Expression analysis of facilitative glucose transporters (GLUTs) in human thyroid carcinoma cell lines and primary tumors

Raffaele Ciampia, Agnese Vivaldi, Cristina Romei, Alberto Del Guerra, Piero Salvadori, Barbara Cosci, Aldo Pinchera, Rossella Elisei

Abstract

Fluorine-18-fluoro-2-deoxy-d-glucose positron emission tomography (FDG-PET) is based on cell capability to take-up glucose. While a significantly higher expression of the glucose transporter GLUT1 has been reported in thyroid tumors only few data are available on the expression of other GLUT isoforms. We studied several GLUT isoforms expression in thyroid tumor cell lines deriving from anaplastic (ARO, FRO), papillary (NPA), follicular (WRO) and medullary (TT) human thyroid carcinoma. GLUT1 and GLUT3 were also studied in 157 human thyroid malignant and benign tissues.

Quantitative Real-time RT-PCR analysis revealed that GLUT1 mRNA levels were higher in less-differentiated cells (ARO, FRO) while GLUT3 mRNA levels were prevalent in well-differentiated cells (NPA, WRO). Accordingly, Western blot showed high expression and correct membrane targeting of GLUT1 protein in ARO and FRO and of GLUT3 protein in NPA and WRO. All cell lines were able to take-up different rates of 3H-deoxy-glucose. The analysis of GLUT1 and GLUT3 mRNA expression in human thyroid tissues showed the prevalence of GLUT1, but not of GLUT3, in malignant with respect to normal tissues. Finally, both GLUT1 and GLUT3 showed a slightly higher expression in anaplastic than in well-differentiated tumors.

In conclusion, we showed that GLUT1 and GLUT3 were the most important glucose transporters in the thyroid tumoral cells. In particular GLUT1 was the most prevalent in less-differentiated cells (ARO and FRO) while GLUT3 was the most prevalent in well-differentiated cells (NPA and WRO). A similar pattern of expression was found for GLUT1 but not for GLUT3 in human thyroid tumors.

1. Introduction

It has been extensively described that tumor cells are characterized by increased requirement for energy that, together with a reduced ability to use oxidative in favor to glycolitic pathways, results in higher glucose requirement (Hatanaka, 1974; Isselbacher, 1972a,b). This is the basis for Fluorine-18-fluoro-2-deoxy-d-glucose (18FDG) positron emission tomography (FDG-PET) (Kresnik et al., 2003) that employs 18FDG, a glucose analog, as tracer. Today, FDG-PET represents one of the most powerful imaging diagnostic tools utilized in the diagnosis of different human tumors (Scott, 2001; Strauss and Conti, 1991). Several reports indicate its usefulness in the follow-up of differentiated thyroid cancers, especially when they are not able to take-up iodine anymore. In these differentiated tumors, a positive FDG-PET has been demonstrated to have an unfavorable prognostic value (Frilling et al., 2001; Grunwald et al., 1999; Plotkin et al., 2002; Stokkel et al., 2006). This finding together with the evidence that most of anaplastic thyroid carcinomas (ATC) are usually well detected by FDG-PET (Conti et al., 1999; Khan et al., 2005) suggests that the lowest is the degree of differentiation of malignant cells the highest is their ability to take-up 18FDG at PET scan.

Facilitative glucose transporters (GLUTs) are transmembrane proteins responsible to introduce glucose into cells using the natural gradient existing between the extracellular and intracellular compartments. Genes encoding for these transporters belong to the solute carrier 2A family and are classified in three groups: class I (GLUT1–GLUT4), class II which includes the fructose transporter GLUT5 and isoforms GLUT7, GLUT9, GLUT11. Class
III (GLUT6, GLUT8, GLUT10, GLUT12 and the H+/myo-inositol transporter HMIT) (Joost et al., 2002; Joost and Thorens, 2001; Macheda et al., 2005). Since the enhanced ability in glucose uptake of tumor cells correlates with an increased expression of GLUTs, these transporters have been characterized in various tumor cell lines and in particular in cells derived from breast cancer (Macheda et al., 2005). Up to date, only two tumoral thyroid cell lines TPC-1 (papillary thyroid carcinoma) and FTC-133 (follicular thyroid carcinoma), have been analyzed for the expression of few GLUT isoforms (Matsuzu et al., 2005). Scanty data are available regarding the expression of GLUT1 in thyroid tumors, generally showing higher expression levels in differentiated thyroid tumors with poor prognosis when compared with well-differentiated tumors, benign tumors and normal thyroid tissues (Haber et al., 1997; Matsuzu et al., 2004; Schonberger et al., 2002). Interestingly, other than GLUT1, also GLUT3 has been shown to have a higher, although not significantly, expression level in thyroid tumors than in normal tissue (Matsuzu et al., 2004).

In the present study we analyzed several GLUT isoforms for their mRNA (GLUT1–4, GLUT6, GLUT8, GLUT10 and GLUT12) and protein (GLUT1 and GLUT3) expression in five cell lines derived from histologically different human thyroid carcinomas (ARO and FRO from ATC, NPA from papillary, WRO from follicular and TT from medullary thyroid carcinomas). We also evaluated glucose uptake levels in all cell lines taking into consideration a relation between the expression of GLUT isoforms and the degree of differentiation of the thyroid tumor cell lines studied. GLUT1 and 3 mRNA expression was evaluated also in 157 samples obtained from different thyroid tumor tissues.

2. Materials and Methods

2.1. Cell lines and human thyroid tissue samples

Experiments were conducted in five human thyroid carcinoma cell lines: FRO and ARO (anaplastic thyroid carcinoma), NPA (poorly differentiated papillary thyroid carcinoma), WRO (follicular thyroid carcinoma) and TT (medullary thyroid carcinoma). Cells were cultured in standard conditions as previously described (Elisei et al., 2005).

A total of 157 human thyroid tissue samples were obtained at surgery and immediately frozen and kept at −80°C until usage. Samples studied were: 37 normal thyroid (NT), 80 papillary thyroid carcinomas (PTC), 22 medullary thyroid carcinomas (MTC), 12 follicular thyroid carcinomas (FTC) and 6 anaplastic thyroid carcinomas (ATC). The 37 NT samples were the contralateral normal thyroid tissues of 37 PTC included in the 80 cases.

2.2. RNA extraction, RT-PCR and Real-time quantitative PCR for GLUTs

Total RNA was extracted from all cell lines and tissue samples using TRIzol Reagent (Invitrogen, Milan, Italy) following manufacturer’s instructions. cDNA was then synthesized and its quality was assessed by RT-PCR for the ubiquitous gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using specific primers and conditions already reported (Elisei et al., 2005). Conventional RT-PCR for genes encoding for GLUT1, GLUT2, GLUT3, GLUT4, GLUT6, GLUT8, GLUT10 and GLUT12 was performed in cell lines using specific primers and conditions as previously described (Matsuzu et al., 2004; Rogers et al., 2003; Stuart et al., 2006). GLUT1, GLUT3, GLUT4, GLUT10 mRNA levels were then quantified by real-time quantitative RT-PCR using the Real-time Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA). Positive detection of GAPDH in all samples demonstrated a good quality cDNA and negative controls, in which cDNA template was omitted, showed no contamination by genomic DNA. As shown in Table 1, the isoforms GLUT1–3 were expressed in all cell lines while GLUT4, GLUT6 and GLUT8 showed extremely weak expression. By contrast, GLUT10 showed quite strong expression in all cell lines except for TT. GLUT12 was expressed in anaplastic thyroid carcinoma cell lines FRO and ARO and medullary thyroid carcinoma cell line TT but its expression was negligible in WRO and NPA.

As shown in Table 1, the quantitative analysis of GLUT1, GLUT3, GLUT4, and GLUT10 mRNA showed high levels of expression of both GLUT1 and GLUT3, but not of GLUT4 and GLUT10, in all cell lines. When comparing the levels of expression of GLUT1 with those of GLUT3 in the cell lines (Fig. 2A), we found that GLUT1 expression levels were significantly higher in ARO and FRO cell lines (p < 0.0001 and p < 0.02, respectively), similar in WRO and NPA and lower in TT cell line. In particular, ARO showed the highest level of expression of GLUT1 and the lowest level of expression of GLUT3 when compared with the other cell lines (p < 0.0003 and p < 0.0001,
Conventional RT-PCR results for GLUT1–4, GLUT6, GLUT8, GLUT10 and GLUT12 genes in thyroid tumor cell lines. All cell lines showed the expression of GLUT1, GLUT2 and GLUT3 genes. GLUT4, GLUT6 and GLUT8 were weakly or not expressed while GLUT10 showed expression in all cell lines but TT. GLUT12 showed strong expression in ARO and TT, a weak expression in WRO and NPA and it was not expressed in WRO and NPA respectively (Fig. 2B). When cell lines where considered according to their degree of de-differentiation (ARO > FRO > WRO ≥ NPA > TT) a mRNA degree of expression levels was observed with a decreasing and increasing pattern of mRNA expression levels of GLUT1 and GLUT3, respectively (Fig. 2B).

### 3.2. GLUT1 and GLUT3 mRNA levels in human thyroid tissue samples

As shown in Fig. 3A, the paired t-test analysis of GLUT1 and GLUT3 mRNA expression in the 37 cases of PTC in which we could analyze both the normal and the tumoral tissues, demonstrated that GLUT1 mRNA was significantly more expressed in tumoral than in contralateral normal thyroid tissues (p < 0.0001) while GLUT3 mRNA was only slightly higher.

#### Table 1

Real-time RT-PCR results for GLUT1, 3, 4 and 10 (ΔCt ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>GLUT1 (ΔCt ± S.D.)</th>
<th>GLUT3 (ΔCt ± S.D.)</th>
<th>GLUT4 (ΔCt ± S.D.)</th>
<th>GLUT10 (ΔCt ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRO</td>
<td>4.33 ± 0.57</td>
<td>7.61 ± 0.60</td>
<td>32.62 ± 0.83</td>
<td>26.83 ± 1.03</td>
</tr>
<tr>
<td>WRO</td>
<td>4.82 ± 0.41</td>
<td>5.02 ± 0.43</td>
<td>31.02 ± 0.62</td>
<td>25.45 ± 0.78</td>
</tr>
<tr>
<td>ARO</td>
<td>1.20 ± 0.27</td>
<td>19.30 ± 0.30</td>
<td>33.32 ± 0.41</td>
<td>25.34 ± 0.51</td>
</tr>
<tr>
<td>NPA</td>
<td>6.04 ± 0.49</td>
<td>5.86 ± 0.51</td>
<td>32.49 ± 0.71</td>
<td>27.42 ± 0.87</td>
</tr>
<tr>
<td>TT</td>
<td>6.37 ± 0.39</td>
<td>4.91 ± 0.40</td>
<td>32.55 ± 0.56</td>
<td>38.72 ± 0.69</td>
</tr>
</tbody>
</table>

* ΔCt is the difference between the threshold cycle (Ct) relative to GLUT gene and the Ct relative to GAPDH used to normalize the results, as described in Section 2. ΔCt values are inversely correlated to mRNA levels.

The comparison of the mRNA levels of the two GLUT isoforms in the different thyroid tumor histotypes (PTC, FTC, MTC and ATC) and in the normal tissues (NT) showed that while in the latter GLUT1 was significantly less expressed than GLUT3, the opposite happened in PTC, FTC, MTC and ATC although this difference was significant only in PTC (Fig. 3B).

As far as the expression of the two isoforms in different histotypes was concerned, both GLUT1 and GLUT3 showed a slightly higher expression in anaplastic than in well-differentiated (PTC and FTC) thyroid tumors (Fig. 3C).

### 3.3. Analysis of GLUT1 and GLUT3 proteins on cell membrane and cytoplasm fractions

GLUT1 and GLUT3 proteins were studied by Western blot analysis on cell membrane and cytoplasm protein fractions. As shown in Fig. 4, GLUT1 protein revealed a very strong band with a molecular weight of 55 kDa in ARO cell membrane and cytoplasm fractions, while a less intense band was observed on both protein fractions of the other anaplastic thyroid carcinoma cell line FRO. On the other hand, GLUT1 protein appeared to be virtually absent on WRO and NPA membranes although the protein was present in the corresponding cytoplasm compartment. GLUT1 protein staining was
not visible in TT cell line, neither in membrane nor in cytoplasm fractions.

The GLUT3 protein, corresponding to a band with molecular weight of 48 kDa, was present in all cytoplasm protein fractions while it was present only in membrane protein fractions obtained from NPA and WRO. As shown in Fig. 4, ARO, FRO and TT cell lines membrane protein fractions were virtually or completely negative for GLUT3.

3.4. 3H-DG uptake experiments

As shown in Fig. 5, the experiments for 3H-deoxy-glucose cell uptake showed different glucose uptake levels in cell lines analyzed. NPA showed the highest level (4000.4 fmol 3H-DG/mg protein) followed by WRO (3567.0 fmol 3H-DG/mg protein), ARO (2300.5 fmol 3H-DG/mg protein) and FRO (2156.4 fmol 3H-DG/mg protein). TT cell line uptake was very low (530.6 fmol 3H-DG/mg protein). The inhibition of the uptake by cytochalasin B demonstrated that the uptake was specifically glucose transporter-mediated in all cell lines. The need to use a higher concentration of cytochalasin B to inhibit the uptake in ARO cell line (1.5 mmol/l vs. 0.05 mmol/l) might be related to the higher levels of expression of the GLUT1.

4. Discussion

In the present study we observed that all thyroid tumors derived cell lines studied (ARO, FRO, NPA, WRO and TT) showed a quite heterogeneous expression pattern for different GLUT isoforms when analyzed by conventional RT-PCR. However, the quantitative Real-time RT-PCR analysis of GLUTs showed that anaplastic carcinoma cell line ARO had a significantly higher amount of GLUT1 when compared with all other cell lines. At variance, both NPA and TT cell lines, arising from more differentiated thyroid carcinomas such as papillary and medullary, showed lower levels of GLUT1. This finding
is in keeping with the previously reported observation that GLUT1 over-expression correlates with more de-differentiated forms of thyroid tumor (Schonberger et al., 2002; Stokkel et al., 2006) that are, indeed, those with a higher ability to take-up 18 FDG (Conti et al., 1999; Khan et al., 2005).

As opposed to GLUT1, quantitative real-time RT-PCR showed that GLUT3 expression levels were very low in anaplastic ARO cell line but significantly higher in all other cell lines, in particular in NPA and FRO cell lines. These results suggest that the expression levels of GLUT1 and GLUT3 have an inverse correlation with the degree of cell differentiation.

The 3H-DG uptake experiments demonstrated that, despite the highest levels of GLUT1 expression and its correct localization on the plasma membrane, anaplastic ARO and FRO cell lines showed lower uptake ability when compared with papillary NPA and follicular WRO cell lines. This discrepancy between GLUT1 expression levels and 3H-DG uptake could be due to a defect of N-glycosylation status as already observed in primary anaplastic thyroid cell lines by other authors (Samih et al., 2003).

Since the level of expression of GLUT1 is lower in both NPA and WRO, their highest ability to take-up 3H-deoxy-glucose could be correlated with both the highest levels of expression of GLUT3 and the correct plasma membrane localization of the protein, which has been demonstrated to have a very high affinity for glucose analogs, especially at low glucose concentrations (Burant and Bell, 1992; Gould et al., 1991). The crucial role of a correct plasma membrane localization of GLUT3 protein for its ability to take-up 3H-deoxy-glucose was clearly demonstrated in TT cell line that expressed GLUT3 mRNA but did not display a plasma membrane protein and were, indeed, unable to incorporate the glucose tracer. The presence of GLUT3 transcript in the absence of a functional protein has been previously reported in other human tumors (Boado et al., 1994; Higashi et al., 1998; Yamamoto et al., 1990).

As far as the GLUT1 and GLUT3 mRNA expression in thyroid tumor is concerned, our data support the results of the few reports that have been published up to date (Haber et al., 1997; Matszu et al., 2004; Schonberger et al., 2002) which showed a significant increase of GLUT1 and a slightly increase of GLUT3 mRNA levels in malignant thyroid tissues when compared to normals. The evidence of a decreasing pattern of mRNA expression levels of GLUT1 in cell lines accordingly to their pattern of de-differentiation was confirmed in human thyroid tumors with ATC showing a higher expression than PTC and FTC. To our knowledge, no data have been yet reported on the relationship between GLUT3 and the tumor differentiation degree. Unfortunately our findings on cell lines were not confirmed in the tumoral tissues that showed a higher level of expression of GLUT3 in ATC than in PTC and FTC. This controversial data are difficult to be explained but we should take into account that the number of ATC was quite small and that not always in vitro models are representative of what really happens in vivo. On this regard, it is well known that cell lines can change their features as consequence of a long term in vitro evolution (van Staveren et al., 2007).

In conclusion, we demonstrated that GLUT1 and GLUT3 are the most important glucose transporters involved in the thyroid tumoral cell metabolism. In particular GLUT1 represents the main GLUT isomorph in the anaplastic ARO and FRO cell lines while GLUT3 appears to be predominant in more differentiated thyroid tumor cell lines such as papillary NPA and follicular WRO. However, the analysis of GLUT1 and GLUT3 mRNA expression in human thyroid tumors confirmed that only GLUT1 was significantly more expressed in malignant than in the corresponding normal tissues and that its expression is higher in more aggressive histotypes.

The clinical meaning and the role in 18-FDG uptake of GLUT3 remains to be established.

**Conflict of interest**

None.

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**References**


