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Regulation of *N*-acetylglucosaminyltransferase V and Asn-linked oligosaccharide β(1,6) branching by a growth factor signaling pathway and effects on cell adhesion and metastatic potential

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Recent evidence demonstrates that the changes in the size of N-linked oligosaccharides that correlate with cell transformation and tumorigenicity are due at least in part to the regulation of expression of a glycosyltransferase involved in the branching of N-linked structures, *N*-acetylglucosaminyltransferase V or GlcNAc-T V. Studies have shown that the increases in GlcNAc-T V expression after oncogenic transformation are most likely caused by direct effects on the GlcNAc-T V promoter by the Ets family of transcriptional activators, which are up-regulated by a cellular proliferation signaling pathway. This pathway begins with growth factor receptors that activate tyrosine kinases at the cell surface and proceeds through *src*, *ras*, and *raf*. Additional evidence for the association between cellular proliferation and GlcNAc-T V expression will be presented, as well as a discussion of the effects of β(1,6) branching on several of the phenotypes of oncogenically transformed cells, including metastatic potential.

***Keywords*: *N*-acetylglucosaminyltransferase V, cell adhesion, metastasis**

Oncogenic transformation affects N-linked oligosaccharide expression

Many studies have demonstrated convincingly that the malignant transformation of a number of cell types correlates with changes in the size of cell surface N-linked oligosaccharides [5, 7, 29–31, 46, 51, 63, 69]. Many of these experiments were begun at a time when the details of cell surface N-linked oligosaccharides were first being elucidated. They showed that these size alterations in N-linked oligosaccharides could be caused by both tumor virus and chemically induced cell transformation. In particular, these changes in oligosaccharide expression correlated strongly not only with the transformed phenotype *in vitro*, but also with cell tumorigenicity, as measured by *in vivo* tumorigenicity assays that quantitated the rate of tumor formation [30]. In one study, revertant cells that showed much lower levels of tumorigenicity were also isolated and these revertants lost the increased size observed for the N-linked oligosaccharides [31]. In subsequent years, detailed oligosaccharide structural analysis revealed that the changes in oligosaccharide expression observed after oncogenic transformation primarily involved an increase in N-linked β(1,6) branches and the carbohydrate residues that are expressed distal to this branch, in particular, the polylactosamines [56, 75]. These oligosaccharide branches are known to be expressed on a number of cell surface glycoproteins, including several adhesion molecules [77]. The consequences of these changes in cell surface oligosaccharide expression will be discussed below, after a detailed description is given of what is now understood concerning the mechanism by which oncogenic transformation causes changes in N-linked β(1,6) branch expression.

Mechanism of increased β(1,6) branching caused by oncogenic transformation

The cause of the increase in the β(1,6) branching in virally transformed BHK cells was demonstrated to be the result of an increase in the specific activity of the glycosyltransferase that synthesizes this branch, *N*-acetylglucosaminyltransferase V, or GlcNAc-T V [4, 76]. The activity of GlcNAc-T V appears to be the rate-limiting event in the expression of other carbohydrate structures distal to its product, so that

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increases in GlcNAc-T V are followed by increased expression of polylactosamine on this branch [56]. GlcNAc-T V from Rous sarcoma virus-transformed BHK (RSV-BHK) cells was shown to have identical enzyme kinetics to that from the non-transformed parental cells, suggesting that the *src* oncogene was most likely up-regulating the number of active enzyme molecules per cell, rather than altering the kinetic properties of the enzyme by post-translational modification [53]. Following cloning of the cDNA encoding this enzyme [64], studies showed that RSV-BHK cells have elevated levels of GlcNAc-T V mRNA as well as increases in activity–both increased about seven-fold suggesting that *src* induces expression of GlcNAc-T V mRNA. This observation has been further confirmed by the observation that GlcNAcT-V activity and mRNA levels in the RSV-BHK cells were reduced to the basal level observed in contact-inhibited BHK cells by the fungal metabolite, herbimycin A, which directly inhibits *src* kinase activity [10]. These results also documented that there was a basal level of GlcNAc-T V expression in these cells that was independent of *src* kinase activity. Thus *src* expression up-regulates GlcNAc-T V activity and cell surface $\beta(1,6)$ branches by increasing GlcNAc-T V message levels. No effect on another GlcNAc transferase, which is active in N-linked oligosaccharide biosynthesis, GlcNAc-T I, could be observed after transformation or herbimycin A treatment, demonstrating the specificity of the effects on GlcNAc-T V.

The control of the expression of the GlcNAcT-V gene appears to be quite complex. The gene itself consists of many exons spanning more than 150 kb [60]. In most mammalian tissues and cell lines examined, there are two mRNAs ranging from about 5.0 kb to about 9.0 kb in length [55], indicating that there is also an alternative use of exons, probably in the non-coding regions. Since the coding region is only about 2.2 kb, it is possible that the GlcNAcT-V mRNA contains a substantial amount of a 5′ untranslated sequence. Utilizing rapid amplification of cDNA ends (RACE) and human brain RNA, known to express large amounts of GlcNAcT-V mRNA of similar size to that found in BHK cells, the 5′ end of the GlcNAcT-V message was identified. The amount of 5′ untranslated sequence was shown to be about 2.9 kb. This region and the upstream promoter were isolated from a genomic library. The 5′ flanking region was shown to have promoter activity when cloned into an enhancer-containing luciferase reporter vector, pGL3E, and transfected into human HepG2 cells. Furthermore, it was possible to show that this fragment, when cloned into a plasmid that did not contain enhancer sequences, could respond to *src* signaling by co-transfecting the HepG2 cells with this reporter plasmid and an *src*-expression plasmid. The *src* co-transformation stimulated luciferase expression by six-to-seven-fold over controls.

A series of deletion experiments were next performed to determine the location of *src*-responsive elements by co-transfecting the cells with the *src*-expression plasmid and smaller fragments of the genomic sequence in the reporter plasmid. These experiments demonstrated that the *src* response element(s) are located in a 400 bp region between bases −659 and −269 upstream from the transcription initiation site. This stretch of DNA contains several motifs known to be recognition sites for transcriptional activation, including three sites that can bind the family of Ets transcriptional activators and one AP-1 site. These factors are known to be activated by the *src* signaling pathway and to stimulate cell proliferation [70–72]. Co-transfection of HepG2 cells with the GlcNAc-T V promoter/reporter plasmid and an expression plasmid encoding *Ets*-2 stimulated transcription from the GlcNAc-T V promoter over four-fold, directly demonstrating that this transcriptional activator can stimulate GlcNAc-T V promoter activity [10]. Furthermore, when a dominant-negative form of *Ets*-2 expression plasmid [3] was co-transfected into HepG2 cells with the GlcNAc-T V promoter/reporter plasmid and the *src* expression plasmid, a large reduction in the induction was observed, indicating that the dominant-negative *Ets*-2 mutant was able to suppress the induction of the GlcNAc-T V promoter by *src*. This result demonstrates that the up-regulation of GlcNAc-T V expression by *src* is most likely due, at least in part, to the utilization of the Ets family of transcriptional activators.

In a very important recent study, a different promoter was found to be used for the expression of GlcNAc-T V in human hepatic bile duct carcinoma cells, HuCC-T1 [39, 60]. The identification of this promoter which is located downstream from the one described above indicates that there are two promoters which can be used in a tissue-specific manner. Our laboratory has additional data to suggest that there is a least a third GlcNAc-T V promoter (Fregien, unpublished observation). The HuCC-T1 promoter also contains Ets-1 binding sites. Co-transfection of the bile duct carcinoma cells with this GlcNAc-T V promoter and an expression plasmid encoding Ets-1 demonstrated that Ets-1 can transcriptionally activate this GlcNAc-T V promoter. In two systems, therefore, Ets sites have now been shown to be involved in the regulation of GlcNAc-T V transcription from two different promoters.

Transformation of various cell types with other oncogenes causes an increase in the cell surface oligosaccharide products of GlcNAc-T V and an increase in GlcNAc-T V specific activity. These oncogenes include *ras*, *fes*/*vps* and others [21, 35, 37, 44]. Neu/her-2 is another oncogene, that is over-expressed in several human carcinomas including breast carcinomas, and can also stimulate the expression of GlcNAc-T V. When the latter oncogene, was co-transfected into HepG2 cells with the smallest fragment of genomic sequence upstream from the GlcNAc-T V promoter that was responsive to *src*, it was also able to significantly stimulate GlcNAc-T V promoter activity (Chen L, Zhang W-J,

Fregien N, Pierce M, unpublished data). This result may explain some of the observations that the cell surface oligosaccharide products of GlcNAc-T V are often over-expressed in malignant breast and colon tissue [22, 28], although another study was not able to confirm this observation [11]. Taken together, these studies argue strongly that the over-expression of GlcNAc-T V and its cell surface products that are associated with oncogenic transformation result from an up-regulation of GlcNAc-T V promoter activity, and that the increase in GlcNAc-T V expression involves the Ets family of transcription activators.

Studies by a large number of laboratories have documented a pathway commonly utilized by *src* and *her-2* to stimulate cell proliferation [15, 27, 49, 73]. This pathway begins with a receptor at the cell surface binding its ligand and the signal generated by this binding reaction passes through *ras* and *raf* on its way to the nucleus where it up-regulates transcriptional activators. In order to determine if the pathway used by *src* and *neu/her-2* to up-regulate GlcNAc-T V promoter activity signals through *raf*, HepG2 cells were co-transfected with the GlcNAc-T V *src*- and *neu*- responsive region, an expression plasmid containing a dominant-negative mutant *raf* [67], and either *src* or *neu/her-2*. This mutant has been used to demonstrate that *raf* activity is utilized in a signaling pathway, since the dominant negative protein can complex with normal *raf* in the HepG2 cells and inhibit signal transduction [57]. The results of the GlcNAc-T V co-transfection experiment demonstrated that the dominant-negative *raf* significantly decreased the effects of both *src* and *neu/her-2* on GlcNAc-T V promoter activity. When the cells were co-transfected with another mutant *raf* that contains an additional mutation that renders the dominant-negative mutant inactive, no effect was seen on the effects of *src* on GlcNAc-T V, demonstrating the specificity of the effect shown by the dominant-negative mutant [6]. At least a portion of the *raf* signaling pathway is utilized, therefore, by *src*, *neu/her-2*, and presumably other oncogenes, as it up-regulates GlcNAc-T V promoter activity. There are multiple pathways by which cellular proliferation can be signaled from cell surface receptors to the nucleus, and it is certainly possible that those that are distinct from the pathway described above may have little or no effect on GlcNAc-T V expression.

Association of GlcNAc-T V expression and rate of cell proliferation

Many oncogenes affect the GlcNAc-T V expression signal through *raf*, and this pathway is also utilized by many growth factor receptors to signal the initiation of cell division. It could be postulated, therefore, GlcNAc-T V activity is expressed by a population of non-transformed cells that are progressing through the cell cycle, *ie* of cells not in the G_0 resting state. The results from two studies suggested that the expression of GlcNAc-T V in HepG2 cells differs between cells that are rapidly dividing and those that have reached confluency and are dividing very slowly [34, 55]. To further test the hypothesis that GlcNAc-T V activity in a population of cells correlates with the percentage of cells in active cell division, we measured the GlcNAc-T V activity of non-transformed BHK cells that were grown to different culture densities. The results from this experiment (see Figure 1) showed that contact-inhibited BHK cells expressed nearly a four-fold reduction in enzyme activity, compared with cells growing under conditions where there was little or no cell contact. Experiments using the *src* tyrosine kinase specific inhibitor, herbimycin A, demonstrated that an RSV-BHK cell GlcNAc-T V activity could be inhibited in most cells to a basal level of GlcNAc-T V activity that was largely independent of their proliferative state. This basal level of expression could then be elevated by increased transcription of the GlcNAc-T V gene resulting from proliferative signals, and the degree of elevation will be proportional to the percentage of the cell population that is not in G_0. In the case of RSV-BHK cells, the expression of GlcNAc-T V was stimulated by about seven-fold over BHK cells grown to a moderate density [10].

Retinoic acid is known to cause changes in the expression of a number of genes, including glycosyltransferases, and in many cases causes cells to reduce their proliferative state and differentiate [12, 15, 16, 43, 58, 59]. Retinoic acid has been shown to inhibit GlcNAc-T V activity [13]; therefore,

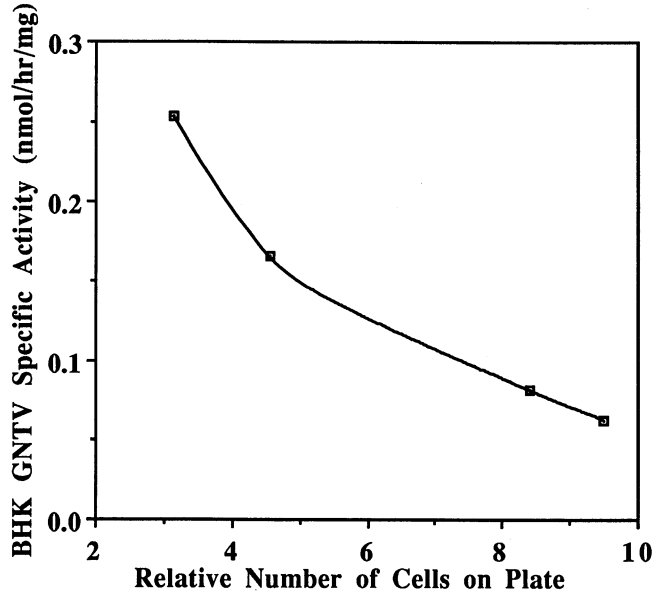

Figure 1. GlcNAc-T V specific activity in BHK cells as a function of cell density. Suspensions of cells were seeded at various densities and allowed to grow for 48 h on 10 cm culture plates. Cells were harvested and specific activity measurements were performed on cell lysates [53]. Cell density was estimated from total protein recovered from each plate.

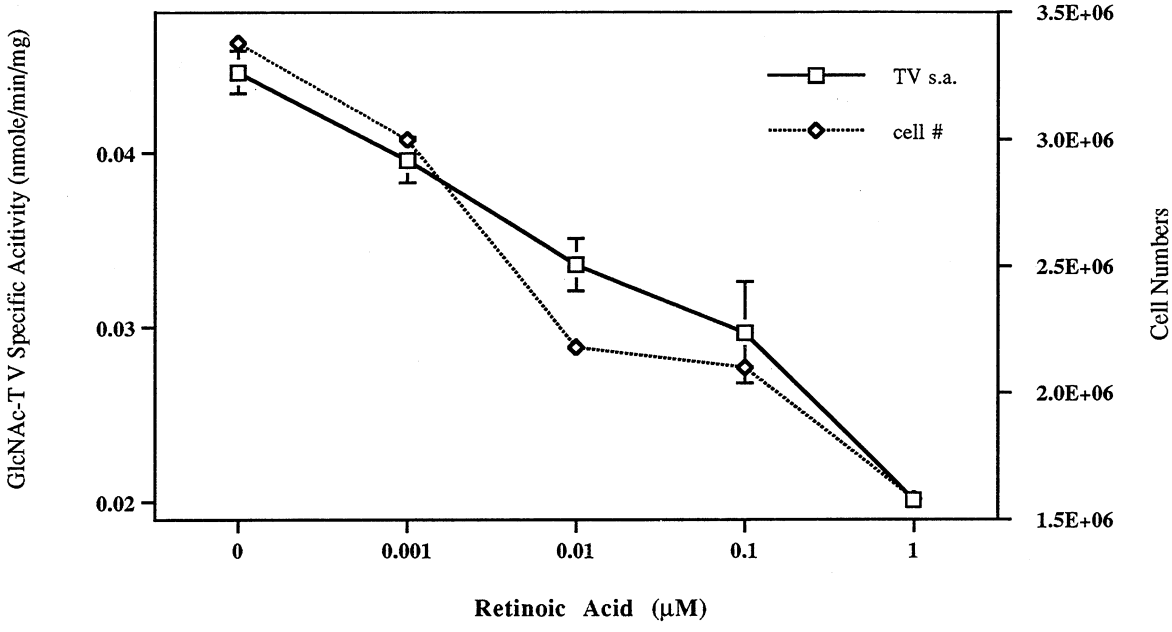

Figure 2. B-16F10 mouse melanoma cells were plated in standard media at a 1×10^4 density in each well of two six-well cell culture plate. Retinoic acid was added at various concentrations, and each concentration was done in duplicate. Cells were cultured for 6 days, and the media with appropriate retinoic acid concentrations was changed after 3 days. Cells were trypsinized and counted with a hemocytometer, and GlcNAc-T V activity assayed as described [53].

we were curious to see if this inhibition correlated with the reduced proliferation when cells were treated with retinoic acid. In order to test this idea, a constant number of B-16-F10 mouse melanoma cells were seeded in culture dishes and grown in the presence of various concentrations of retinoic acid for 6 days, and then the GlcNAc-T V activities were measured. As expected, the number of cells per culture after this treatment decreased in proportion to the increasing concentration of retinoic acid [50]. The activity of GlcNAc-T V (Figure 2) was also observed to decrease as a function of the proliferative rate of the treated cells. Further experiments demonstrated that the decrease in activity was caused by a reduction in mRNA for GlcNAc-T V (data not shown). Although the mechanism for the effects of retinoic acid on depressing GlcNAc-T V mRNA levels has not yet been demonstrated, retinoic acid does decrease the rate of cell proliferation. Therefore, the proliferative signaling pathway may be affecting GlcNAc-T V transcriptional activity.

A recent study [48] on the effects of the negative growth factor, TGF-β, on melanoma cell proliferation and GlcNAc-T V activity, however, found different effects to those described for the positive growth factors that signal through *raf*. Although the growth of melanocytes and melanoma cells was inhibited by TGF-β, and the proliferation of B-16F1 mouse melanoma cells was inhibited about 1.5-fold at 72 h, GlcNAc-T V activity was shown to increase in the treated cells. After 72 h GlcNAc-T V activity and mRNA levels increased by about 1.7-fold in the TGF-β-treated cells, and the mechanism of GlcNAc-T V regulation was shown to involve effects on GlcNAc-T V mRNA half-life. Thus, in the case of a 'negative' growth factor such as TGF-β, when cell proliferation is inhibited, there is an elevation in GlcNAc-T V activity, and the mechanism of up-regulation of activity is distinct from the effects on the GlcNAc-T V promoter.

For many cell types, increases in the rates of proliferation are associated with an elevation in the expression of GlcNAc-T V oligosaccharide products at the cell surface, namely, the glycoconjugates with N-linked β(1,6) branches and distal oligosaccharides. Cells that differ in their rates of tumorigenesis, therefore, should differ in the levels of GlcNAc-T V cell surface products. This difference has been observed in older studies that correlated cell tumorigenicity with the presence of larger cell surface N-linked oligosaccharides [31, 35]. Moreover, previous studies showed an increase in levels of cell surface N-linked oligosaccharide β(1,6) branching, as assayed by the binding of the lectin L-phytohemagglutinin (L-PHA), correlated with the progression of malignant disease in the mammary gland, the colon and in hepatocarcinogenesis [36, 41, 47]. It remains to be determined, however, how general this association is, and whether it is useful clinically as a predictor of cell proliferative rate. *In situ* hybridization experiments on developing mouse embryos suggested an association of GlcNAc-T V mRNA expression and cells that are active in

migration [33]. Preliminary results from further experiments of GlcNAc-T V mRNA levels, GlcNAc-T V activity, and L-PHA staining of β(1,6) products in mouse neuromorphogenesis do not, however, appear to completely support this correlation (unpublished observations). It appears that in a few cell types there are other mechanisms of GlcNAc-T V mRNA regulation, such as translational control, and these mechanisms are now under investigation.

In oncogenically-transformed cultured cells, there does appear to be a general association between the rate of proliferation and GlcNAc-T V activity, but for at least one other enzyme in the N-linked pathway, GlcNAc-T I, this association is not observed. Other studies have shown for some *ras*-transformed cell types there are increases in other glycosyltransferase activities, notably α(2,6) sialyltransferase [42]. In this case, *ras* transformation of Rat1 and NIH 3T3 fibroblasts upregulates the mRNA levels of the enzyme, but has no effect on the α(2,3) sialyltransferase levels in these cells [68]. In fact, FR3T3 cells actually show a decrease in α(2,3) sialyltransferase activity after *ras* transformation [17]. Oncogenic transformation can obviously have multiple, coordinated effects on glycosyltransferase activities [26]. Why are the activities of these glycosyltransferases increased after transformation? In the case of GlcNAc-T V, which has been studied in more detail than the rest, it is important to know whether the increase of GlcNAc-T V activity and cell surface β(1,6) branching observed as cells increase their rates of proliferation, especially after transformation, cause changes in cell adhesion? Results from recent experiments coupled with those from earlier studies, suggest that alterations of cell surface β(1,6) branching may in some cases influence cell adhesion and metastatic potential.

Association of N-linked β(1,6) branching, adhesive behavior and metastatic potential

Studies have used cytotoxic L-PHA to select for mutant metastatic tumor cells that have reduced levels of GlcNAc-T V activity and cell surface β(1,6) branching, and the metastatic potential of these mutant lines have been compared with the metastasis of the lines from which they were derived [19, 23, 24, 45]. Reduced N-linked β(1,6) branching was associated with reduced metastatic potential, even though the growth rates of the parental and mutant cells were similar. Reduced metastatic potential was also evident when cells were treated with glycosylation inhibitors such as swainsonine that inhibited complex N-linked oligosaccharide expression (reviewed in [20]). Taken together these and other results [62, 74], show an association between complex N-linked oligosaccharide expression, in particular β(1,6) branches, and metastatic potential. It is important to be careful however, before concluding that there is a cause and effect relationship when comparing with a parental line cells selected with cytotoxic agents.

After the cloning of GlcNAc-T V [61, 64], it was possible to transfect cells directly with an expression plasmid encoding GlcNAc-T V, select for clones with increased GlcNAc-T V activity, and determine if these cells demonstrated altered adhesive properties and cell migration [18]. A mink lung epithelial cell line, Mv1Lu, was utilized for these experiments and three clones that expressed four-, nine- and 15-fold increased GlcNAc-T V activity were selected. The clones also displayed increased sensitivity of L-PHA cytotoxicity, confirming that they expressed increased β(1,6) branching on the cell surface, and demonstrated increased tumorigenicity, assayed by subcutaneous injection of cells in nude mice. The parental Mv1Lu cells showed contact inhibition of growth when grown to confluency. The GlcNAc-T V-transfectants, however, showed an altered 'transformed cell' morphology and formed foci, as is often observed for oncogenically-transformed cells. All of the cell lines apparently showed similar growth rates in serum-containing culture media. Using a scratch-wounding technique, confluent cultures of parental, control-transfected, and GlcNAc-T V-transfected cells were studied for their rates of migration into scratched areas of the culture plates. The results of this experiment showed that the three GlcNAc-T V-transfected clonal lines all displayed a three- to 10-fold increase in their rates of migration over control transfectants. Adding serum to the medium during this assay increased the rate at which the parental cells migrated into the wounded area. The conclusion drawn from this experiment was that GlcNAc-T V over-expression reduced the serum-growth requirements of the contact-inhibited Mv1Lu cells to rapidly migrate into the wounded area. Significantly, the GlcNAc-T V transfectants all adhered less well to fibronectin or collagen type IV-coated plastic wells than did the parental cells. The GlcNAc-T V transfectants appeared to express unaltered levels of the $\alpha 5\beta 1$ and $\alpha v\beta 3$ integrins, but the αV, α5, and β1 subunits showed lower mobility on SDS-PAGE gels than those integrins from the parental cells, suggesting that they express higher levels of N-linked β(1,6) oligosaccharides. The conclusion drawn from these studies is that overexpression of GlcNAc-T V activity in Mv1Lu cells has a profound influence on the migration and adhesive properties of the cells. This can be explained at least in part by the alteration of the adhesive properties of two integrins, caused by increases in their β(1,6) branched oligosaccharides. Thus, increases in GlcNAc-T V expression can affect rates of cell migration on plastic and adhesion to plates coated with either collagen type IV or fibronectin.

Other studies have shown that $\alpha 4\beta 1$ (laminin receptor) and $\alpha 5\beta 1$ (fibronectin RGD receptor) integrins require minimal N-linked oligosaccharides present in order to function [2, 81]. These experiments, however, do not address the issue of whether differences in branching *per se* (*eg* changes from biantennary to triantennary or tetraantennary) affect the binding activities of particular integrins. A recent study

showed that *ras*-transformation of 3T3 fibroblasts caused a specific increase in $\alpha 6\beta 1$ integrin (laminin receptor) expression, and the $\beta 1$ chain showed extensive $\beta(1,6)$ branching [38]. The transformed cells migrated towards laminin while the non-transformed 3T3 cells did not. Treatment of the transformed cells with swainsonine or deoxymannojirimycin, complex N-linked oligosaccharide inhibitors, caused an increase in the migration towards laminin. By contrast, 3T3 cells transformed with c-*jun* showed no increase in laminin receptor expression and a smaller increase in cell surface $\beta(1,6)$ structures. The conclusion from this study was that the $\alpha 6\beta 1$ integrin on the *ras*-transformed cells could mediate migration towards laminin-1 independent of the glycosylation state of the integrin.

Other changes in the levels of particular oligosaccharide structures expressed on N-linked carbohydrates can affect the adhesion of cells to extracellular matrix molecules. For example, a lectin-resistant variant of B-16 mouse melanoma cells showed increases $\alpha(1,3)$ fucosylation of distal GlcNAc residues on N-linked oligosaccharides, including the laminin receptor, and this variant showed lower rates of adhesion to laminin, and decreased metastatic potential when assayed by several methods [40, 52]. Recent results suggested that simple over-expression of the human Fuc-T IV fucosyltransferase in the parental B-16 cells, and resultant increase in $\alpha(1,3)$-linked fucose on GlcNAc residues, caused decreased adhesion to laminin (Chein W, Pierce M, unpublished data). Both the lectin-resistant variant and transfected B-16 cells also demonstrated lower levels of $\alpha(2,3)$ and $\alpha(2,6)$-linked sialic acid residues on their N-linked oligosaccharides, so the precise cause of the changes in adhesion is not certain. In another study, over-expression of the $\alpha(1,3)$ galactosyltransferase in mouse melanoma cells caused a reduction in the metastatic potential of these cells [32]. Flow cytometry data suggested that sialic acid residues were significantly reduced in the transfected cells. Many other studies have demonstated correlations between sialylation and metastatic potential [54, 65]. In summary, the precise effects on cell adhesion or metastatic potential seen when N-linked oligosaccharide structures are changed are not clear; nonetheless, there is strong evidence that changes in oligosaccharide structures do affect cell adhesive properties.

A completely novel strategy was employed by Taniguchi's laboratory to determine if changes in N-linked oligosaccharide branching on melanoma cells could affect their metastatic behavior [80]. Since GlcNAc-T V III competes directly with GlcNAc-T V in the Golgi for oligosaccharide substrates, and since oligosaccharides branched by GlcNAc-T III are not then substrates for GlcNAc-T V (and vice versa), over-expression of GlcNAc-T III should reduce or eliminate GlcNAc-T V expression on N-linked oligosaccharides. Mouse melanoma B-16F-hm cells were transfected with *GlcNAc-T III* gene and a clone was selected with high levels of GlcNAc-T III activity and $\beta(1,4)$-bisected GlcNAc structures. The GlcNAc-T III transfectant showed no expression of the native $\beta(1,6)$ branched N-linked oligosaccharides and also reduced sialic acid expression. This transfectant, moreover, showed significantly reduced metastatic potential, assayed by tail vein injection and lung colony formation. A subsequent study showed that E-cadherin, expressed on the melanoma cell surface and responsible for cell-cell homotypic adhesion, contained GlcNAc-T III-branched oligosaccharides [78]. Intriguingly, the GlcNAc-T III-branched E-cadherin showed a significantly longer half-life on the cell surfaces of the transfected cells, and consequently, there were higher levels of E-cadherin on the surface of the transfected cells, compared to controls. Using an antibody specific for E-cadherin, cell–cell aggregation assays demonstrated that the GlcNAc-T III-transfectants showed higher rates of homotypic adhesion than did the control cells, and this difference in rate was inhibited by the E-cadherin antibody. Thus, greater cell–cell aggregation, and consequently lower metastatic potential, resulting from the more stable E-cadherin on the transfectants was caused, in turn, by the over-expression of the $\beta(1,4)$bisected GlcNAc oligosaccharides.

In a related study, GlcNAc-T III was transfected into a line of human erythroleukemia cells, K562, which normally do not produce GlcNAc-T III [79]. These cells expressed $\beta(1,4)$ bisected GlcNAc on their surfaces, and expressed decreased sialic acid and $\beta(1,6)$ branches. The transfectants were then shown to be resistant to natural killer (NK) cell killing *in vitro* using a Cr^{2+}-release assay. When injected into mice, control cells did not form colonies in the spleen, presumably because they were killed by native NK cells. The transfectants, however, formed spleen colonies. When nude mice were used for this experiment, both control and transfectant cells formed colonies. These results argue that the transfectants survived and colonized the spleen *in vivo* because they were resistant to NK cell killing, and they are consistent with an earlier study that suggested that changes in N-linked oligosaccharide expression can significantly affect killing by NK cells [1] and recognition by macrophages [66].

Conclusion

Altering the Asn-linked branching patterns of some cell types can clearly affect their adhesive properties. In the case of the Mv1Lu cells [18], over-expression of GlcNAc-T V and increases of $\beta(1,6)$ branching on the cell surface appears to be sufficient to cause significant decreases in adhesion to two extracellular matrix proteins coated on plastic and increases in rates of cell migration. In the context of oncogenically transformed cells that have increased rates of division, the increased expression of GlcNAc-T V may be a necessary (but not sufficient) step in the cascade of adhesive changes that result in increases in invasive and metastatic potential. In particular cell types such as Mv1Lu,

GlcNAc-T V over-expression may be sufficient to alter adhesive properties. In other cells, reduction of GlcNAc-T V expression may actually promote particular adhesive properties and thereby affect invasiveness and metastatic potential.

Experiments that utilize inducible GlcNAc-T V expression-constructs to investigate how changes in $\beta(1,6)$ branching can alter adhesive properties should allow a detailed understanding of the mechanisms causing effects on cell adhesion. In addition, reagents such as antisense cDNA or ribozymes that inhibit selectively GlcNAc-T V expression in transformed cells could determine if inhibition of $\beta(1,6)$ branching of cell surface glycoproteins can affect the invasive and metastatic potential of tumor cells. Studies are needed to test directly the hypothesis that alterations in the degree of $\beta(1,6)$ branching of Asn-linked oligosaccharide on various cell adhesion molecules affect their abilities to bind ligands. These molecules include the integrins, cadherins, and carcinoembryonic antigen (CEA) which functions as a homotypic adhesion molecule. Since polylactosamine expression is often increased as a result of the increase of GlcNAc-T V expression, and since these structures are ligands for the galectin family, homotypic adhesion or adhesion to matrix proteins such as laminin may be regulated by changes in GlcNAc-T V activity [25]. Galectin-1, for example, can mediate adhesion between the polylactosamine chains expressed on cell surfaces and those expressed on laminin, independent of integrin-dependent adhesion [82]. In order to understand the functional consequences of the changes in GlcNAc-T V activity, investigations are also underway to produce transgenic mice that lack GlcNAc-T V expression, as well as mice that show ectopic, over-expression of this enzyme. The results of these experiments should significantly increase our understanding of the function of Asn-linked $\beta(1,6)$ branched oligosaccharides in regulating cell adhesion.

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