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MOLECULAR MECHANISMS OF THE INCREASED GLUCOSE TRANSPORT IN TRANSFORMED FRTL-5 RAT THYROID CELLS

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SUMMARY We investigated the molecular mechanisms involved in the glucose carrier (Glut 1) regulation in FRTL-5 cells and two derived transformed clones (SRC and Ki-Mol cells). When compared to the wild-type strain, SRC and Ki-Mol cells showed an increase in both glucose consumption and uptake (about 60 fold), associated with 6-8 fold higher Glut 1 mRNA levels. Transcriptional studies revealed a 2- to 3 fold increased activation of the gene in the transformed cells, suggesting that transcription alone cannot fully account for the higher Glut 1 gene expression. Western blot studies showed an increase of the Glut 1 protein in SRC and Ki-Mol cells, associated with a different gel migration pattern and a disparate distribution rate between the plasma membrane and the microsomal fraction. These data indicate that the higher rate of glucose uptake observed in SRC and Ki-Mol cells is associated to an increase in Glut 1 gene expression, and that also changes in the subcellular distribution and probably in the structure of Glut 1 protein are present.

The neoplastic transformated phenotype is associated to several changes in the biochemical characteristics of the cell. Among these changes, there is an increased rate of glucose transport and consumption (1-3); although this feature has been described since decades (1) and considered one of the most typical biochemical features of cancer cells, the molecular mechanisms involved in this process are still poorly understood.

A family of glucose transporter genes has been recently identified and cloned (4,5); in human neoplastic tissues, two glucose transporter mRNA species, namely Glut 1 and Glut 3 have been found to be highly overexpressed (6-8). However, there is no agreement regarding the mechanism of regulation of the glucose carrier gene and its transcript (8,9). Glucose enters thyroid cells by a facilitated transport mechanism that is regulated by TSH (10,11), an experimental evidence obtained in rat FRTL-5 cells, a non transformed thyroid cell line which maintains most thyroid specific functions in the presence of TSH (12). When FRTL-5 cells are infected with either the murine Rous sarcoma virus (MRSV) or Kirsten murine sarcoma virus (Ki-MSV), expressing the oncogenes v-src and ki-ras respectively, they acquire a transformed neoplastic phenotype (clones SRC and Ki-Mol) (13).

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We have used this in-vitro model to investigate the molecular characteristics of glucose transport and Glut 1 expression changes, by comparing these parameters in transformed versus non-transformed cells.

MATERIALS AND METHODS

Cell culture: FRTL-5 rat thyroid cells were routinely cultured in 100 mm Petri dishes with Coon's modified F12, supplemented with 5% calf serum, and with the addition of insulin (10 μg/ml), transferrin (5 μg/ml), and TSH (5 mU/ml) ("3H medium"). The two derived clones, SRC and Ki-Mol cells (13), were propagated in the same culture medium, with no TSH ("2H medium"). All the cell lines were maintained in a 5% CO_2 humified incubator at 37C.

Glucose consumption: Glucose consumption was calculated by comparing the glucose concentration in the incubation medium at time 0 and after 24 h of incubation. Glucose was measured by the glucose oxidase method, using a glucose analyzer (Beckman, USA).

Glucose uptake: The hexose transport was studied with the use of the non-metabolizable glucose analogue [^{3}H]2deoxy-D-glucose (10). Experiments were performed in Krebs Ringer HEPES (20mM), pH 7.4, at 22C in the presence of 1 μCi/ml of the tracer (specific activity: 17 Ci/mmol; Amersham). Incubations were stopped by 3 rapid washes with ice-cold PBS, containing 100 mM D-Glucose. Cells were solubilized in 0.1N NaOH and aliquots counted for radioactivity in a liquid scintillation beta-counter.

Northern blot analysis: total RNA was extracted from thyroid cells using a guanidine thyocyanate method (14). Samples of total RNA (20 μg) were fractionated on a formaldehyde-agarose gel and transferred on a Zeta probe nylon filter (Bio-Rad, Milan, Italy). The membrane was hybridized with a [^{32}P]random-primed-probe (pGT25L) (15), containing sequences of the Glut 1 cDNA (kindly provided by Dr. Mueckler). Autoradiographs were quantitated by scanning densitometry (model 620 video densitometer, Bio-Rad).

"Run-off" nuclear transcriptional assay: 5 μg of linearized Glut 1 gene plasmid (pGT25L) and controls (pUC12, rat ferritin H, human ß-actin, provided by Dr. Frunzio, Naples, Italy) bound to nitrocellulose were hybridized with [^{32}P]labelled run-off transcripts from nuclei isolated from each clone of cells. The procedure and conditions have already been reported (16).

Western blot analysis: Cells were homogenized with a glass Dounce homogenizer (20 strokes) in 10 mM Tris/2mM EDTA/250 mM sucrose in the presence of protease inhibitors (2 mM PMSF, 2 mg/ml bacitracin).
The homogenate was centrifuged at 10,000xg for 15 min at 4C, and the supernatants were then separated and centrifuged at 100,000xg for 1h at 4C to obtain the microsomal fraction. Plasma membranes (10,000xg pellet, 50 μg) or microsomal-related proteins were separated on a 7.5% polyacrylamide gel and electroblotted on nitrocellulose. Block of non-specific binding was obtained by the use of 3% gelatin. The membrane was then exposed to a rabbit polyclonal antibody against the C-terminal region of the human glucose carrier (17) (provided by Dr. Baldwin, London, UK), followed by incubation with [^{125}I]Protein A (Amersham, Milan, Italy) (10^5 cpm/ml) and autoradiography.

RESULTS AND DISCUSSION

Following transformation with oncogenic viruses, both SRC- and Ki-Mol clones showed a dramatic increase in glucose consumption from the culture medium with respect to untransformed FRTL5 thyroid cells (FRTL-5: 0.27, SRC: 4.72, Ki-Mol: 5.95 μg

glucose/μg DNA/min^{-1}) (fig. 1). Although glucose consumption has been speculated to parallel cell proliferation (18), we found that this was not the case. Cell doubling time, evaluated by cell counting, was strikingly different for Ki-Mol (18 h) and SRC clones (48 h), being the latter similar to parental FRTL-5 cells (42 h) (data not shown). In our cell models, therefore, glucose consumption was more generally related to transformation and the neoplastic phenotype, rather than to cell replication.

A major step in the regulation of glucose metabolism is represented by its uptake via a specific carrier (19). To determine whether the increase in glucose utilization observed in the two transformed clones reflected an activation of its carrier-mediated transport, we studied the uptake of labelled 2-deoxyglucose. In both transformed cell lines we found at the initial uptake rate an approximately 60-fold increase (fig. 1), indicating that in these transformed cells glucose enters the cell through a more efficient glucose carrier system. These findings are in agreement with previous studies, reporting that an increased glucose uptake, together with morphological changes, follows oncogenic transformation (20,21).

In both SRC and Ki-Mol cells, the increase of glucose uptake was associated to an increase in the cell content of Glut 1 glucose transporter mRNA. As determined by densitometric analysis of Northern blot hybridization studies, in comparison to parental FRTL-5, Glut 1 mRNA was increased 6-7 fold in SRC, and 7-8 fold in Ki-Mol cells (fig. 2).

Glut 1 gene expression may be regulated by both transcriptional and/or post-transcriptional mechanisms. Induction of Glut 1 transcription has been demonstrated in fibroblasts exposed to transforming oncogenes and fetal calf serum (7,22), while an increase of both transcription and Glut 1 mRNA stability has been reported in PDGF treated 3T3 cells (23). Similarly, both mechanisms are involved also in the Glut 1 increase observed in L6 myocytes following insulin treatment or glucose deprivation (24). To investigate whether in the two transformed clones the increase of Glut 1 mRNA steady state levels was due to an

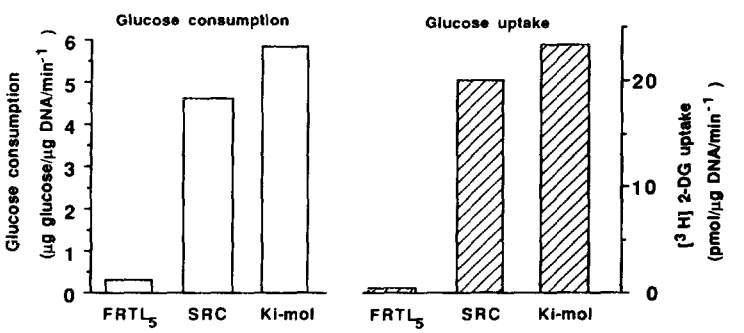

Fig. 1. Glucose consumption and 2-deoxy-D-glucose uptake in FRTL-5 cells and SRC and Ki-Mol clones. Cells were cultured in Coon's F12 medium (1.82 g/L glucose), supplemented with serum and with the addition of insulin and transferrin ("2H medium"). Glucose levels in the medium were measured at 24 h. Initial [^{3}H]2-deoxy-D-glucose uptake rate was measured at 5 min at 22 C.

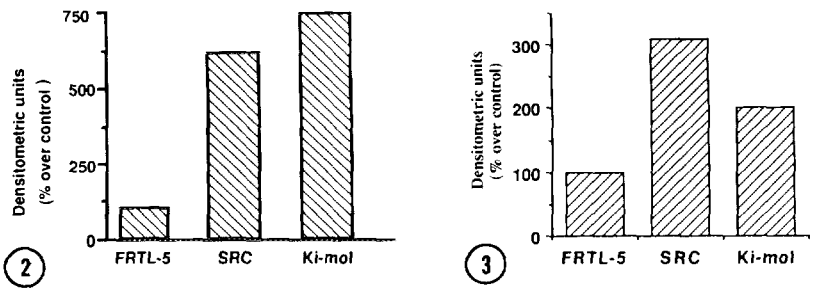

Fig. 2. Glut 1 mRNA levels in FRTL-5 cells and transformed clones SRC and Ki-Mol. Northern blot analysis was performed with total RNA (20 µg) extracted from cells exposed to "2H" medium. Quantitation of results by densitometric scanning is shown.

Fig. 3. Nuclear transcription of Glut 1 gene. Glut 1 transcriptional activity was determined in FRTL-5 and SRC and Ki-Mol transformed clones cultured in "2H medium" by run-off assay, as described (16). Quantitation of results by densitometric scanning is shown.

activation of gene transcription, studies were carried out using the run-off assay. A 2- to 3 fold increase of Glut 1 gene transcription was observed in the transformed cells (fig. 3). These findings indicate that transcription alone cannot fully account for the increased gene expression in SRC and Ki-Mol cells.

The mechanism of Glut 1 protein increase in transformed cells is not univocous: when transformed by src oncogene, both chicken embryo fibroblasts (CEFs) and rat fibroblasts increase their rates of glucose uptake (9,25). However, in CEFs cells, src reduces the degradation of the carrier protein, whereas in rat fibroblasts src increases the transporter biosynthesis, with little or no effect on degradation (26). Furthermore, the src oncogene can stabilize a human glucose transporter expressed in chicken cells, but not in rat cells (27), suggesting that the mechanism active in the regulation is intrinsic to the cell machinery and enviroment, rather than to the gene itself. When we measured by immunoblot studies the Glut 1 content in the transformed thyroid cells we found a 6- to 7 fold increase versus the non-transformed cells. This figure correlates well with the observed increase in mRNA levels (fig.4), suggesting that protein biosynthesis may play a major role in Glut 1 overexpression in these cells. When, in these immunoblotting studies, we separately analyzed plasma membranes and microsomes, we found that in transformed cells the Glut 1 phenotype in plasma membranes largely overexceeded that in the microsomal fraction, suggesting that in these cells, the glucose carrier translocates more efficiently to the plasma membrane (Fig. 4). A recruitment of Glut 1 glucose transporters from an intracellular pool to the plasma membrane following exposure to insulin or cytosolic cAMP has already been described (28-30).

In these studies we also found that Glut 1 migration in the gel was different in each cell clone studied, (apparent molecular weights: FRTL5: 55 kD; SRC: 60 kD; Ki-Mol: 62 kD) (Fig. 4), suggesting that post-translational processes and changes in intrinsic activity may, in addition, be part of the complex regulation of Glut 1 expression and function in transformed cells. Interestingly, similar differences in size of Glut 1 were found between

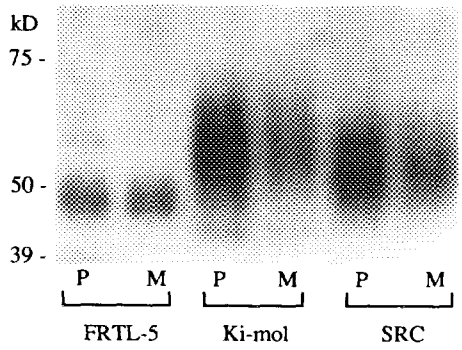

Fig. 4. Western blot analysis of the Glut 1 protein. Plasmamembranes (P) and microsomes (M) were prepared from normal and transformed cells cultured in standard conditions ("2H medium"). The technical procedure is described in the Methods.

myoblasts and myotubes (31) and during rat brain differentiation in vivo (32). In both cases the developmental regulation has been attributed to heterogenous complex N-linked glycosylation.

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