A Comparison of Glycosyltransferase Activities and Malignant Properties in Normal and Transformed Cells Derived from BALB/c Mice

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SUMMARY

The ability of suspensions of BALB/c cells to catalyze the incorporation of nucleotide sugars into complex polysaccharides has been compared. These cells have previously been characterized for concanavalin A-induced agglutinability, tumorigenicity, and malignancy. All of the cell lines tested catalyze transfer of the sugar moieties of cytosine 5'-monophosphate N-acetylneuraminic acid, uridine 5'-diphosphate galactose, uridine 5'-diphosphate N-acetylglucosamine, uridine 5'-diphosphate N-acetylglucosaminyltransferase, and increased sialic acid transfer to desialized cell surface glycosyltransferases. While some transformed lines exhibit alterations in transferase levels, others cannot be distinguished from normal cells. Normal cells, transformed cells that cause tumors that regress, and transformed cells that cause tumors that kill an immunologically competent host show growth-dependent changes in transferase activities. Determining the ability to catalyze carbohydrate transfer is insufficient for predicting the tumorigenic and malignant properties of a cell line.

INTRODUCTION

A number of investigators have reported altered glycosyltransferase levels associated with transformation. Observations of transformed cells have included decreases in the ability to catalyze sialic acid transfer to glycoproteins and glycolipids (7-10), decreased galactose and N-acetylgalactosamine transfer to glycolipids (5, 6, 12), loss of contact-induced increases in galactose transfer to glycolipids (11), and increased sialic acid transfer to desialized cell surface glycopeptides (20). Recently, glycosyltransferases have been postulated to be directly involved in the biology of cell-to-cell interactions. Rosman (16) suggested that glycosyltransferases can be exposed on the surfaces of cells and that the interaction between a particular transferase of one cell and a specific potential glycosyl acceptor of another could regulate cell adhesions and contact inhibition. Evidence supporting the postulated function for cell surface glycosyltransferases has been published. Roth and White (18) studied galactosyltransferases of BALB/c 3T3 and 3T12 cells and presented evidence supporting a cell surface location for the activity. In those studies normal cells, in contrast to transformants, appeared to require cell-to-cell contact before transferase activities could be detected (18). In recent work, Yogeeswaran et al. (21) reported that suspended normal cells can catalyze carbohydrate transfer from exogeneous nucleotide sugars to glycolipids attached to glass beads. Transformed cells had decreased abilities to catalyze carbohydrate transfer to glycolipid acceptors. Bömmann (2), in studies of DNA and RNA virus-transformed cells, extended these observations and further implicated an involvement of glycosyltransferases detected in suspended cells with cancer. In those experiments, transformed cells were observed to have elevated glycosyltransferase activities. In other studies, Bömmann et al. (3) have reported that high metastatic as compared with low metastatic melanoma cell lines from C57 mice have elevated cell surface glycosyltransferases. It was suggested that these surface transferases might function in adhering the cells to new implantation sites (secondary metastases). Finally, recent studies by Bömmann and Hall (4) have indicated that human malignant neoplastic tissues, compared to normal or benign tissues, have elevated glycoprotein: sialyltransferase levels detected in cell suspensions.

In unrelated studies, Podolsky et al. (15) have suggested that galactosyltransferases on the surfaces of cells are the cellular concanavalin A-binding sites. In those studies, a direct correlation was observed between intact cell-associated galactosyltransferase activity and the concentration of concanavalin A, which causes agglutination. Glycosyltransferases detected in intact cells have been the subject of a recent review (17).

We have recently reported studies of lectin-stimulated agglutination and tumorigenic properties of a variety of BALB/c fibroblasts (19). The normal and spontaneous or viral transformants could be divided into 3 categories: (a) normal, nontumorigenic; (b) tumor producing, but tumors regress when cells are injected into immunocompetent BALB/c mice; (c) tumor producing and malignant, in all cases leading to death of an animal receiving tumor cells. Concanavalin A-stimulated agglutinability could usually, but not always, predict tumorigenicity but failed to predict whether a tumorigenic cell line would cause a regressing tumor or a progressing tumor that kills. In the results reported in this communication, we have determined whether measuring glycosyltransferase levels detected in suspended BALB/c cells was capable of predicting con-
canavalin A agglutinability, tumorigenicity, or malignancy.

**MATERIALS AND METHODS**

**Cell Lines.** A description of the various cell lines and their methods of production and cell growth has been published (19). A31, SVT2, and 3T12 cells were gifts of Dr. G. Todaro, NIH, Bethesda, Md. A31 is a clone of BALB/c 3T3 cells. KMSV cells were a gift of Dr. L. Culp, Case Western Reserve University, Cleveland, Ohio. PBC cells are low-passage BALB/c fibroblasts prepared from 16-day gestation embryos. Both 3T3 and PBC cells are normal and do not produce tumors when injected into 3-week-old immunocompetent BALB/c mice. 3T12 and SVT2 cells are spontaneously and SV40 transformed BALB/c fibroblasts. Both lines cause tumors that regress when 5 × 10⁶ cells are injected into 3-week-old immunocompetent BALB/c mice. 2°KMSVT and 3T12T are tumor cell lines produced from tumors caused by injecting KMSV and 3T12 cells into BALB/c mice (19). The KMSV cells have been transformed by murine sarcoma virus but do not produce virus or viral particles, and they have been shown to lack murine leukemia virus antigens (1). Both 2°KMSVT and 3T12T cells produce progressively growing malignant tumors that kill any animal receiving from 10⁴ to 5 × 10⁶ cells within 30 days. We have previously published studies of the concanavalin A-stimulated agglutinability of these cell lines. The concentration of concanavalin A causing half-maximal agglutinability was: A31, > 1000 µg/ml; PBC, 600 µg/ml; 2°KMSVT, 600 µg/ml; 3T12, 3T12T, and SVT2, 50 µg/ml.

**Substrates.** CMP-[14C]NeuNAc (229 mCi/m mole), UDP-[14C]Gal (254 mCi/m mole), UDP-[14C]GalNAc (51.5 mCi/m mole), UDP-[14C]GlcNAc (56.5 mCi/m mole), UDP-[14C]Glc (227 mCi/m mole), and GDP-[14C]Man (160 mCi/m mole) were purchased from New England Nuclear, Boston, Mass. Ethanol used for storing substrates was removed prior to their use.

**Glycosyltransferase Measurements.** Methods for cell preparation, conditions for glycosyltransferase measurements, assay procedures for glycoproteins and glycolipids, and characterization of reaction products have been described (13). Cells approximately half-confluent on as-scribed (13). Cells approximately half-confluent on are replated at 10° for 16 hr. We have also observed that primary hamster cell lines have elevated abilities to catalyze glycosyltransfer as compared with suspended established hamster cell lines (L. M. Patt, unpublished observation). Three of the cell lines showed a growth effect on glycosyltransferase activities.

**RESULTS AND DISCUSSION**

In an effort to correlate biochemical aspects of the cell surface with changes leading to cancer, we have studied several normal, spontaneously transformed, and virally transformed cell lines derived from inbred BALB/c mice (19). The properties of several of the cell lines are summarized in "Materials and Methods." All of the cell lines tested can catalyze transfer of N-acetylneuraminic acid, galactose, N-acetylgalactosamine, N-acetylglucosamine, glucose, and mannose from nucleotide sugar donors to both glycolipids and glycoproteins (Tables 1 and 2). The highest activities we have observed are found in the normal PBC cells. Apparently, the process of establishment leads to a lowering of glycosyltransferase levels detected in this system. We have also observed that primary hamster cell lines have elevated abilities to catalyze glycosyltransfer as compared with suspended established hamster cell lines (L. M. Patt, unpublished observation). Three of the cell lines showed a growth effect on glycosyltransferase activities.
Specific activities of glycoprotein glycosyltransferases of suspended normal and transformed cells

Reactions in a total volume of 100 μl of 0.15 M NaCl-0.02 M Tris-HCl, pH 7.4, contained 10^6 A31, 3T12, 3T12T, 2°KMSVT cells, or 2 x 10^6 SVT2 cells; 25,000 cpm of the appropriate nucleotide sugar; and the optimum concentration of inorganic ions as follows: CMP-NeuNAc, 5 mM Mn^2+; UDP-Gal, 5 mM Mn^2+; UDP-GalNac, 5 mM Mn^2+; UDP-GlcNAc, 15 mM Mg^2+; UDP-Glc, 15 mM Mg^2+; and GDP-Man, 15 mM Mg^2+. Reactions were incubated for 1 hr at 37°C and incorporation into glycoprotein was determined as described in “Materials and Methods.” The values shown represent the activity detected in from 1 to 5 cell preparations, each assayed in triplicate.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>A31</th>
<th>PBC</th>
<th>SVT2 (cpm/mg)</th>
<th>Growth</th>
<th>Confluent</th>
<th>2°KMSVT (protein)</th>
<th>Growth</th>
<th>Confluent</th>
<th>Growth</th>
<th>Confluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMP-NeuNAc</td>
<td>1022 ± 96</td>
<td>2207 ± 58</td>
<td>3569 ± 260</td>
<td>1270 ± 281</td>
<td>2180 ± 32</td>
<td>564 ± 32</td>
<td>1745 ± 181</td>
<td>1283 ± 65</td>
<td>2060 ± 56</td>
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<tr>
<td>UDP-Gal</td>
<td>903 ± 46</td>
<td>1617 ± 130</td>
<td>4554 ± 183</td>
<td>811 ± 200</td>
<td>583 ± 20</td>
<td>608 ± 44</td>
<td>1679 ± 263</td>
<td>206 ± 19</td>
<td>635 ± 204</td>
<td></td>
</tr>
<tr>
<td>UDP-GalNac</td>
<td>590 ± 64</td>
<td>851 ± 175</td>
<td>2233 ± 20</td>
<td>705 ± 98</td>
<td>390 ± 31</td>
<td>612 ± 34</td>
<td>647 ± 21</td>
<td>210 ± 36</td>
<td>217 ± 47</td>
<td></td>
</tr>
<tr>
<td>UDP-GlcNAc</td>
<td>775 ± 206</td>
<td>725 ± 54</td>
<td>2283 ± 163</td>
<td>996 ± 56</td>
<td>511 ± 206</td>
<td>334 ± 55</td>
<td>269 ± 40</td>
<td>207 ± 22</td>
<td>228 ± 33</td>
<td></td>
</tr>
<tr>
<td>UDP-Glc</td>
<td>452 ± 100</td>
<td>987 ± 20</td>
<td>3314 ± 104</td>
<td>537 ± 20</td>
<td>366 ± 29</td>
<td>367 ± 84</td>
<td>495 ± 192</td>
<td>417 ± 39</td>
<td>427 ± 16</td>
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</tr>
<tr>
<td>GDP-Man</td>
<td>3101 ± 262</td>
<td>7511 ± 1452</td>
<td>12392 ± 738</td>
<td>2657 ± 570</td>
<td>4641 ± 211</td>
<td>1153 ± 305</td>
<td>3580 ± 438</td>
<td>2614 ± 404</td>
<td>3082 ± 1173</td>
<td></td>
</tr>
</tbody>
</table>

a Growth, cells harvested before extensive cell-to-cell contacts; Confluent, cells prepared just at the time of extensive cell-to-cell contact.

The values shown represent the activity detected in from 1 to 5 cell preparations, each assayed in triplicate.

Specific activities of glycolipid glycosyltransferases of suspended normal and transformed cells

Reactions in a total volume of 100 μl of 0.15 M NaCl-0.02 M Tris-HCl, pH 7.4, contained 10^6 A31, 3T12, 3T12T, 2°KMSVT cells, or 2 x 10^6 SVT2 cells; 25,000 cpm of the appropriate nucleotide sugar; and the optimum concentration of inorganic ions as follows: CMP-NeuNAc, 5 mM Mn^2+; UDP-Gal, 5 mM Mn^2+; UDP-GalNac, 5 mM Mn^2+; UDP-GlcNAc, 15 mM Mg^2+; UDP-Glc, 15 mM Mg^2+; and GDP-Man, 15 mM Mg^2+. Reactions were incubated for 1 hr at 37°C and incorporation into glycolipid was determined as described in “Materials and Methods.” The values shown represent the activity detected in from 1 to 5 cell preparations, each assayed in triplicate.

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<tr>
<th>Substrate</th>
<th>A31</th>
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<th>SVT2 (cpm/mg)</th>
<th>Growth</th>
<th>Confluent</th>
<th>2°KMSVT (protein)</th>
<th>Growth</th>
<th>Confluent</th>
<th>Growth</th>
<th>Confluent</th>
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<tr>
<td>CMP-NeuNAc</td>
<td>931 ± 83</td>
<td>1973 ± 135</td>
<td>3100 ± 736</td>
<td>858 ± 361</td>
<td>1278 ± 90</td>
<td>836 ± 127</td>
<td>1851 ± 288</td>
<td>1258 ± 92</td>
<td>1209 ± 93</td>
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<tr>
<td>UDP-Gal</td>
<td>338 ± 19</td>
<td>1253 ± 247</td>
<td>2879 ± 130</td>
<td>561 ± 140</td>
<td>370 ± 10</td>
<td>332 ± 26</td>
<td>1297 ± 478</td>
<td>276 ± 15</td>
<td>332 ± 98</td>
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<tr>
<td>UDP-GalNac</td>
<td>113 ± 50</td>
<td>326 ± 167</td>
<td>331 ± 120</td>
<td>710 ± 380</td>
<td>114 ± 23</td>
<td>380 ± 32</td>
<td>592 ± 70</td>
<td>124 ± 30</td>
<td>103 ± 65</td>
<td></td>
</tr>
<tr>
<td>UDP-GlcNAc</td>
<td>328 ± 193</td>
<td>481 ± 181</td>
<td>1104 ± 97</td>
<td>665 ± 240</td>
<td>173 ± 99</td>
<td>108 ± 39</td>
<td>145 ± 32</td>
<td>145 ± 10</td>
<td>142 ± 25</td>
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</tr>
<tr>
<td>UDP-Glc</td>
<td>624 ± 19</td>
<td>1699 ± 102</td>
<td>3477 ± 633</td>
<td>1447 ± 200</td>
<td>1377 ± 53</td>
<td>192 ± 29</td>
<td>422 ± 26</td>
<td>136 ± 66</td>
<td>315 ± 50</td>
<td></td>
</tr>
<tr>
<td>GDP-Man</td>
<td>2515 ± 197</td>
<td>6741 ± 582</td>
<td>8031 ± 559</td>
<td>883 ± 199</td>
<td>480 ± 73</td>
<td>1340 ± 150</td>
<td>2705 ± 150</td>
<td>1305 ± 215</td>
<td>3255 ± 515</td>
<td></td>
</tr>
</tbody>
</table>

a Growth, cells harvested before extensive cell-to-cell contacts; Confluent, cells prepared just at the time of extensive cell-to-cell contact.

The values shown represent the activity detected in from 1 to 5 cell preparations, each assayed in triplicate.

compared with confluent cells. The changes in enzyme levels determined was independent of the cell saturation density, because 3T12 and 3T12T cells lack contact inhibition of growth. The elevation in transferase activities appears to occur just as cells reach confluency. Cells that have been maintained for long periods of time at confluency have transferase levels more similar to growing cells. These results are in contrast to those of Podolsky et al. (15). How intact cells catalyze transfer of CMP-NeuNAc, UDP-Gal, UDP-GalNac, UDP-GlcNAc, UDP-Glc, and GDP-Man is unknown. The evidence rules out the possibility that the substrates are transported into the cellular pools of nucleotide sugars (13). These observations do not eliminate the possibility that nucleotide sugar is transported into cellular compartments or vesicles (Golgi derived?) where the sugar moiety is rapidly transferred to glycoproteins and glycolipids. The recent observation of carbohydrate transfer from endogenous or exogenous nucleotide sugar suggests that cellular complex polysaccharide synthesis may routinely occur on the cell surface (21). We have previously suggested that the use of whole cells suspended in the presence of nucleotide sugars allows the study of in vivo complex polysaccharide biosynthesis. We were unable to obtain evidence supporting the hypothesis that cell surface glycosyltransferases play a direct role in contact inhibition or cell-to-cell interactions (13). In addition, measuring
glycosyltransferase levels in suspended cells does not allow a prediction of whether a cell line is normal or tumorigenic or causes tumors that regress or grow progressively.

REFERENCES

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