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The control of sialyltransferase activity in tumor-cell lines derived from different tissues is multifactorial

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Abstract The activities of the sialyltransferase enzymes and the resulting expression of sialoglycoproteins were examined in tumor cells derived from different tissues in order to gain a greater understanding of the factors controlling the cell glycosylation state. Cell–cell contact, which is dependent on cell confluency state, was shown to influence glycosylation in the neurally-derived mouse neuro-2A neuroblastoma and the C6 glioma cell lines. Both showed a relatively high level of cell sialyltransferase activity under sub-confluent conditions with activity decreasing upon the formation of cell–cell contacts associated with confluency. A parallel decrease in the expression of sialoglycoproteins, as determined by lectin blot analysis, was also observed under these conditions. In contrast, the H411e hepatoma cell line showed an increase in enzyme activity with confluency with the susceptibility of the enzyme in this cell line to glucocorticoid induction only being detected in sub-confluent cell cultures. The number of trypsinisation cycles of the cells was also shown to affect the enzyme activity of the neuro-2A and C6 cells with an increase in enzyme activity coincident with passage number being observed in the neuro-2A cells, and a decrease in the C6 glioma cell line. Trypsinisation had no effect on enzyme activity in the H411e cells. These results demonstrate that the control of sialyltransferase activity in tumor cells is multifactorial with the tissue of origin playing a key role.

Key words: Sialyltransferase; Glycoprotein; Neuroblastoma; Hepatoma; Glioma; Sialic acid

1. Introduction

Tumor cells express altered glycosylation characteristics with an over-expression of the negatively charged sialic acid sugar in particular being associated with an increase in tumor metastatic potential [1]. The final stage in the biosynthetic pathway of sialoglycoconjugates is partly controlled by the sialyltransferase (sialyl-T) enzymes, which belong to the glycosyltransferase enzyme family, and which catalyse the transfer of activated sialic acid (CMP-Neu5Ac) to terminal positions on the oligosaccharide chains of glycoproteins and glycolipids at the level of the Golgi. Removal of sialic acid from the glycoconjugate can subsequently be effected by the action of the neuraminidase enzyme which is located primarily in the lysosome [2] but which has also been demonstrated at the level of the plasma membrane [3]. Sialic acid can be joined onto a number of acceptor sugars including galactose, *N*-acetyl galactosamine and *N*-acetyl glucosamine in both α2,3 and α2,6 linkages, with each transfer being catalysed by an individual sialyl-T isozyme [4]. Sialic acid can in addition be transferred to sialic acid residues in α2,8 linkages to form polysialic acid chains (PSA) of up to 50 residues in length, which are expressed in a developmentally regulated manner on the neural cell adhesion molecule, NCAM [5]. The expression of protein-bound sialic acid is altered in certain tumor cell lines where it acts to influence cell adhesivity and consequently the invasive or metastatic potential of the cell [6]. Tumor cells with significant metastatic potential tend to have a high sialyl-T activity [7], and treatment of adenocarcinoma cells with an enzyme inhibitor was demonstrated to decrease metastasis [8].

While the role of cellular transcription factors and oncogenes [9], and also external agents such as glucocorticoids and cytokines [10–12] in the control of sialyl-T activity have been well characterised, the role of cell–cell interaction in this process has not been addressed. As cell adhesion molecule (CAM) mediated cell–cell contact can activate a cellular signalling mechanism [13] and has previously been demonstrated to influence the expression of cell surface adhesion ligands [14], it was of interest to examine the effect of cell–cell contact on both basal sialyl-T expression in cell lines derived from different tissues and also to observe the enzyme susceptibility to regulation by external factors such as corticosteroids under various states of cell confluency.

2. Materials and methods

2.1. Cell culture

Neuro-2A mouse neuroblastoma cells, C6 rat glioma cells and H411e rat hepatoma cells were cultured, as previously described, in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (FCS), 1% (v/v) penicillin and 1% (v/v) streptomycin [14]. Cells were split (passaged) by trypsinisation and reseeded at a density of 5×10^5 cells (neuro-2A and C6) or 7.5×10^5 cells (H411e) per 75 cm^2 flask and the cell confluency status monitored by nuclear counting using the crystal violet stain [15]. At specific stages, the cells were harvested by scraping and resuspended by sonication in water containing protease inhibitors. The protein content of the samples was determined by the method of Lowry et al. [16].

2.2. Sialyltransferase assay

Total sialyltransferase activity was determined as previously described [3] using cytidine-5-monophosphate-4,5,6,7,8,9-^{14}C-*N*-acetylneuraminic acid (CMP-^{14}C-Neu5Ac, Radiochemical Centre, Amersham; specific activity 293 mCi/mmol) as the sialic acid donor asialofetuin as an exogenous acceptor.

2.3. Glycoprotein analysis

Glycoconjugate expression was determined by lectin blot analysis as previously described [17]. Briefly, 50 μg protein samples were separated by discontinuous SDS polyacrylamide gel electrophoresis and transferred to PVDF membranes (Millipore) by electroblotting. Glycoproteins containing sialic acid attached to Gal residues in α2,3-linkages and

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sialic acid attached to Gal/GalNAc residues in α2,6-linkages were detected using the *Maackia amurensis* and *Sambucus nigra* lectins respectively and visualised using the DIG Glycan Differentiation Kit (Boehringer Mannheim).

3. Results and discussion

Sialoglycoproteins play different roles in particular tissues within the body. In the nervous system, for example, the majority of sialoglycoproteins are membrane-bound and play a key role in the mediation of cell–cell interactions [18]. In the liver, the majority of sialoglycoproteins are soluble proteins which are released upon stimulation, for example, as part of the acute-phase immune response [19].

In this study, we have examined the activity of the sialyl-T enzyme, as well as the expression of sialoglycoproteins, in a number of tumor cell lines derived from different tissues with a particular interest in the role of cell contact on these parameters. In the embryonic nervous system in vivo, there is a developmentally-regulated decrease in sialyl-T activity which corresponds well with the onset of cell–cell adhesion and synaptogenesis [3,20,21] with subsequent alterations in the developmental expression of sialoglycoproteins causing an upset in neuronal patterning with consequent mental deficits [22,23]. Sialyl-T activity decreased significantly in parallel with the attainment of confluency in both the neuro-2A neuroblastma and C6 glioma cell lines (Fig. 1). This parallels the time course previously observed in vivo [24] and was accompanied in the neuro-2A cells by a general decrease in the expression of both α2,3- and α2,6-linked sialoglycoproteins (Fig. 2). Interestingly, although the enzyme activity is highest on day 1 after seeding, the expression of sialoglycoproteins is low and does not reach a maximum until day 2. This is probably due to the cleavage of cell surface glycoproteins during the trypsinisation procedure, which is followed by a delay in the re-expression of these proteins. Altered neuraminidase activity may also be responsible for this anomaly.

In contrast to the neuronal cell lines, sialyl-T activities in the H411e hepatoma cell line increased in parallel with cell confluency state (Fig. 1). This may reflect the differing role of the enzyme and the sialoglycoprotein substrates in the two cell lines derived from distinct tissues. Sialoglycoproteins play a pivotal role in cell–cell interaction in the neural cell lines, with the cells in a deadhesive state expressing relatively high levels of sialic acid in the early developmental stage. In the hepatoma cells, however, sialoglycoproteins are mainly secreted by the cell in response to certain stimuli, such as inflammation and it is likely, therefore, that the cells will be required to maintain a significant enzyme activity in the mature confluent state.

Although previous studies have demonstrated sialyl-T activity to be inducible by the synthetic glucocorticoid dexamethasone in hepatoma cells [11,25], enzyme induction in the H411e cells was shown to be influenced by the confluency state of the cells, with significant induction only being observed in non-confluent cell cultures (Fig. 3). As an increase in the release

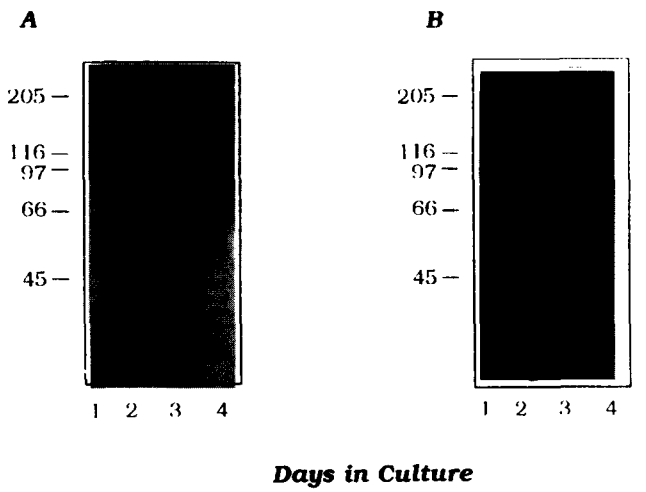

Fig. 2. The effect of cell confluency state on the expression of (A) α2,3- and (B) α2,6-linked sialoglycoproteins in Neuro-2A cells. The migration of molecular weight standards is indicated.

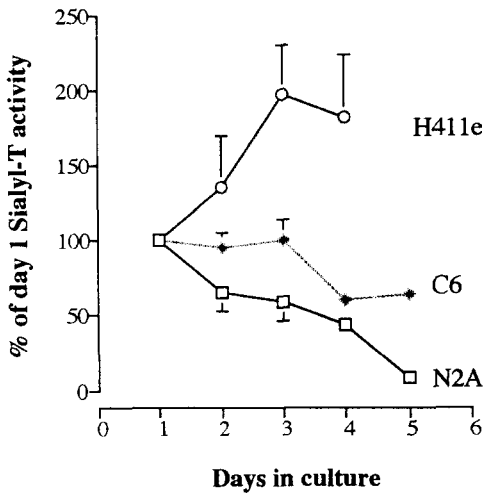

Fig. 1. The effect of increasing cell confluency state on total cellular sialyltransferase activity. Activities are expressed as a percentage of the activity per mg protein on day 1 post-plating and represent mean ± S.E.M. ($n = 3$).

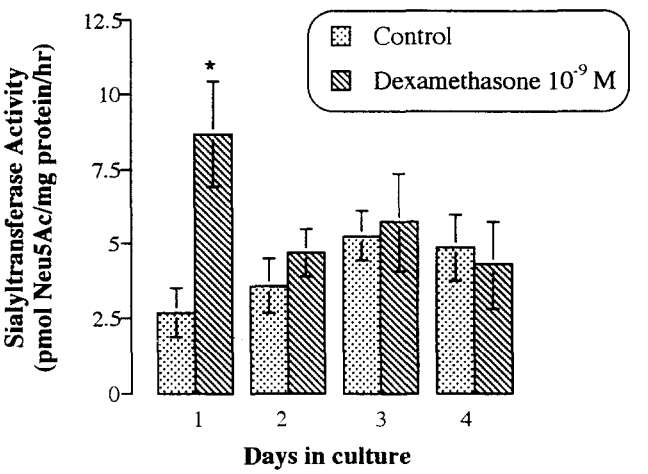

Fig. 3. The effect of cell confluency on dexamethasone induction of sialyl-T activity in H411e cells. The cells were plated at day 0 and were treated with dexamethasone (10^{-9} M) for 18 h prior to harvesting on days 1, 2, 3 and 4 post-plating. Results are expressed as mean ± S.E.M. ($n = 3$). *indicates $P < 0.05$ vs. control (Students t-test).

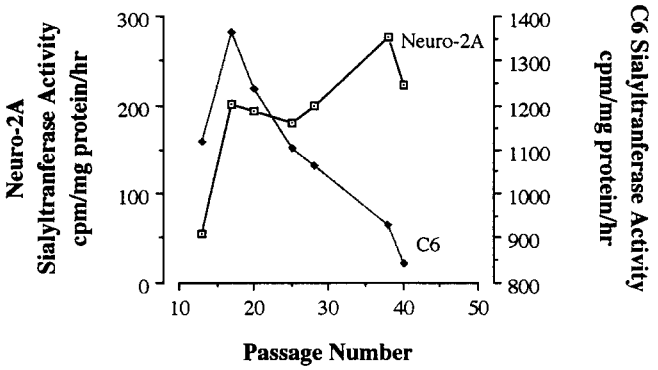

Fig. 4. The effect of increasing passage number on the sialyl-T activities in Neuro-2A and C6 cells (n = 1).

of acute-phase glycoproteins from the liver in response to agents such as corticosteroids have been characterised in vivo, this loss of dexamethasone-stimulated enzyme induction in confluent hepatoma cell cultures may reflect the tumorogenic nature of the cells with cell-contact inhibition altering the responsiveness of the cells to external stimuli.

These results support previous studies which have demonstrated that tumor cell lines may display modified characteristics in culture which include alterations in the cell surface glycosylation state [7], sialic acid being particularly identified as a sugar residue whose expression is upset in tumor cells subsequent to alterations in sialyl-T activity [26,27]. Therefore, while tumor cells may retain certain characteristics of the parent tissue in vitro, others may be lost with these changes being particularly exacerbated by factors such as increasing durations of time in culture. In order to examine this, we investigated the effect of increasing passage number on cellular sialyl-T activity. Again, there was a particular variability between the three cell lines examined. An increase in sialyl-T activity was observed in the neuro-2A cells with the C6 cells showing a decrease in enzyme activity with increasing time in culture (Fig. 4). No effect of culture duration was observed on the sialyl-T activity of the H411e cell line (data not shown). Therefore, these variations may reflect the different phenotypes of the cells or may also be explained by their metastatic states. However, the results do suggest that cells in culture over extended periods of time may display significantly altered glycosylation characteristics and this factor should be considered when studies on protein glycosylation are being carried out.

While this study has demonstrated that tumor cell lines reflect certain characteristics of the parent tissue such as cell-contact inhibition of neural cell glycosylation, care should be taken before directly extrapolating the results to the in vivo situation.

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