Multiple Heparan Sulfate Chains Are Required for Optimal Syndecan-1 Function

(Received for publication, April 4, 1998, and in revised form, August 11, 1998)

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Syndecans have three highly conserved sites available for heparan sulfate attachment. To determine if all three sites are required for normal function, a series of mutated syndecans having two, one, or no heparan sulfate chains were expressed in ARH-77 cells. Previously, we demonstrated that expression of wild-type syndecan-1 on these myeloma cells mediates cell-matrix and cell-cell adhesion and inhibits cell invasion into collagen gels. Here we show that to optimally mediate each of these activities, all three sites of heparan sulfate attachment are required. Generally, an increasing loss of syndecan-1 function occurs as the number of heparan sulfate attachment sites decreases. This loss of function is not the result of a decrease in either the total amount of cell surface heparan sulfate or syndecan-1 core protein. In regard to cell invasion, cells expressing syndecan-1 bearing a single heparan sulfate attachment site exhibit a hierarchy of function based upon the position of the site within the core protein; the presence of an available attachment site at serine 47 confers the greatest level of activity, while serine 37 contributes little to syndecan-1 function. However, when all three heparan sulfate chains are present, significantly greater biological activity is observed than is predicted by the sum of the activities occurring when the chains act individually. This synergy provides a functional basis for the evolutionary conservation of the three heparan sulfate attachment sites on syndecans and supports the idea that molecular heterogeneity, which is characteristic of proteoglycans, contributes to their functional diversity.

Heparan sulfate is the most ubiquitous glycosaminoglycan (GAG) on cell surfaces. These long, highly diverse carbohydrate polymers are negatively charged and are most often found covalently linked to protein in the form of proteoglycans. Heparan sulfate binds to many extracellular effector proteins including insoluble extracellular matrix molecules, soluble peptide growth factors, and cell adhesion molecules (1). It is through these interactions that heparan sulfate mediates or regulates activities occurring when the chains act individually. Here we show that to optimally mediate each of these activities, all three sites of heparan sulfate attachment sites are required. Generally, an increasing loss of syndecan-1 function occurs as the number of heparan sulfate attachment sites decreases. This loss of function is not the result of a decrease in either the total amount of cell surface heparan sulfate or syndecan-1 core protein. In regard to cell invasion, cells expressing syndecan-1 bearing a single heparan sulfate attachment site exhibit a hierarchy of function based upon the position of the site within the core protein; the presence of an available attachment site at serine 47 confers the greatest level of activity, while serine 37 contributes little to syndecan-1 function. However, when all three heparan sulfate chains are present, significantly greater biological activity is observed than is predicted by the sum of the activities occurring when the chains act individually. This synergy provides a functional basis for the evolutionary conservation of the three heparan sulfate attachment sites on syndecans and supports the idea that molecular heterogeneity, which is characteristic of proteoglycans, contributes to their functional diversity.

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* This work was supported by National Institutes of Health Grant CA 68494 (to R. S.) and National Research Service Award CA 71145 (to J. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

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Cells expressing syndecan-1 having less than three heparan sulfate attachment sites show a reduction in biological activity that in general is relative to the number of chains present. These results provide a basis for the conservation of all three attachment sites on syndecans and imply that the structural heterogeneity common to proteoglycans serves as a mechanism for modulating their function.

EXPERIMENTAL PROCEDURES

Cell Culture and Mutagenesis—The EcoRI–HindIII fragment of clone 4-29b (kindly provided by Dr. M. Bernfield), containing the coding region of murine syndecan-1 (13), was excised from the pGEM-3Z vector and ligated into the EcoRI–HindIII sites of pBluescript II KS (+) (Stratagene, La Jolla, CA). This was used as the template for oligonucleotide-directed mutagenesis (TransformTM site-directed mutagenesis kit, CLONTECH, Palo Alto, CA), which was used to generate seven mutagenic constructs of syndecan-1 in which serines 37, 45, and 47 were converted to alanine in all possible combinations (Fig. 1). Following mutagenesis, the syndecan-1 coding region was excised at the BamHI–Sall sites and ligated into pCND3A (Invitrogen, San Diego, CA) at the BamHI–XhoI sites. All mutagenic constructs were confirmed by manual sequence analysis using the dyeoxy method with the T7 Sequenase Quick Denaturation plasmid sequencing kit (Amersham Pharmacia Biotech). ARH-77 human B lymphoid cells were obtained from the American Type Culture Collection (Rockville, MD). These cells express little, if any, endogenous syndecan-1 (14). ARH-77 cells transfected with a pMAmneo vector containing a murine syndecan-1 cDNA (clone B23P; Ref. 11) were used as a wild-type syndecan-1-expressing cell line in all assays. Cells were maintained in culture as described previously (11). ARH-77 cells were transfected with the vector containing mutant syndecan-1 constructs or vector alone (neomycin control) by liposome-mediated transfection according to the manufacturer's instructions (Life Technologies, Inc.). Cells expressing the syndecan-1 molecules were sorted by “panning” using a monoclonal antibody 281.1 (that recognizes the syndecan-1 core protein) immobilized to 100-mm tissue culture dishes. Upon release of cells from the plates, expression of syndecan-1 was confirmed by immunofluorescence staining.

Western Blotting—Culture media conditioned by cells expressing syndecan-1 were brought to a final concentration of 2 m urea and 50 mM sodium acetate. Syndecan-1 was isolated by ion exchange and affinity chromatography as described previously (15). Equivalent aliquots of proteoglycan, still bound to the beads, were either 1) untreated or 2) digested twice with chondroitinase ABC (as described above) and then heparitinase (Seikagaku, Tokyo, Japan). Proteoglycans were then eluted and analyzed on 4–12% acrylamide, Tris/glycine gel (Novex, San Diego, CA) and transferred to nitrocellulose membranes (Bio-Rad) using a dot-blotting apparatus (Millipore, Bedford, MA). Syndecan-1 was isolated by extracting cells as described above. After centrifugation, the supernatant was brought to 6M urea and 50 mM sodium acetate, pH 6.0, and subjected to DEAE and 281.2 affinity chromatography (15). Proteoglycans were eluted from the affinity beads with 0.5% (w/v) triethanolamine hydrochloride, 15 volumes of 1 M Tris-HCl, pH 7.0, and desalted over an excellocelle column (Pierce) into ACE running buffer (0.1 m sodium acetate, 50 mM sodium MOPS (Fluka Biochemika, Ronkonkoma, NY), pH 7.0). Approximately 50,000 cpm of labeled material was analyzed on a 1% agarose gel having nine parallel lanes of agarose containing decreasing amounts of type I collagen (15, 17). After electrophoresis, the gel was soaked in 5% acetic acid for 4 h and dried overnight with forced air at 42 °C, and the labeled material was imaged using a PhosphorImager.

Assay for Cell Aggregation—Cells were placed into wells of a 24-well plate in aggregation buffer (Hank's balanced salt solution, 1 mM calcium chloride, 1 mM magnesium sulfate, 1% bovine serum albumin, and 10 mM HEPES buffer) and rotated on a gyratory shaker (100 revolutions/min) at 37 °C for 60 min (12). The cells were then removed and placed onto a glass slide, and the number of cells in aggregates lying within a central rectangular area of 6.2 mm² were counted. Aggregates were defined as containing four or more cells.

RESULTS

Production of Cells Bearing Syndecan-1 Having Mutated Heparan Sulfate Attachment Sites—To determine if all three heparan sulfate attachment sites are essential for syndecan-1 biological activity, we generated a series of seven constructs by performing oligonucleotide-directed mutagenesis on a murine syndecan-1 cDNA. Serine residues at position 37, 45, and/or 47 were changed to alanine, thereby preventing the addition of GAG chains at those sites. Mutations were confirmed by DNA sequencing, and each construct was separately transfected into the human B lymphoid cell line ARH-77. The mutated syndecan-1 molecules are capable of bearing no, one, or two heparan sulfate chains at the N-terminal attachment sites, whereas wild-type syndecan-1 is capable of expressing three heparan sulfate chains (Fig. 1). The two GAG attachment sites within the syndecan-1 core protein located at serines 210 and 220 (adjacent to the cell membrane) were not deleted, because they are reported to bear only chondroitin sulfate chains (18). The biological activity of syndecan-1 in the invasion, adhesion, and aggregation assays resides in its heparan sulfate chains (11, 12, 19). Thus, although chondroitin sulfate chains are present on the syndecans, they probably do not influence the functions being assessed in the present study.

Characterization of Mutants—Deletion of GAG attachment sites should yield syndecan-1 molecules having a smaller modal molecular size than wild-type syndecan-1. To confirm this, syndecan-1 purified from the media of cells bearing the mutated proteoglycans was examined by Western blotting. The relative mobilities reflect the deletion of one, two, or all three N-terminal GAG attachment sites and their associated chains (Fig. 2A). Wild-type syndecan-1 appears as a broad smear
characteristic of proteoglycans bearing multiple GAG chains. The triple deletion mutant (TDM) also appears as a smear because it bears GAG chains at serines 210 and 220. Upon heparitinase digestion, all of the syndecans migrate with a similar pattern and as relatively small molecules (Fig. 2B).

This indicates that, as previously reported (18), the majority of N-terminal attachment sites on syndecan-1 are substituted with heparan sulfate chains. Otherwise, if substantial chondroitin sulfate was present at each of the three sites, the proteoglycan remaining after heparitinase digestion would show a pattern similar to that observed for intact proteoglycans (Fig. 2A). The possible exception is the syndecan-1 having a single attachment site at serine 37. This proteoglycan has an intact size very similar to the TDM, suggesting that the serine 37 attachment site may not always bear a GAG chain.

To further confirm successful deletion of GAG attachment sites, we analyzed the levels of cell surface heparan sulfate per syndecan-1 core protein. As expected, with loss of GAG attachment sites, a reduction in the amount of heparan sulfate per core protein occurs relative to wild-type syndecan-1 (Fig. 3A). Even when a single site is deleted (leaving two attachment sites), a dramatic effect on the amount of heparan sulfate per core protein is seen, with all single site deletion mutants having at least a 50% decrease in heparan sulfate per core protein relative to wild-type syndecan-1. When sites at 37 and 45 or...
Deletion of Heparan Sulfate Attachment Sites Diminishes Syndecan-1-mediated Cell Adhesion to Collagen—Cells transfected with wild-type syndecan-1 bind tightly to collagen and therefore do not pellet upon centrifugation (Fig. 4). In contrast, neomycin- and TDM-transfected cells form a tight pellet indicating lack of strong binding to collagen. This demonstrates that syndecan-1 is responsible for cell adhesion to collagen and that the heparan sulfate chains present within the N-terminal region of the core protein are required for this binding. Treatment of cells with heparitinase prior to the assay abolishes cell binding, also indicating that adhesion is mediated through the heparan sulfate chains (data not shown; see Ref. 19). Cells bearing single or double GAG deletions exhibit an intermediate range of adhesion; when one of the three chains is deleted, only a very slight rim of pelleting is present, and when two of the three chains are deleted, a broad ring of pelleted cells is present but much less distinct than the pellet seen with neomycin- or TDM-transfected cells. Analysis of cells bearing the other heparan sulfate attachment mutants indicates that among all three single deletion mutants the extent of cell pelleting was similar and that among all three double deletion mutants the extent of pelleting was similar (data not shown). It is interesting that cells bearing the mutant having a single attachment site at position 37 and the TDM have almost identical amounts of heparan sulfate per core protein and total heparan sulfate on the cell surface, yet the cells differ in their behavior on collagen. This finding underscores the importance of having heparan sulfate chains positioned in the N-terminal region of the core protein. Taken together, these data show that all three heparan sulfate attachment sites are essential for optimal syndecan-1-mediated binding of cells to collagen and that the extent of binding is determined by the number, not the position, of the heparan sulfate chains.

Deletion of Heparan Sulfate Attachment Sites Promotes Cell Invasion—As expected, cells expressing the TDM were highly invasive in collagen gels as compared with cells expressing wild-type syndecan-1, which do not invade (Fig. 5A). This confirms that the heparan sulfate chains at these sites are required for inhibition of invasion and that the chondroitin sulfates at serines 210 and 220 probably do not contribute to this inhibitory activity. Deletion of a single attachment site at either serine 37 or 45 results in only a small (16 and 9%, respectively) increase in cell invasion when compared with wild-type syndecan-1. However, removal of serine 47 results in a significant (35%) increase in invasion, suggesting that this site is the most influential in regard to the anti-invasive effect of syndecan-1. Data from the double deletion mutants also supports this, because cells bearing syndecan-1 with only the serine 47 attachment site were much less invasive (39%) than mutants having a single attachment site at either serine 37 or 45 (82 and 69%, respectively). While these results demonstrate that all three heparan sulfate chains are required for optimal inhibition of cell invasion by syndecan-1, having a heparan sulfate chain attached at serine 47 clearly is the most influential single site on syndecan-1 for regulating this behavior.

Closer analysis of the data in Fig. 5A reveals that the resulting degree of invasion of cells bearing the TDM (105% invasion as a percentage of control) is greater than would be predicted by adding the percentage increase in invasion of the three single deletion mutations (16% + 9% + 35% = 59%). This predicted value is significantly lower than the actual value (p < 0.05) and supports the conclusion that all three GAG attachment sites are required for optimal syndecan-1 functional activity.

As a control, cells having equal amounts of syndecan-1 core protein were harvested and analyzed for their invasion into gels. Results in Fig. 5B show that the invasive behavior of these cells is similar to that observed with unsorted cells (Fig. 5A), indicating that differences in cell invasion are not due to differences in the levels of syndecan-1 at the cell surface.
To determine if the basis for variability in the invasive behavior between the mutants is due to differences in their affinity for type I collagen, we analyzed several mutated proteoglycans by ACE. Wild-type syndecan-1, syndecan-1 with an attachment deletion at position 47, and syndecan-1 with deletions at positions 37 and 47 all have similar affinities for collagen (Fig. 6), although they vary greatly in their ability to inhibit cell invasion (Fig. 5A). Both a low and high affinity population are present in all of the samples, with the high affinity fraction containing approximately 50–55% of the proteoglycan. Of the mutants examined, all forms bearing at least one heparan sulfate chain show a shift in proteoglycan mobility at a collagen concentration of 40–160 nM, with the $K_d$ falling somewhere in this range. The similarity in affinity among the GAG attachment mutants and wild-type syndecan-1 is consistent with previous work showing that commercial heparin, individual heparan sulfate chains of syndecan-1, and intact syndecan-1 all have similar affinities for type I collagen (15, 20). Thus, variability in the invasive behavior of cells bearing the various mutants is not likely to be due to differences in individual proteoglycan affinity for type I collagen.

**Deletion of Heparan Sulfate Attachment Sites Diminishes Cell-Cell Adhesion—**Expression of wild-type syndecan-1 on ARH-77 cells induces cell-cell adhesion in a heparan sulfate-dependent manner (12). Overall, deletion of a single attachment site decreases the ability of syndecan-1 to mediate cell-cell adhesion, deletion of two chains decreases adhesion further, and removal of all three sites abolishes cell-cell adhesion (Fig. 7). Interestingly, over half of the adhesive ability of syndecan-1 was still present when only a single heparan sulfate attachment site was available at position 45 or 47. This is in contrast to the poor adhesion that occurs when only a single attachment site is available at position 37. Comparison of mutant behavior in aggregation assays with the data in Fig. 3 indicates that the extent of syndecan-1-mediated cell-cell adhesion may be determined by the amount of heparan sulfate present at the cell surface.

**DISCUSSION**

Because the three N-terminal GAG attachment sites of syndecans are highly conserved, a series of mutated syndecans having two, one, or none of these sites were expressed in ARH-77 cells, and their behavior was analyzed in a series of functional assays. Generally, loss of one heparan sulfate chain results in a slight to moderate decrease in cell binding to collagen, inhibition of invasion, and cell-cell adhesion; loss of two chains results in a more substantial decrease in these functions; and elimination of all three chains results in total loss of function (Table I). We conclude that the three N-terminal GAG attachment sites on syndecan-1 are essential for maximal heparan sulfate-mediated biological activity. This correlation provides a functional basis for the evolutionary conservation of these three heparan sulfate attachment sites found throughout the syndecan family of proteoglycans.

As has been demonstrated with syndecan-4 and with syndecan-1 chimeras (4, 10), we find that all three N-terminal GAG chains enhance syndecan-1 function.
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attachment sites can bear heparan sulfate chains and that all three sites are essential for ensuring the maximal expression of heparan sulfate per core protein (Fig. 3A). Also, as with both syndecan-1 chimeras and perlecan, our data indicate that coupling occurs across the heparan sulfate sites of wild-type syndecan-1, because the three sites acting together bear more heparan sulfate than predicted by the behavior of each site acting independently (10, 21). Our findings lend functional import to this synergistic phenomenon that occurs across GAG attachment sites; i.e. as the number of heparan sulfate attachment sites increases, the overall biological activity of syndecan-1 increases.

In addition, we also find that individual heparan sulfate attachment sites do not influence all functional activities equally. Removal of attachment sites at positions 45 and 47 (leaving a single attachment site at position 37) results in a syndecan-1 with a very low heparan sulfate per core protein ratio and low levels of total cell surface heparan sulfate. This suggests that when attachment sites are absent at both positions 45 and 47, the site at position 37 is poorly substituted with heparan sulfate. This form of syndecan-1 does not inhibit invasion well, nor is it very effective in mediating cell aggregation (Figs. 5A and 7). Thus, the site at position 37 appears to be the least influential in regulating syndecan-1 functional activity. Another striking observation is the apparent role of attachment position 47, which has the greatest impact among the three attachment sites on inhibiting invasion as demonstrated with both single and double heparan sulfate chain deletions (Fig. 5A).

In contrast, adhesion of cells to collagen is not substantially influenced by heparan sulfate chain position; rather, the number of heparan sulfate chains is the most important determining factor. Surprisingly, in regard to cell-cell adhesion, syndecan-1 bearing only a single heparan sulfate chain at position 45 or 47 can promote extensive aggregation. These results on cell adhesion to collagen and cell-cell adhesion together support an important conclusion, that even when relatively low amounts of heparan sulfate-bearing syndecan-1 are present on the cell surface they can have substantial impact on cell adhesion. This is possibly due to mobility of syndecan-1 in the plane of the membrane, which allows focal concentration of the heparan sulfate-bearing molecules at sites where they interact with ligands.

The behavior of cells bearing the TDM supports several important conclusions. First, the presence of heparan sulfate on the N-terminal GAG attachment sites is absolutely required for syndecan-1-mediated cell adhesion to collagen, inhibition of cell invasion, and cell-cell adhesion. Second, chondroitin sulfate on the attachment sites near the plasma membrane does not mediate cell adhesive activity in any of the assays utilized in this study. Third, at least some heparan sulfate may be present on the attachment sites near the plasma membrane (positions 210 and 220). Low levels of heparan sulfate have also been found in chimeric proteoglycans containing these syndecan-1 GAG attachment sites (10). However, we do not know if heparan sulfate is present at these sites on wild-type syndecan-1 transfecants or if substitution of heparan sulfate at these sites is a consequence of removing the three N-terminal attachment sites.

Mutations of GAG sites on syndecans have not as yet been related to any specific pathological state. Therefore, other than providing a functional explanation for the conservation of the three heparan sulfate attachment sites, what have we learned from this work? First, it has been shown both in vivo and in vitro that syndecan-1 exhibits a molecular polymorphism due to differences in the number and size of its heparan sulfate and chondroitin sulfate chains (5). Specifically, syndecan-1 present on simple epithelia has more heparan sulfate chains (two or three chains present) and larger heparan sulfate chains than does syndecan-1 on stratified epithelial cells (only one heparan sulfate chain). Because these cell types have different adhesive requirements (simple epithelia bind tightly to underlying extracellular matrix and under normal conditions are unable to migrate, while stratified epithelia form extensive cell-cell adhesive interactions and often migrate away from the extracellular matrix (e.g. stratified keratinocytes)), it was speculated that the different syndecan forms have distinct functions (5). Results from our present study fully support this notion. In cells expressing syndecan-1 mutants lacking all three heparan sulfate chains, cell migration (invasion) increases and adhesion to extracellular matrix (collagen) decreases relative to cells expressing wild-type syndecan-1. Moreover, when only a single heparan sulfate chain is present on syndecan-1 in our cells (similar to syndecan-1 on stratified epithelia), the cells are invasive and bind extracellular matrix poorly but still exhibit extensive heparan sulfate-dependent cell-cell adhesive interactions. Thus, it is likely that tissue-specific differences in the

![Diagram](image)

**FIG. 7. Reducing the number of heparan sulfate chains on syndecan-1 weakens cellular aggregation.** Cells expressing wild-type or mutated syndecan-1 were assayed for their ability to aggregate using a rotation-mediated aggregation assay (12). Values represent the means ± S.E. (n = 3) of the percentage of cells in aggregates.

**TABLE I Summary of mutant syndecan-1 functional activity**

| Number of HS attachment sites | Cell binding to collagen | Invasion as percentage of control | Percentage of cells in aggregates |
|-----------------------------|--------------------------|----------------------------------|---------------------------------|
| 3                           | +++                      | <1                               | 86                              |
| 2                           | ++                       | 20                               | 68                              |
| 1                           | +                        | 63                               | 39                              |
| 0                           | −−                       | 105                              | 3                               |

Functional activity of syndecan-1 decreases as the number of heparan sulfate attachment sites is reduced, and removal of all three HS attachment sites abolishes syndecan-1 activity. Cell binding to collagen (Fig. 4) was graded visually on a scale ranging from tight cell adhesion (++++) to no adhesion (−−). For invasion and aggregation assays, the mean activity of the transfectants expressing syndecan-1 having either two or one HS attachment site was calculated from data shown in Fig. 5A (invasion) and from Fig. 7 (aggregation). These are shown along with values obtained for cells expressing either wild-type syndecan-1 (three HS attachment sites) or TDM (no HS attachment sites).
number of heparan sulfate chains present on syndecan-1 at least in part account for the differing adhesive capacities and behavior of simple and stratified epithelial cells. Second, syndecans exhibit extensive heterogeneity even when expressed within the same cell type as indicated by their broad smearing pattern on SDS-polyacrylamide gel electrophoresis. Studies of L cells transfected with wild-type syndecan-4 show the proteoglycans produced have one, two, or three heparan sulfate chains as well as varying numbers of chondroitin sulfate chains (and some isoforms have only chondroitin sulfate chains) (4). This finding, together with our present results, indicates that within some tissues, or even on a single cell surface, there are probably syndecans that, due to their differing number of heparan sulfate chains, have distinct functional capacities. This dichotomy of function among syndecans could be important, for example, on migrating cells; chondroitin sulfate-containing syndecan could localize to the leading edge of the cell (chondroitin sulfate proteoglycans have been shown to promote cell migration (22, 23)) and heparan sulfate-containing syndecan to the trailing edge in order to anchor the cell. Were such a mechanism in place, the relative amount of heparan sulfate-syndecan versus chondroitin sulfate-syndecan could regulate the balance between migration and tight adhesion.

Last, when heparan sulfate attachment sites were deleted on glypican, a glycosylphosphatidylinositol-anchored heparan sulfate proteoglycan, it was found that the molecules sorted to different cell surface compartments (i.e. apical, basolateral) based on the number of heparan sulfate chains present on the core protein (24). This finding led the authors to speculate that modulation in the number of heparan sulfate chains controls the subcellular expression and therefore the functional availability of glypican. Thus, modulation of cell behavior via molecular heterogeneity of cell surface proteoglycans may not be restricted solely to the syndecans; rather, it may be a widespread mechanism to facilitate the multifunctional nature of these molecules.

Acknowledgments—We thank Allison Theus for technical assistance, Jeff Woodliff and John Theus for flow cytometry, and Dr. Arthur Lander and Dr. James San Antonio for helpful advice on running ACE gels.

REFERENCES
1. Stringer, S. E., and Gallagher, J. T. (1997) Int. J. Biochem. Cell Biol. 29, 709–714
2. Bernfield, M., Kokenyesi, R., Kato, M., Hinkes, M. T., Spring, J., Gallo, R. L., and Lose, E. J. (1992) Annu. Rev. Cell Biol. 8, 365–393
3. Carey, D. J. (1997) Biochem. J. 327, 1–16
4. Shworak, N. W., Shirakawa, M., Mulligan, R. C., and Rosenberg, R. D. (1994) J. Biol. Chem. 269, 21204–21214
5. Sanderson, R. D., and Bernfield, M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 9562–9566
6. Bacic, P. C., Acaster, C., and Goetinck, P. F. (1994) J. Biol. Chem. 269, 696–703
7. Spring, J., Paine-Saunders, S. E., Hynes, R. O., and Bernfield, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3334–3338
8. Rosenblum, N., Botelho, B., and Bernfield, M. (1995) Biochem. J. 309, 69–76
9. Rapraeger, A., Jalkanen, M., Endo, E., Koda, J., and Bernfield, M. (1985) J. Biol. Chem. 260, 11046–11052
10. Zhang, L. J., David, G., and Esko, J. D. (1995) J. Biol. Chem. 270, 27127–27135
11. Liebersbach, B. F., and Sanderson, R. D. (1994) J. Biol. Chem. 269, 20013–20019
12. Stanley, M. J., Liebersbach, B. F., Liu, W., Anhalt, D. J., and Sanderson, R. D. (1995) J. Biol. Chem. 270, 5077–5083
13. Saunders, S., Jalkanen, M., O’Farrell, S., and Bernfield, M. (1989) J. Cell Biol. 108, 1547–1556
14. Dhodapkar, M. V., Abe, E. A., Theus, A., Lacy, M., Langford, J. K., Barlogie, B., and Sanderson, R. D. (1998) Blood 91, 20013–20019
15. Sanderson, R. D., Turnbull, J. E., Gallagher, J. T., and Lander, A. D. (1994) J. Biol. Chem. 269, 13100–13106
16. David, G., Bai, X. M., Van der Schueren, B., Cassiman, J.-J., and Van den Berghe, H. (1992) J. Cell Biol. 119, 961–975
17. Lee, M. K., and Lander, A. D. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2768–2772
18. Kokenyesi, R., and Bernfield, M. (1994) J. Biol. Chem. 269, 12304–12309
19. Ridley, R. C., Xiao, H., Hata, H., Woodliff, J., Epstein, J., and Sanderson, R. D. (1993) Blood 81, 767–774
20. San Antonio, J. D., Karsowsky, M. J., Gay, S., Sanderson, R. D., and Lander, A. D. (1994) Glycobiology 4, 327–332
21. Dolan, M., Horchar, T., Rigatti, B., and Hassell, J. R. (1997) J. Biol. Chem. 272, 4316–4322
22. Faassen, A. E., Schrager, J. A., Klein, D. J., Oegema, T. R., Coughman, J. R., and McCarthy, J. B. (1992) J. Cell Biol. 116, 521–531
23. Iida, J., Meijne, A. M. L., Oegema, T. R., Jr., Yednock, T. A., Kovach, N. L., Furcht, L. T., and McCarthy, J. B. (1998) J. Biol. Chem. 273, 5555–5562
24. Mertens, G., VanderSchueren, B., vandenBerghe, H., and David, G. (1996) J. Cell Biol. 132, 487–497