Differences in the Expression of Glucose Transporter Protein Isoforms in Human Retinoblastoma Cell Lines

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Abstract: We investigated the expression of glucose transporter (GLUT) protein isoforms in two human retinoblastoma cell lines, Y79 and WERI-Rbl, by Western blotting analysis with anti-GLUT1, 2, 3, and 4 antibodies. GLUT1 and GLUT4 proteins were detected in Y79, whereas GLUT1 and GLUT3 proteins were found in WERI-Rbl. GLUT2 protein was not detected in Y79 or WERI-Rbl. Our findings are of interest because (1) the expression of GLUT protein isoforms in the two retinoblastoma cell lines was different, and (2) GLUT4 protein, the insulin-sensitive GLUT isoform, was detected in Y79. This suggests that these cell lines have different mechanisms of glucose transport. Jpn J Ophthalmol 1997;41:226-230 © 1997 Japanese Ophthalmological Society

Key Words: Glucose transporter, retinoblastoma, WERI-Rbl, Y79.

Introduction

Retinoblastoma is a malignant tumor derived from the human retina that requires glucose as an energy source for proliferation. The uptake of glucose into retinoblastoma cells is mediated by glucose transporter (GLUT) protein. Of the seven GLUT protein isoforms, GLUT1, 2, 3, and 4 proteins are involved in the transport of glucose across plasma membranes.1

GLUT protein isoforms identified in the retina are: GLUT1 protein in various species,2-10 GLUT2 protein in the rat,11 and GLUT3 protein in humans.6 However, the GLUT4 protein has never been detected in the retina.

The isoforms of GLUT protein expressed in human retinoblastoma cells have not been clarified. To our knowledge, there has been only one report that stated GLUT1 protein was not found in the primary retinoblastoma tissues; the other GLUT protein isoforms were not investigated.12

Materials and Methods

Cell Culture

The Y79 cell line was purchased from Dainippon Pharmaceuticals (Osaka, Japan), and the WERI-Rbl cell line from American Type Culture Collection (Rockville, MD, USA). Both cell lines were maintained in suspension cultures in RPMI 1640 medium with 10% fetal bovine serum, 2 mmol/L glutamine, and antibiotics under humidified 5% CO₂ and 95% air at 37°C.

Sample Preparation

Cells were homogenized in SVE solution (0.25 mol/L sucrose, 1 mmol/L EDTA, 0.1% ethanol, pH 7.3) containing 1 mmol/L phenylmethylsulfonylfluoride and 2 µg/mL leupeptin. The homogenates were centrifuged at 1000 × g for 10 minutes and the supernatant was centrifuged at 100,000 × g for 30 minutes. The resultant pellet was suspended in SVE solution and used as the crude membrane fraction for Western blotting analysis.
Table 1. Primary Antibodies for Western Blotting Analysis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen</th>
<th>Dilution</th>
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<tbody>
<tr>
<td>GLUT1</td>
<td>Carboxy terminus of rat GLUT1&lt;sup&gt;25&lt;/sup&gt;</td>
<td>1:5000</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Carboxy terminus of human GLUT2&lt;sup&gt;26&lt;/sup&gt;</td>
<td>1:1000</td>
</tr>
<tr>
<td>GLUT3</td>
<td>Carboxy terminus of human GLUT3&lt;sup&gt;27&lt;/sup&gt;</td>
<td>1:500</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Carboxy terminus of rat GLUT4&lt;sup&gt;28&lt;/sup&gt;</td>
<td>1:500</td>
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</tbody>
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Western Blotting

The proteins of the crude membrane fractions separated by sodium dodecyl sulfate-polyacrylamide gel (10% polyacrylamide) electrophoresis were transferred onto nitrocellulose membranes. These membranes were incubated with primary and secondary antibodies. All primary antibodies (Table 1) were obtained from Eastacres Biologicals (Southbridge, MA, USA). GLUT proteins were detected with a chemiluminescence reagent (Runaissance) from DuPont (Boston, MA, USA).

Detection of Nonglycosylated GLUT Protein

Tunicamycin inhibits glycosylation of protein. To detect GLUT proteins under nonglycosylated conditions, cells were cultured in the media containing 1 txg/mL tunicamycin for 24 hours and Western blotting analysis was done as described above.
Figure 4. Western blotting in Y79, WERI-Rbl, and rat muscle with anti-GLUT4 antibody.

Table 2. Expression of GLUT Isoforms in Y79 and WERI-Rbl

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Y79</th>
<th>WERI-Rbl</th>
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<tbody>
<tr>
<td>GLUT1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GLUT2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GLUT3</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>GLUT4</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ : detected. - : not detected.

Results

Isoforms of GLUT Protein

A 45kDa GLUT1 protein was detected in rat brain (positive control), Y79, and WERI-Rbl (Figure 1). A 60kDa GLUT2 protein was detected in rat liver (positive control), but not in Y79 or in WERI-Rbl (Figure 2). A 47kDa GLUT3 protein was detected in human white blood cells (positive control) and WERI-Rbl, but not in Y79 (Figure 3). A 50kDa GLUT4 protein was detected in rat skeletal muscle (positive control) and Y79, but not in WERI-Rbl (Figure 4).

Detection of Nonglycosylated GLUT Protein

Nonglycosylated GLUT proteins were newly identified after tunicamycin treatment: a 40kDa GLUT1

Figure 5. Western blotting in Y79 and WERI-Rbl treated with 1 µg/mL tunicamycin for 24 hours with (A) anti-GLUT1, (B) anti-GLUT3, and (C) anti-GLUT4 antibody.
forms differs in the two retinoblastoma cell lines, protein was found in Y79 and WERI-Rb1 (Figure 5A), a 41kDa GLUT3 protein in WERI-Rb1 (Figure 5B) and a 46kDa GLUT4 protein in Y79 (Figure 5C).

Discussion

In this article we identified the expression of GLUT protein isoforms in Y79 and WERI-Rb1. Our results are summarized in Table 2. We also detected isoforms of GLUT protein under nonglycosylated conditions.

It was of interest that the expression of GLUT3 and GLUT4 proteins differed in the two cell lines: GLUT3 protein was found in WERI-Rb1, but not in Y79; whereas GLUT4 protein was detected in Y79, but not in WERI-Rb1. GLUT 3 protein, the major neuronal GLUT isoform, has been found in the normal human retina. In this study, GLUT3 protein was expressed in WERI-Rb1, but not in Y79. Based on these findings, Y79 may have fewer characteristics of neuronal cells than WERI-Rb1.

GLUT4 protein is known to be expressed in muscle cells and adipocytes. Several recent studies showed that GLUT4 protein is found in the central nervous system, especially in the cerebellum and in a few cases of human astrocytic tumors. In the retina, no GLUT4 protein has been detected. GLUT4 protein, not found in the normal retina, was expressed in Y79. This suggests that GLUT4 protein was newly expressed during the canceration of the cells.

GLUT4 protein is also known to be regulated by insulin. The expression of GLUT4 protein in Y79 seems to be related to the presence of an insulin receptor in Y79, whereas GLUT4 protein was detected in Y79, but not in WERI-Rb1. GLUT 3 protein, the major neuronal GLUT isoform, has been found in the normal human retina.6 In this study, GLUT3 protein was expressed in WERI-Rb1, but not in Y79. Based on these findings, Y79 may have fewer characteristics of neuronal cells than WERI-Rb1.

GLUT4 protein was newly expressed during the canceration of the cells.

We report here that the expression of GLUT isoforms differs in the two retinoblastoma cell lines, suggesting that the mechanism of glucose transport into retinoblastoma cells is specific for each type.

References