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O-linked Glycosylation Modifies CD44 Adhesion to Hyaluronate in Colon Carcinoma Cells

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CD44 alternative splicing patterns differ between normal and malignant tissue, and accordingly, modulation of CD44 splicing has received the most attention in studies that have examined the role of CD44 in tumor progression. Many investigators have examined functional differences between individual CD44 alternative splice variants. However, specific CD44 isoforms function uniquely depending on the type of cell on which they are expressed, thereby suggesting that additional tissue-specific mechanisms regulate CD44 function. In the present study we have demonstrated that colon carcinoma cells modify CD44 with O-linked glycosyl groups, and blockade of this glycosylation enhances their CD44-mediated adhesion to hyaluronate. This enhancement is attributable principally to CD44H (CD44s) rather than high molecular weight CD44 variants. Use of site-directed mutant *CD44*H cDNA transfectants demonstrated that CD44 O-linked glycosylation modulates interaction between hyaluronate and the B loop domain of CD44. The influence of glycosylation on CD44 function in colon carcinoma cells is specific to the presence of O-linked sugars; inhibition of N-linked glycosylation had minimal influence on CD44 function. These findings indicate that O-linked glycosylation may be as important as alternative splicing in the regulation of CD44 function and the broad spectrum of biological processes attributed to it, including normal development, tumor metastases, and lymphocyte function.

CD44 is a group of related cell surface glycoproteins expressed by a variety of normal and malignant tissues, and may play a role in several biological processes, including lymphocyte activation (1, 2), cell motility (3, 4), tumor growth regulation (5, 6), growth factor presentation (7), and tumor metastases (8, 9). Significant protein heterogeneity results from alternative splicing of 10 exons that encode a portion of the extracellular domain (10). Most tumors display different CD44 alternative splicing patterns than their normal tissue counterparts (11-18). This finding is significant because individual CD44 alternative splice variants possess different functional characteristics, such as their ability to bind to hyaluronate (19-21), promote cell motility (4, 21), and enhance metastatic potential (8, 9). Other mechanisms in addition to alternative splicing may play important roles in CD44 regulation. CD44 undergoes extensive post-translational modification, including N- and O-linked glycosylation and substitution with high molecular weight glycosaminoglycans (22-24). In the present study we have examined how CD44 function is influenced by protein glycosylation and our results suggest that CD44 glycosylation may be as functionally important as alternative splicing in the regulation of CD44 interaction with extracellular matrix.

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Abbreviations used in this paper: phenyl-α-GalNAc: Phenyl-N-Acetyl-α-D-Galactosaminide; mAb: monoclonal antibody; BSA: bovine serum albumin; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; DMSO, dimethyl sulfoxide; MTT, 1-[4,5-dimethylthiazol-2-yl]-3,5-diphenylformazan.

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MATERIALS AND METHODS

Cell lines. The human colon carcinoma cell lines KM12L4 (25) and SW620 were generous gifts from Drs. Isaiah Fidler and Lee Ellis, respectively (MD Anderson Cancer Center, Houston, TX). HT29 human colon carcinoma cells were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in DMEM-F12 supplemented with 8% FCS. To block O-linked glycosylation of CD44, cells were grown for 4 days in DMEM-F12/8% FCS/G418 in the presence of phenyl-α-GalNAc[2] (Sigma Chemical Co.). Phenyl-α-GalNAc was dissolved in acetone and added to a final concentration of 2 mM. An equivalent amount of acetone was added to the media of control cells. N-linked glycosylation was inhibited by growth of the cells for 4 days in DMEM-F12/8% FCS/G418 in the presence of tunicamycin (Sigma Chemical Co.) Tunicamycin was dissolved in 0.1 N NaOH and added to a final concentration of 0.75 μg/ml. An equivalent amount of 0.1 N NaOH was added to the media of control cells.

Antibodies. The mAbs F10-44-2 (Biodesign International, Kennebunk, ME) and BU52 (Binding Site, Inc., San Diego, CA) are directed against epitopes common to all CD44 isoforms. mAb BRIC 235 blocks CD44 binding to hyaluronate and was a generous gift from Dr. D. J. Anstee (International Blood Group Reference Laboratory, Bristol, U.K.).

Western blot and immunofluorescence. Western blot analysis was performed using F10-44-2 and immunofloures-cence was performed using BU52 as previously described (26).

Adhesion assays. Adhesion assays were performed as previously described (5). Briefly, cells were harvested with 5 mM EDTA in PBS and added to 96 well plates that had been coated with hyaluronate. Adhesion was allowed to proceed for one hour at 4°C. Plates were sealed with parafilm, vortexed briefly, inverted, and centrifuged. Unattached cells were shaken off and the number of viable cells was estimated using MTT (27,28). All experiments were performed in triplicate.

Transfection. SW620 colon carcinoma cells were grown to 25% confluence. 40 μl of lipofectamine (Life Technologies, Inc.) and 10μg of plasmid DNA were added to the cells in serum-free media. After overnight incubation, medium with 8% fetal calf serum was added for 48 hours. G418 was then added to a final concentration of 1.5 mg/ml to select for drug-resistant colonies. Colonies were screened by FACS analysis and by surface labeled immunoprecipitation.

RESULTS

CD44 expressed on human colon carcinoma cells have O-linked oligosaccharide substitution. We first examined two human colon carcinoma cell lines for O-linked glycosylation of CD44. We have previously reported that KM12L4 and HT29 human colon carcinoma cells do not express CD44H, but do express higher molecular weight CD44 isoforms that contain epitopes corresponding to exons V3, V6, and V8-10 (5). We have established stable tranfectants of these two cell lines by introduction of CD44H cDNA (designated with the suffix ΔH) or vector without cDNA (designated with the suffix Δneo) (5).

To determine the nature of CD44 post-translational modification in these cells, the cells were grown for 4 days in phenyl-α-GalNAc, an inhibitor of O-linked glycosylation, at a concentration that had no effect on cell viability. This compound blocks the addition of O-linked carbohydrates without affecting cell growth, cell viability, protein synthesis, N-linked glycosylation, or GAG synthesis (29). All of the CD44 isoforms expressed by KM12L4 and HT29 cells demonstrate a shift in molecular mass after exposure to phenyl-α-GalNAc (Fig. 1). Four days of incubation in phenyl-α-GalNAc blocked CD44 O-linked glycosylation more effectively than 2 days of incubation (data not shown). KM12L4 cells substitute CD44 more heavily with O-linked oligosaccharides than do HT29 cells.

Effect of O-linked glycosylation on CD44 adhesion to hyaluronate. We then examined whether these differences in O-linked oligosaccharide substitution patterns influence CD44H function. Cells grown in the presence of phenyl-α-GalNAc were tested for adhesion to hyaluronate. KM12L4Δneo and HT29Δneo cells grown in phenyl-α-GalNAc displayed minimal enhancement of hyaluronate binding compared to cells grown in solvent alone (Fig. 2). Because KM12L4Δneo and HT29Δneo express predominantly high molecular weight CD44 isoforms and virtually no detectable CD44H, these results suggest that the influence of O-linked glycosyl modification on hyaluronate adhesion by high molecular weight CD44 isoforms is minimal.

KM12L4ΔH and HT29ΔH are transfectants that express CD44H. We have previously reported that expression of CD44H by HT29ΔH dramatically enhances cellular adhesion to

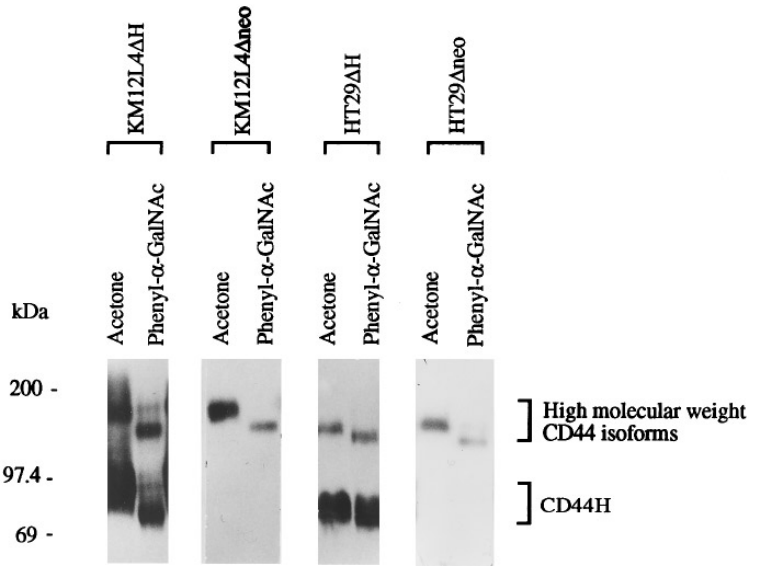

FIG. 1. Analysis of O-linked oligosaccharide substitutions on CD44. CD44 from KM12L4 and HT29 ransfectants grown for 4 days in the presence of either phenyl-α-GalNAc or solvent (acetone) were analyzed by Western blot to detect shifts in molecular mass. Shifts in molecular mass were detected after exposure of the cells to phenyl-α-GalNAc. Molecular weight standards are shown at the left in kDa.

hyaluronate, whereas expression of CD44H by KM12L4ΔH cells does not enhance their adhesion to hyaluronate (5). Interestingly, growth of KM12L4ΔH and HT29ΔH transfectants in the presence of phenyl-α-GalNAc significantly enhanced adhesion of both transfectants to hyaluronate (Fig. 2). This enhancement in adhesion was blocked by pre-treatment of the cells

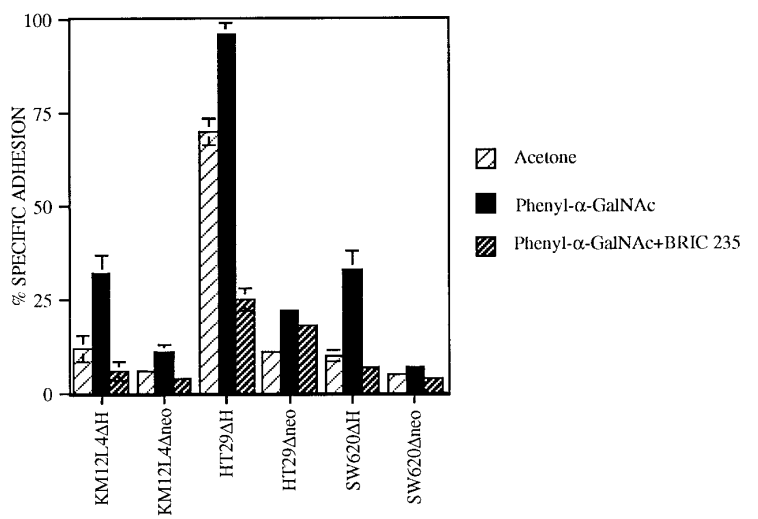

FIG. 2. Inhibition of O-linked oligosaccharide substitution on CD44 enhances adhesion to hyaluronate by KM12L4, HT29, and SW620 transfectants. Adhesion to hyaluronate of CD44H cDNA transfectants (designated ΔH) and control transfectants (designated Δneo) grown in the presence of either phenyl-α-GalNAc or solvent (acetone) was measured. Adhesion was also measured in the presence or absence of anti-CD44 mAb BRIC 235 (10 μg/ml). Data are presented as the mean ± S.D. of triplicate experiments.

with the anti-CD44 mAb BRIC 235, indicating that the enhancement in hyaluronate binding was mediated by CD44 rather than other cell surface adhesion molecules. These data support the hypothesis that O-linked glycosylation reduces CD44H adhesion to hyaluronate.

However, the presence of high molecular weight CD44 isoforms in addition to CD44H on KM12L4 and HT29 transfectants precluded an accurate assessment of how much of the enhancement of HA binding in response to phenyl-α-GalNAc treatment was mediated through its effect on CD44H versus high molecular weight isoforms. Accordingly, we identified a human colon carcinoma cell line, SW620, that does not express any CD44 isoforms. We established stable transfectants that express CD44H (designated SW620ΔH) as well as control transfectants (SW620Δneo) that had been tranfected with vector only. Cell surface CD44H expression was confirmed in SW620ΔH cells by FACS analysis and immunoprecipitation of cell surface labeled proteins (data not shown). CD44H expressed on SW620ΔH transfectants demonstrated a shift in molecular mass after growth in phenyl-α-GalNAc (data not shown), indicating that SW620ΔH cells also post-translationally modify CD44H by O-linked glycosylation.

These cells were then examined for their adhesion to hyaluronate. As expected, SW620Δneo cells, which do not express any cell surface CD44 did not effectively bind to hyaluronate (Fig. 2). Furthermore, growth of these cells in the presence of phenyl-α-GalNAc did not enhance their adhesion to hyaluronate. In contrast, the adhesion of SW620ΔH cells to hyaluronate was enhanced markedly by growth of the cells in the presence of phenyl-α-GalNAc. This enhancement was blocked by pre-treatment of the cells with mAb BRIC 235. The absence of CD44 expression on SW620Δneo cells combined with the absence of enhanced SW620Δneo hyaluronate adhesion after growth with phenyl-α-GalNAc support the hypothesis that the phenyl-α-GalNAc-mediated enhancement of SW620ΔH cell adhesion to hyaluronate was mediated principally by CD44H and not another cell surface protein. The enhancement of hyaluronate adhesion displayed by SW620ΔH transfectants that express only CD44H, combined with the minimal enhancement noted in treated cells that express only high molecular weight CD44 isoforms (KM12L4Δneo and HT29Δneo) suggest that the effect of O-linked glycosylation on hyaluronate adhesion was mediated principally by CD44H rather than high molecular weight CD44 variants.

Inhibition of O-linked glycosylation does not increase cell surface CD44. These experiments raised the possibility that growth of the cells in the presence of phenyl-α-GalNAc increased their adhesion to hyaluronate by increasing total cell surface CD44. To address this hypothesis, we performed FACS analysis with mAb BU52 to measure cell surface CD44 on cells grown either in the presence or absence of phenyl-α-GalNAc. No increase in cell surface CD44 expression was detected on cells exposed to phenyl-α-GalNAc, indicating that phenyl-α-GalNAc did not increase total cell surface CD44 expression (data not shown).

Identification of the CD44 domain responsible for enhanced hyaluronate binding after blockage of O-linked glycosylation. Because mAb BRIC 235 blocked CD44 adhesion to hyaluronate and also blocked the enhancement of adhesion to hyaluronate measured in phenyl-α-GalNAc-treated cells, we formulated the hypothesis that reduction in CD44 O-linked glycosylation affected its binding to hyaluronate through modulation of the B loop domain (30, 31). To test our hypothesis we used a site-directed mutant CD44H, in which amino acid 41 within the B loop has been changed from arginine to alanine. This alteration dramatically reduces the mutant CD44H adhesion to hyaluronate.

Surface expression of mutant CD44H on HT29 and SW620 tranfectants (designated HT29Δ41R/A and SW620Δ41R/A, respectively) was confirmed by Western blot and FACS (data not shown). HT29Δ41R/A, and SW620Δ41R/A transfectants demonstrated the same hyaluronate binding patterns as did the control HT29Δneo and SW620Δneo cells, confirming that the mutant CD44H binds to hyaluronate poorly. Cells expressing mutant CD44H grown

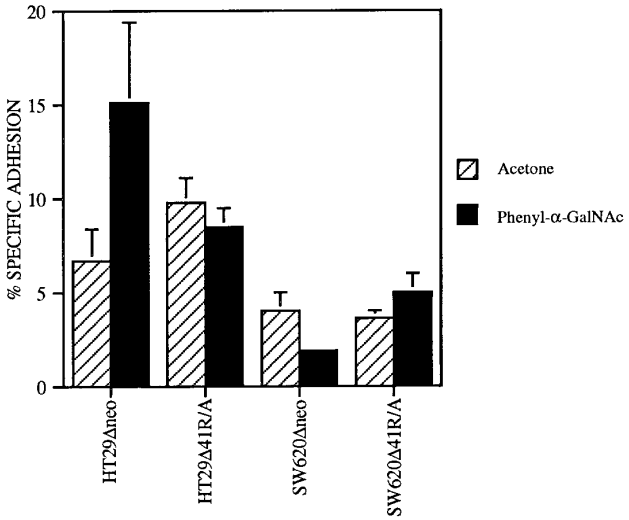

FIG. 3. Phenyl-α-GalNAc treatment of HT29 and SW620 cells expressing mutant CD44H (designated HT29Δ41R/A and SW620Δ41R/A) does not enhance adhesion to hyaluronate. Site-directed mutation of the *CD44*H cDNA that changes arginine 41 to alanine in the B loop domain significantly reduces its ability to bind to hyaluronate. Transfectants expressing this mutant form of CD44H (designated with the suffix Δ41R/A) were grown for 4 days in the presence of either phenyl-α-GalNAc or solvent (acetone). Inhibition of O-linked glycosylation did not enhance hyaluronate adhesion by mutant CD44H Data are presented as the mean $\pm$ S.D. of triplicate experiments.

in the presence of phenyl-α-GalNAc did not display enhanced binding to hyaluronate (Fig 3). These results indicate that O-linked oligosaccharide substitution on CD44H modulated its interaction with hyaluronate through its B-loop domain.

Effect of N-linked glycosylation on CD44 adhesion to hyaluronate. The influence of O-linked glycosylation on CD44 function was evident in each of the colon carcinoma cell lines that we examined. CD44 also undergoes N-linked glycosylation and Western blot analysis revealed a minor shift in the CD44 molecular mass in cells exposed to tunicamycin (data not shown). We measured minor increases in hyaluronate binding of KM12L4, HT29, and SW620 transfectants after growth in the presence of tunicamycin (Fig. 4). The magnitude of the shift in molecular mass and the magnitude of the enhancement in hyaluronate binding was smaller in cells exposed to tunicamycin than in cells exposed to phenyl-α-GalNAc.

DISCUSSION

CD44 expression has been characterized in many types of tumors (11-18, 32, 33). Tumor cell expression of CD44 modulates tumorigenicity and metastastic potential, and moreover, this influence of CD44 expression on tumor phenotype differs between different tumors (8, 9). These studies suggest that CD44 function is regulated and that this regulation occurs in a tissue-specific manner. The regulation of CD44 interaction with hyaluronate has been clearly demonstrated in hematopoietic cell lines (19), and the abundance of hyaluronate in the extracellular matrix suggests that CD44 interaction with hyaluronate may be regulated in many other cell types. This hypothesis is supported by the finding that not all cells that express cell surface CD44H are capable of binding to hyaluronate (19, 20, 34, 35).

CD44 alternative splicing has received the most attention in studies of regulation of CD44 function. In contrast, we have focused our studies on a separate and distinct CD44 regulatory mechanism, CD44 post-translational modification. We have previously reported that keratan sulfate modification of CD44 modulates its adhesion to hyluronate (5). In the present study

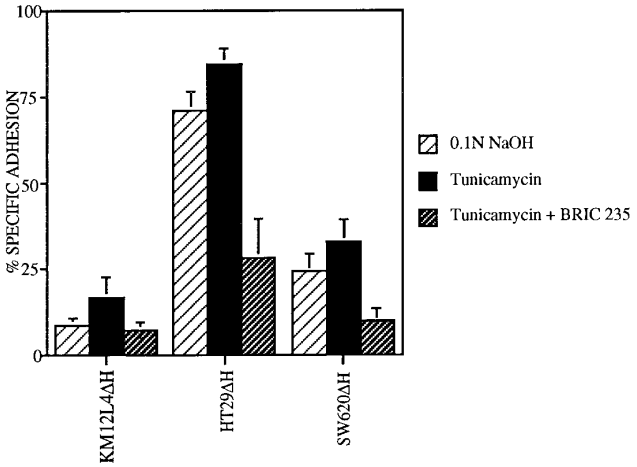

FIG. 4. Effect of N-linked CD44 glycosylation on its adhesion to hyaluronate. Transfectants grown in the presence of either tunicamycin or solvent were tested for hyaluronate adhesion. Minimal enhancement of hyaluronate adhesion was detected in cells treated with tunicamycin. Data are presented as mean ± S.D. of triplicate experiments.

we have demonstrated that differences between cells in their O-linked glycosylation of CD44 modulates their adhesion to hyaluronate. Studies with mutant CD44H indicate that these glycosyl groups modulate interaction between hyaluronate and the B loop domain of CD44H.

The effects of phenyl-α-GalNA are unlikely to be confined to CD44; growth of the colon carcinoma cells in the presence of phenyl-α-GalNAc inhibits O-linked glycosylation of most if not all glycosylated structures on the cell surface. However, we carefully controlled for this lack of treatment specificity. First, we demonstrated that the enhanced cellular adhesion to hyaluronate detected in treated cells could be abrogated by the CD44-specific mAb, BRIC 235. Second, we examined SW620 that express no cell surface CD44 and demonstrated no enhancement in hyaluronate adhesion after treatment with phenyl-α-GalNAc. However, once these cells were transfected to express CD44H (SW620ΔH), they demonstrated enhanced hyaluronate adhesion after treatment with phenyl-α-GalNAc. And lastly, phenyl-α-GalNAc treatment had no effect on cells transfected to express the site-directed mutant CD44H, in which the single amino acid mutation disrupts interaction between the B loop domain and hyaluronate. In concert, these data strongly suggest that the functional effect of phenyl-α-GalNAc is on CD44 itself. Nonetheless, these data do not completely eliminate the possibility that the treatment alters glycosylation of an unrelated structure that then modulates the function of CD44. Presently, there are no published data to suggest that such a hypothetical protein exists.

Significant differences in glycosylation machinery that exist between cell lines suggest that the influence of glycosylation on CD44 function differs between cell lines and tumor types. Accordingly, it is not possible to extrapolate results obtained with human colon carcinoma cell lines to other tumor cell lines (7, 36, 37). Furthermore, additional regulatory mechanisms such as the degree of glycosaminoglycan substitution, phosphorylation status, or interactions with other cell surface proteins differ between tumor cell lines.

The mechanism by which glycosylation of CD44 affects its adhesion to hyaluronate has not yet been elucidated. The cluster of basic amino acids in the B loop domain appears to be critical for its interaction with hyaluronate (38); site-directed mutagenesis of this domain dramatically reduces the ability of CD44H to bind hyaluronate (30). Because CD44 adhesion to hyaluronate can be influenced by mAb IRAWB 14 (19) or by inclusion of alternatively

spliced exons in the membrane proximal region (34, 39), it is tempting to speculate that CD44 glycosylation may modulate hyaluronate adhesion via changes in protein conformation. Alternatively, the negatively charged glycosyl groups may obscure exposure of the positively charged residues that comprise the hyaluronate binding site.

In the current study we have demonstrated that O-linked glycosylation of CD44H reduces its adhesion to hyaluronate in three separate colon carcinoma cell lines of different origins. O-linked glycosylation of high molecular weight CD44 isoforms, and N-linked glycosylation of CD44 appears to influence hyaluronate binding to a much lesser degree. Our previous studies tightly link regulations of colon carcinoma growth with its expression of CD44H and ability to bind hyaluronate (5). Furthermore, cell surface hyaluronate correlates with metastatic potential in experimental metastases models (40). The impact of glycosylation on CD44 function is significant, and may be as functionally important as *CD44* alternative splicing. Closer examination of this CD44 regulatory mechanism in investigations of development, tumor progression, tumor metastases, and lymphocyte function will likely reveal it to be an important regulator for these biological processes.

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REFERENCES

1. Arch, R., Wirth, K., Hofmann, M., Ponta, H., Matzku, S., Herrlich, P., and Zöller, M. (1992) *Science* **257,** 682–685.
2. Huet, S., Groux, H., Caillou, B., Valentin, H., Prieur, A. M., and Bernard, A. (1989) *J. Immunol.* **143,** 798–801.
3. Weber, G. F., Ashkar, S., Glimcher, M. J., and Cantor, H. (1996) *Science* **271,** 509–512.
4. Thomas, L., Byers, R., Vink, J., and Stamenkovic, I. (1992) *J. Cell Biol.* **113,** 971–977.
5. Takahashi, K., Stamenkovic, I., Cutler, M., Saya, H., and Tanabe, K. K. (1995) *Oncogene* **11,** 2223–2232.
6. Bartolazzi, A., Peach, R., Aruffo, A., and Stamenkovic, I. (1994) *J. Exp. Med.* **180,** 53–66.
7. Bennett, K. L., Modrell, B., Greenfield, B., Bartolazzi, A., Stamenkovic, I., Peach, R., Jackson, D. G., Spring, F., and Aruffo, A. (1995) *J. Cell Biol.* **131,** 1623–1633.
8. Günthert, U., Hofmann, M., Rudy, W., Reber, S., Zöller, M., Haubmann, I., Matzku, S., Wenzel, A., Ponta, H., and Herrlich, P. (1991) *Cell* **65,** 13–24.
9. Sy, M. S., Guo, Y. J., and Stamenkovic, I. (1991) *J. Exp. Med.* **174,** 859–866.
10. Screaton, G. R., Bell, M. V., Jackson, D. G., Cornelis, F. B., Gerth, U., and Bel, J. I. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 12160–12164.
11. Tanabe, K. K., Ellis, L. M., and Saya, H. (1993) *Lancet* **341,** 725–726.
12. Matsumura, Y., and Tarin, D. (1992) *Lancet* **340,** 1053–1058.
13. Kaufmann, M., Heider, K. H., Sinn, H. P., von Minckwitz, G., Ponta, H., and Herrlich, P. (1995) *Lancet* **345,** 615–619.
14. Heider, K.-H., Dämmrich, J., Skroch-Angel, P., Müller-Hermelink, H.-K., Vollmers, H. P., Herrlich, P., and Ponta, H. (1993) *Cancer Res.* **53,** 4197–4203.
15. Gansauge, F., Gansuage, S., Zobywalski, A., Scharnweber, C., Link, K. H., Nussler, A. K., and Beger, H. G. (1995) *Cancer Res.* **55,** 5499–5503.
16. Nagasaka, S., Tanabe, K. K., Bruner, J. M., Saya, H., Sawaya, R. E., and Morrison, R. S. (1995) *J. Neurosurg.* **82,** 858–863.
17. Fox, S. B., Fawcett, J., Jackson, D. G., Collins, I., Gatter, K. C., Harris, A. L., Gearing, A., and Simmons, D. L. (1994) *Cancer Res.* **54,** 4539–4546.
18. Salmi, M., Grön-Virta, K., Sointu, P., Grenman, R., Kalimo, H., and Jalkanen, S. (1993) *J. Cell Biol.* **122,** 431–442.
19. Lesley, J., He, Q., Miyake, K., Hamann, A., Hyman, R., and Kincade, P. W. (1992) *J. Exp. Med.* **175,** 257–266.
20. van der Voort, R., Manten-Horst, E., Smit, L., Ostermann, E., van der Berg, F., and Pals, S. T. (1995) *Biochem. Biophys. Res. Comm.* **214,** 137–144.
21. Culty, M., Shizari, M., Thompson, E. W., and Underhill, C. B. (1994) *J. Cell. Physiol.* **160,** 275–286.

22. Carter, W. G., and Wayner, E. A. (1988) *J. Biol. Chem.* **263,** 4193–4201.
23. Bennet, K. L., Jackson, D. G., Simon, J. C., Tanczos, E., Peach, R., Modrell, B., Stamenkovic, I., Plowman, G., and Aruffo, A. (1995) *J. Cell Biol.* **128,** 687–698.
24. Stamenkovic, I., Amiot, M., Pesando, J. M., and Seed, B. (1989) *Cell* **56,** 1057–1062.
25. Morikawa, K., Walker, S. M., Nakajima, M., Pathak, S., Jessup, J. M., and Fidler, I. J. (1988) *Cancer Res.* **48,** 6863–6871.
26. Takahashi, K., Stamenkovic, I., Cutler, M., Dasgupta, A., and Tanabe, K. K. (1996) *J. Biol. Chem.* **271,** 9490–9496.
27. Denizot, F., and Lang, R. (1986) *J. Immunol. Methods* **89,** 271–277.
28. Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D., and Mitchell, J. B. (1987) *Cancer Res.* **47,** 936–942.
29. Kuan, S.-F., Byrd, J. C., Basbaum, C., and Kim, Y. S. (1989) *J. Biol. Chem.* **264,** 19271–19277.
30. Peach, R. J., Hollenbaush, I., Stamenkovic, I., and Arrufo, A. (1993) *J. Cell. Biol.* **122,** 257.
31. Yang, B., Yang, B. L., Savani, R. C., and Turley, E. A. (1994) *EMBO* **13,** 286–296.
32. Jalkanen, S., Joensuu, H., Soderstrom, K. O., and Klemi, P. (1991) *J. Clin. Invest.* **87,** 1835–1840.
33. Wielenga, V. J. M., Heider, K.-H., Offerhaus, J. A., Adolf, G. R., van den Berg, F. M., Ponta, H., Herrlich, P., and Pals, S. T. (1993) *Cancer Res.* **53,** 4754–4756.
34. Bartolazzi, A., Jackson, D., Bennett, K., Aruffo, A., Dickinson, R., Shields, J., Whittle, N., and Stamenkovic, I. (1995) *J. Cell Science* **108,** 1723–1733.
35. Dougherty, G. J., Cooper, D. L., Memory, J. F., and Chiu, R. K. (1994) *J. Biol. Chem.* **269,** 9074–9078.
36. Lesley, J., English, N., Perschl, A., Gregoroff, N., and Hyman, R. (1995) *J. Exp. Med.* **182,** 431–437.
37. Katoh, S., Zheng, Z., Oritani, K., Takaichi, S., and Kincade, P. W. (1995) *J. Exp. Med.* **182,** 419–429.
38. Yang, B., Zhang, L., and Turley, E. A. (1993) *J. Biol. Chem.* **268,** 8617–8623.
39. Jackson, D. G., Bell, J. I., Dickinson, R., Timans, J., Shields, J., and Whittle, N. (1995) *J. Cell Biol.* **128,** 673–685.
40. Zhang, L., Underhill, C., and Chen, L. (1995) *Cancer Res.* **55,** 428–433.