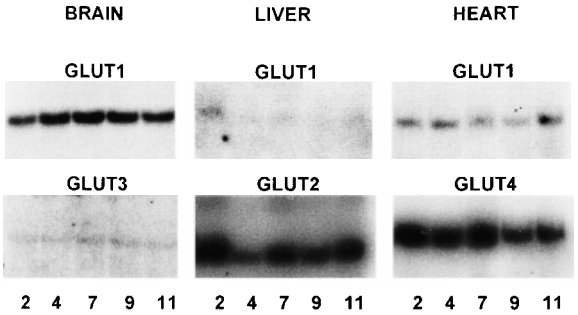

Fig. 3. Expression of mRNA levels of glucose transporters in mice brain, liver, and heart during the growth of EAT. EAT cells (10^7) were inoculated into mice on day 0. The brains, livers and hearts of the animals were collected at the indicated days. The organs were rinsed with ice-cold phosphate-buffered saline and stored in liquid nitrogen. Total RNA were extracted for Northern blot analysis as described under Methods. Each group consisted of at least 10 mice.

the host were unaffected. Fig. 3 shows that no changes in GLUT1 mRNA levels were observed in the brain, liver and heart of the mice from day 2 to day 11 after tumor implantation (Fig. 3, top row). After load normalizing, the levels of GLUT3 mRNA in the brain, of GLUT2 in the liver and of GLUT4 in the heart was also found to have remained constant during the course of EAT growth (Fig. 3, bottom row).

We had previously shown that the rate of glucose transport in vivo in EAT cells varies linearly with the glucose concentration in the blood and peritoneal fluid [Chan et al., 1983]. The effect of serum glucose concentration on the expression of GLUT1 and GLUT3 mRNA was studied. Serum glucose in normal mice, and in mice fasted for 24 h and 48 h were determined to be 284 ± 18 mg/dl ($n = 10$), 222 ± 16 mg/dl ($n = 10$), and 194 ± 9 mg/dl ($n = 10$) respectively. EAT cells (10^7) harvested from day-9 tumors were inoculated into these animals. Cells were re-collected 3 h after inoculations and analysed for glucose transporter mRNA. Figure 4 shows that after load normalizing, GLUT3 mRNA dropped significantly in cells inoculated into nonfasting but remained at a high level (50–70% of levels in day-9 cells before inoculation) in cells inoculated in fasted animals. However, for GLUT1 mRNA, cells inoculated in both nonfasting and fasting animals showed significant drops of about 80%.

DISCUSSION

Six isoforms of glucose transporters have been reported in mammalian cells [Gould and Hol-

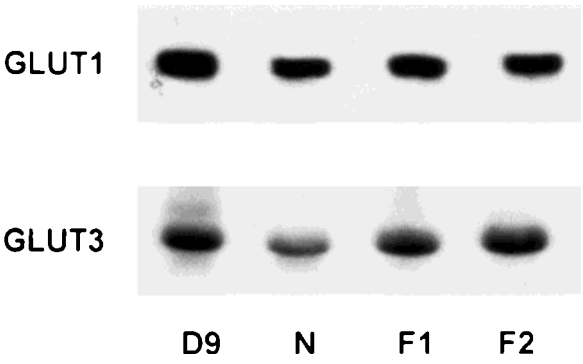

Fig. 4. Expression of mRNA levels of GLUT1 and GLUT3 in EAT cells inoculating in normal and fasting mice. EAT cells (10^7) harvested at day 9 (D9) were inoculated into normal mice (N), mice after fasting for 24 h (F1) and mice after fasting for 48 h (F2). Each group consisted of at least 10 mice. The cells were collected 3 h after inoculation and total RNA were extracted for Northern blot analysis.

man, 1993; Mueckler, 1994]. They show varying degrees of cell and tissue specificity. GLUT1 is expressed in most tissue types and is thought to be responsible for housekeeping levels of glucose transport [Mueckler, 1994]. GLUT3 is a brain-type and testis-type glucose transporter with a low K_m for glucose [Yano et al., 1991; Gould et al., 1991]. Overexpression of GLUT1 and GLUT3 has recently been reported in certain human tumors, including gastrointestine tumour, brain, head-and-neck, bladder, breast, kidney, lung, and ovary [Boado et al., 1994; Mellanen et al., 1994; Nagamatsu et al., 1993; Yamamoto et al., 1990; Younes et al., 1996] as well as in experimental tumor cell lines, such as osteosarcoma [Thomas et al., 1996]. GLUT1 and GLUT3 isoforms have been postulated responsible for the changes associated with malignant transformation [Merrall et al., 1993; While and McCubrey, 1995].

In the present study, we found that EAT cells possess both GLUT1 and GLUT3 isoforms (Fig. 1). The mRNA of GLUT1 and GLUT3 increased in parallel with tumor growth from day 1 to day 9 post-implantation (Fig. 2). In a previous communication [Chan et al., 1983], we reported that the rate of glucose uptake by EAT cells rises during tumor development. The increase in GLUT mRNA in EAT cells during tumor growth reported here would provide explanation for the change. In particular, as tumor growth is accompanied by a continuous decline in glucose levels in serum and ascitic fluid of the EAT-bearing mice [Chan et al., 1983; Choy et al., 1988], the increase in GLUT3 transporter, with high glucose binding affinity [Mueckle, 1994], would afford the tumor cells an advantage over the normal cells from brain, heart and liver, where the expression of glucose transporters remained unchanged throughout tumor development (Fig. 3).

The significance of the GLUT3 transporter in the cellular uptake of glucose under hypoglycaemic conditions is especially evident in the experiment described in Figure 4. When EAT cells from 9-day-old tumor, fully developed with regard to both GLUT1 and GLUT3 type transport, were inoculated into normal mice and exposed to the relatively high glucose level, GLUT3 expression dropped within 3 h. By contrast, in cells inoculated into fasted mice, where serum glucose had been depleted, the GLUT3 expression remained high. In both normal and fasted mice, newly inoculated EAT cells exhibited similar drops in levels of GLUT1 mRNA (about 80% of levels in day 9 cells before inoculation), reflecting the general housekeeping role of GLUT1 in glucose transport in tumor cells.

The mechanisms by which the expression of glucose transporters are activated during tumor growth are unknown. Anoxia due to poor perfusion surrounding the tumor cells may be one of the causes [Clavo et al., 1995]. We have hypothesized that hypoglycaemia with a resultant PRPP depletion in tumor cells might be another cause [Fung et al., 1986; Choy et al., 1988; Fung et al., 1996]. In the experiments reported here the observation that the two types of GLUT in EAT cells respond differently to glucose deprivation and replenishment suggest that more than one mechanism are operative. The exact pathway leading to the expression of mRNA of GLUT1 and GLUT3 in tumor cells under different glucose environments warrants further study.

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