CD44H Regulates Tumor Cell Migration on Hyaluronate-coated Substrate

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Abstract. CD44 is a broadly distributed cell surface glycoprotein expressed in different isoforms in various tissues and cell lines. One of two recently characterized human isoforms, CD44H, is a cell surface receptor for hyaluronate, suggesting a role in the regulation of cell-cell and cell-substrate interactions as well as of cell migration. While CD44H has been shown to mediate cell adhesion, direct demonstration that CD44H expression promotes cell motility has been lacking. In this work we show that a human melanoma cell line, stably transfected with CD44H, displays enhanced motility on hyaluronate-coated surfaces while transfectants expressing an isoform that does not bind hyaluronate, CD44E, fail to do so. Migration of CD44H-expressing transfectants is observed to be blocked by a soluble CD44-immunoglobulin fusion protein as well as by anti-CD44 antibody, and to depend on the presence of the cytoplasmic domain of CD44. However, cells expressing CD44H cytoplasmic deletion mutants retain significant binding capacity to hyaluronate-coated substrate. Taken together, our results provide direct evidence that CD44H plays a major role in regulating cell migration on hyaluronate-coated substrate.

THE cell surface glycoprotein CD44 is a polymorphic molecule as a result of differential usage of a series of exons encoding a portion of the extracellular domain and cell lineage-specific glycosylation (Stamenkovic et al., 1991; Brown et al., 1991; Hoffman et al., 1991; Gunthert et al., 1991; Stamenkovic et al., 1989). Two human isoforms of 85 and 150 kD have recently been characterized. The 85-kD isoform, CD44H, is broadly distributed in hematopoietic cells, fibroblasts, and numerous tumors of both mesenchymal and neuroectodermal origin (Stamenkovic et al., 1989; Quackenbush et al., 1990), while expression of the 150-kD isoform, CD44E, appears restricted to subsets of epithelial cells (Stamenkovic et al., 1991; Brown et al., 1991). In addition to differences in their polypeptide sequences and posttranslational modifications, the two isoforms have been shown to differ functionally. Whereas CD44H displays high affinity for hyaluronate (Aruffo et al., 1990; Culty et al., 1990; Miyake et al., 1990; Sy et al., 1991), CD44E does not mediate attachment to surface-bound hyaluronate, and its ligands remain to be identified (Stamenkovic et al., 1991; Sy et al., 1991).

As the principal cell surface receptor for hyaluronate, CD44H plays an important role in both cell-cell and cell-substrate adhesion (Stamenkovic et al., 1991; Aruffo et al., 1990; Culty et al., 1990; Miyake et al., 1990; Sy et al., 1991; Miyake et al., 1990*b*; St. John et al., 1990). However, mediating cell adhesion is likely to be only a part of the function of CD44H. The observations that the cytoplasmic tail of CD44 colocalizes with cytoskeletal proteins (Jacobson et al., 1984*a*,*b*; Kalomiris and Bourguignon, 1989; Lacy and Underhill, 1987; Tarone et al., 1984) and that hyaluronate accumulates in areas of cell migration, including developing limb buds (Toole, 1982), and sites of inflammation (Weigel, et al., 1989) and tumor invasion (Knudson et al., 1984; Knudson et al., 1989; Knudson and Knudson, 1990), are consistent with the notion that CD44H may play a major role in regulation of cell motility. Although such a function has often been alluded to (Toole, 1982; Turley et al., 1991), direct evidence for a role in motility has been lacking. To determine whether expression of either of the two isoforms of CD44 influences cell motility, CD44H and CD44E, as well as two CD44H cytoplasmic deletion mutants, were stably expressed in a human CD44-negative melanoma cell line and transfectants tested for motility on and adhesion to a variety of substrates. The choice of melanoma cells was prompted by observations that melanoma cell invasiveness (Hart et al., 1991), metastatic proclivity (Turley and Tretiak, 1985) and motility (Thomas, L., and H.R. Byers, unpublished observations) correlate with CD44 expression. Our results show that expression of CD44H promotes melanoma cell migration on hyaluronate-coated surfaces while expression of CD44E has no significant effect. The observed enhancement of cell migration is shown to depend on the presence of both the extracellular and the cytoplasmic domain of CD44H, while adhesion to hyaluronate-coated substrate is predominantly determined by the extracellular domain.

Materials and Methods

cDNA Clones and Cytoplasmic Deletion Mutants

cDNA clones encoding CD44H (Stamenkovic et al., 1989) and CD44E (Stamenkovic et al., 1991) were isolated previously and production of soluble CD44-immunoglobulin fusions has been described (Aruffo et al., 1990). The cytoplasmic deletion mutants CD44HΔ1 AND CD44HΔ2 were generated by synthetic oligonucleotide-primed amplification of sequences encoding the extracellular, transmembrane, and truncated cytoplasmic domains of CD44H in polymerase chain reactions (PCR). A 5′ oligonucleotide possessing a HindIII endonuclease restriction site was designed as follows: CGC GGG AAG CTT ATG GAC AAG TTT TGG TGG CACGCA GCC TGG.

A 3′ oligonucleotide primer complementary to the reverse sequence of CD44H starting at residue 297 (CD44HΔ1) was designed to contain a stop codon downstream from residue 297 and an XhoI endonuclease restriction site as follows: CGC GGG CTC GAG TTA CTG CCC ACA CCT TCG ACT GTT GAC.

Similarly, a second 3′ oligonucleotide primer complementary to the reverse sequences of CD44H starting at residue 305 (CD44HΔ2) was designed to contain a stop codon downstream from residue 305 and an XhoI restriction site as follows: CGC GGG CTC GAG TTA ACT GTT GAT CAC TAG CTT CCC CTT CTG.

PCR cycles were conducted along the following pattern: 94°C/1 min; 60°C/2 min; 72°C/3 min for 30 cycles. Amplified sequences were subjected to Hind III and Xho I digestion and ligated to HindIII/XhoI-cut CDM8 vector (Seed, 1987).

Stable Transfectants and CD44 Expression

The cell line RPM-MC was derived from a recurrent cutaneous melanoma and has been described previously (Byers et al., 1991). Melanoma cells stably expressing CD44 variants were generated by cotransfecting the various CD44 constructs and the pSV2neo plasmid, bearing the neomycin resistance gene, by electroporation. 50 μg of plasmid DNA containing a given CD44 construct and 1 μg of pSV2Neo DNA were added to 0.4 ml of a 10^7/ml cell suspension in PBS in 4-mm electroporation chambers. Cells were electroporated at 250 V/960 μF using a Biorad Gene Pulser (Richmond, CA). Immediately after electroporation, cells were incubated on ice for 10 min and seeded onto 24-well plates at 2×10^5 cells/well in DME supplemented with 20% FBS (Gibco Laboratories, Grand Island, NY). After 48 h, the medium was replaced with DME/10% FBS containing 800 μg/ml of G418 (Gibco Laboratories). Three to four weeks after transfection individual clones were picked, seeded onto fresh plates, expanded, tested for CD44 expression by indirect immunofluorescence, and subcloned by limiting dilution to obtain homogeneous cell lines expressing high levels of the various CD44 isoforms and mutants.

To identify CD44-expressing clones, cells were lifted from plates with EDTA and incubated for 45 min at 4°C with the anti-CD44 mAb F10–44–2 (Accurate Chemicals Corp., Westbury, NY) at a 1:500 dilution. Cells were washed in PBS, incubated with affinity-purified, fluorescein-conjugated goat-antimouse antibody (Cappel, Malvern, PA), washed, and analyzed on a FACScan cytometer (Becton Dickinson, Mountainview, CA).

Production of Soluble CD44

Production of soluble CD44-Ig and GMP140-Ig fusion proteins (called CD44 or GMP140 receptor globulins [CD44Rg]) has been described previously (Aruffo et al., 1990; Aruffo et al., 1991). Briefly, CD44-Ig and GMP140-Ig-containing expression vectors were introduced into COS cells by the DEAE-Dextran method (Aruffo et al., 1990) and supernatants were harvested 5–8 d after transfection. CD44Rg and GMP140Rg were purified on trisacryl protein A beads (Pierce Chemical Co., Rockford, IL), dialyzed against PBS overnight, and the concentration was determined in an ELISA assay. In blocking experiments, purified CD44Rg and GMP140Rg were used at 25 μg/ml.

Attachment Assays

24-well tissue culture plates (Falcon, Becton-Dickinson, Lincoln Park, NJ) were incubated overnight at 4°C with a 5-mg/ml solution of hyaluronic acid (Sigma Chemical Co., St. Louis, MO) or chondroitin-6-sulfate (Sigma Chemical Co.) in PBS. Wells were washed with PBS, incubated for 12 h with heat-denatured BSA (Sigma Chemical Co.), washed and used for attachment experiments. Control wells were treated with heat-denatured BSA only. Subconfluent cultures of CD44 transfectants were radiolabeled with ^{3}H-thymidine overnight at 37°C, detached with EDTA, and seeded at 10^5 cells/well. Cells were allowed to adhere to substrate for 30 min at 4°C, and nonadherent cells were washed away with PBS. These conditions were found to produce the least nonspecific attachment. Adhesion cells were lysed with 0.1% SDS and the corresponding radioactivity was determined. The fraction of attached cells for each cell line was deduced by calculating the ratio of the radioactivity displayed by attached cells to the radioactivity released from the total number of cells initially seeded. All experiments were done in triplicate; statistical analysis was performed using a *t* test.

Attachment Inhibition Assays

Hyaluronate-coated wells were incubated with CD44Rg or GMP140Rg (25 μg/ml) for 30 min at RT before seeding of cell lines, washed with PBS, and subjected to seeding with $CD44H_{high}$ transfectants. In antibody blocking assays, transfectants were preincubated for 30 min at 4°C with anti-CD44 mAb KM201 (Miyake et al., 1990), purchased from ATCC or with a control, anti-HLA class I mAb, W6/32 (ATCC), before seeding on substrate-coated wells. 1 ml of undiluted hybridoma supernatant was used in each case. Incubation, washing, and evaluation of attached cells were done as above.

Migration Assays

Coated substrates were derived following a slight modification of the procedures of Goodman et al. (1989). Briefly, sterile glass coverslips were incubated for 6 h at 4°C in 35-mm petri dishes containing 0.5 or 5 mg/ml hyaluronic acid or chondroitin sulfate in PBS. Coverslips were then washed with PBS, incubated overnight with heat-denatured BSA, and washed with PBS. Control coverslips were treated with heat-denatured BSA only. Subconfluent cultures of the various CD44 transfectants were lifted off culture plates with EDTA, and one drop of a suspension of 10^6 cells was placed in the center of the coated coverslips immersed in DME/10%FBS gauged to obtain a cell density ranging between 0.4 and 1.0 cells/$10^4 \mu m^2$. After a minimum of 4 h of incubation at 37°C in 5% atmospheric CO_2, cell migration was studied over a 3-h period under a Nikon Diaphot inverted microscope with a 10× phase-contact objective, in an attached, hermetically sealed plexiglass Nikon NP-2 incubator at 37°C. Cell migration was recorded using a Dage-MTI 65DX video camera and a Hitachi TLC1550 time-lapse video cassette recorder. Image analysis was performed by playing back video images, digital saving of images at 0, 1, 2, and 3 h, and planar morphometry determination of the migration rate. Migration rate was defined by the algebraic sum of all the two-dimensional migration distances and expressed in μm/h. Migration of at least 35 cells was analyzed for each experimental condition. Image analysis was performed with a Microcomp image analysis system (Southern Micro Instruments, Atlanta, GA), a Numonic digitablet, a high resolution video monitor (Sony, New York, NY) and an IBM-compatible computer (Samsung S500) equipped with a video card (PC version plus frame grabber; Imaging Technology, Woburn, MA). Data were saved as an MS-DOS (Microsoft) file and translated through a network (TOPS, Sun Microsystem Co., Berkeley, CA) to a Macintosh SE for statistical analysis using a *t* test. Normalized migration paths were obtained, using graphic computer software, by "grabbing" individual cell paths and "dragging" them without rotation so that the origin of each path was superimposed on one central point.

Migration Inhibition Assays

Cell suspensions of CD44 transfectants were placed on sterile coverslips coated with a 5 mg/ml solution of hyaluronic acid as described above. The medium was removed, coverslips were washed in PBS, and medium (DME) containing 25 μg/ml of CD44Rg, GMP140Rg, anti-CD44 mAb KM201, or anti-HLA mAb (undiluted supernatants) was added to petri dishes containing the coverslips. The preparation was allowed to reequilibrate for 25 min at 37°C, following which migration pathways of cells were recorded and analyzed as described above.

Results

Development of Stable Human Melanoma Transfectants Expressing Wild Type or Truncated CD44 Isoforms

Previously described cDNA clones encoding CD44H (Sta-

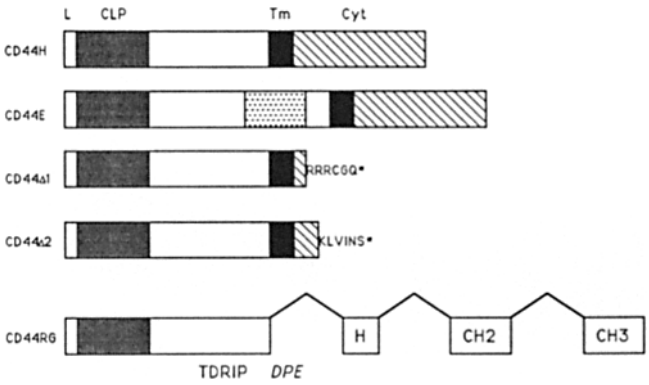

Figure 1. CD44 constructs. Each of the constructs containing CD44-specific cDNA sequences is illustrated schematically. CD44H and CD44E represent the entire, unmodified cDNA sequences encoding the 85- and 150-kD isoforms, respectively. The leader peptide (*L*), the cartilage link protein-homologous region (*CLP*), the transmembrane region (*Tm*), and the cytoplasmic domain (*cyt*) are indicated. CD44HΔ1 is a cytoplasmic deletion mutant lacking the entire cytoplasmic domain with the exception of the six residues indicated; the asterisk indicates a stop codon. CD44HΔ2 is a second cytoplasmic deletion mutant containing the first 16 cytoplasmic residues. The last six amino acids and the inserted stop codon are indicated. CD44Rg represents the CD44-immunoglobulin fusion protein. Antibody exons are boxed and introns are represented by connecting lines. H, CH2, and CH3 denote the hinge, and constant regions CH2 and CH3 respectively. Amino acid sequences predicted at the fusion are indicated below.

menkovic et al., 1989) and CD44E (Stamenkovic et al., 1991) as well as two CD44H cytoplasmic deletion mutants, CD44HΔ1 and CD44HΔ2, were stably expressed in the human melanoma cell line RPM-MC (Byers et al., 1991) which does not constitutively express CD44 (Fig. 1). CD44HΔ1 and two cytoplasmic deletion mutants were derived by synthetic oligonucleotide-primed amplification of cDNA sequences encoding the entire extracellular and transmembrane domains and truncated intracytoplasmic segments of CD44H. A stop codon was introduced at the 3′ end of the truncated sequences (Fig. 1), and oligonucleotide primers were designed to include restriction sites allowing directional subcloning into the CDM8 vector (Seed, 1987).

Each of the constructs was introduced by electroporation into the melanoma cells. cDNA clones were cotransfected with pSV2Neo and transfectants were selected for neomycin resistance. Each of the resulting transfectant clones was tested for CD44 expression by indirect immunofluorescence using the F-10-44-2 anti-CD44 mAb (Table I), and 5 clones were retained for further experiments. The cell line MC-C, transfected with the CDM8 and pSV2Neo vectors was used as a negative control. It showed no reactivity with anti-CD44 mAb (mean fluorescence intensity (MFI: 3.0). Two cell lines, MC-44H$_{high}$ and MC44H$_{low}$, transfected with CD44H-specific cDNA were selected for high (MFI: 385) and low (MFI: 83) levels of surface CD44 expression, respectively, and used to determine the role of CD44H in cell motility. To determine the requirement for the cytoplasmic domain of CD44H in adhesion and motility, two cell lines, MC-44HΔ1 and MC-44HΔ2, expressing cytoplasmic deletion mutants of CD44H, were derived. MC-44HΔ1 and MC-44HΔ2 express truncated forms of CD44H, containing only the first 6 and 16 cytoplasmic residues, respectively (Fig. 1). Both cell lines were selected for high expression of truncated CD44H (respective MFIs of 440 and 375), comparable to surface CD44 expression of MC-44H$_{high}$ (Table I). A cell line obtained as a result of transfection of CD44E, MC-44E, expressing high (MFI: 400) levels of CD44E was selected for the study of the role of CD44E in cell migration.

Table I. CD44 Expression in Melanoma Cell Transfectants

Cell line	Transfected DNA	Mean fluorescence intensity
MC-C	pCDM8 / psv2Neo	3
MC-44H$_{high}$	CD44H / psv2Neo	385
MC-44H$_{low}$	CD44H / psv2Neo	83
MC-44E	CD44E / psv2Neo	400
MC-44HΔ1	CD44HΔ1 / psv2Neo	440
MC-44HΔ2	CD44HΔ2 / psv2Neo	375

Each of the transfectants used in the present experiments is indicated, along with the transfected wild type or mutant CD44 cDNA (all cDNA inserts were in pCDM8) and the corresponding level of surface expression as assessed by indirect immunofluorescence. Reactivity of CD44 transfectants was determined using the mAb F-10-44-2 and expressed as mean fluorescence intensity.

Attachment of CD44H and CD44E Melanoma Transfectants to Hyaluronate-coated Surfaces

CD44 transfectants were radiolabeled with ^{3}H-thymidine and tested for attachment to hyaluronate-coated plates. Cells were seeded onto plastic microtiter wells coated with 5 mg/ml hyaluronate, heat-denatured BSA or chondroitin-6-sulfate, allowed to attach to substrate for 30 min, and nonadherent cells removed by washing. Adherent cells were lysed, incorporated radioactivity was measured, and the number of attached cells calculated from the ratio of residual radioactivity to radioactivity incorporated by the initial number of seeded cells. MC-44H$_{high}$ cells displayed 10-fold higher degree of attachment to hyaluronate substrates than MC-C controls while the MC-44E cell line failed to exhibit significant binding (Fig. 2). This result confirmed previous observations using lymphoma CD44 transfectants (Sy et al., 1991), as well as findings that CD44+ but not CD44− melanomas bound hyaluronate-coated substrates (Thomas, L., and H. R. Byers, unpublished observations). Attachment of CD44H transfectants was effectively blocked by preincubation of transfectants with anti-CD44 mAb KM201 (which recognizes both human and murine CD44) confirming previous observations of Miyake et al. (1990), and by preincubating hyaluronate-coated wells with CD44Rg (Fig. 2).

Migration of CD44H and CD44E Melanoma Transfectants on Hyaluronate Substrates

The principal goal of this study was to determine whether the capacity of CD44H to bind hyaluronate may promote cell migration on appropriate substrate. Migration rate of the different transfectants on hyaluronate-coated surfaces was measured by image analysis after video-microscopic recording of 3-h migration pathways (Fig. 3). Transfectants expressing CD44H or CD44E at different levels were compared to CD44-negative MC-C cells for migration on hyaluronate, heat-denatured BSA and chondroitin-6-sulfate-

1. *Abbreviation used in this paper*: MFI, mean fluorescence intensity.

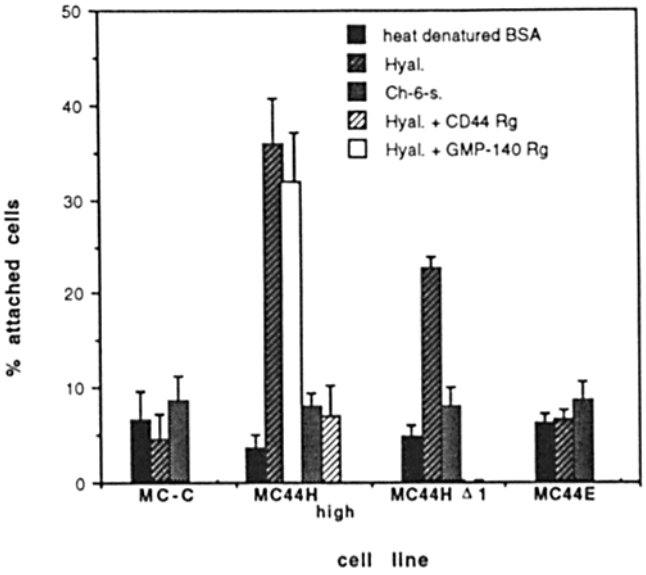

Figure 2. CD44 transfectant attachment to substrate. Attachment is represented as the percentage of seeded cells adhering to substrate after incubation and removal of nonadherent cells. 10^5 cells from each cell line were seeded per well. Substrates are denoted by shading: *Hyal*, hyaluronate; *Ch-6-s*, Chondroitin-6-sulfate. All experiments were done in triplicate and standard errors are shown.

coated surfaces. The baseline migration rate of all transfectants on heat-denatured BSA substrates was statistically homogeneous (8.8 ± 1.9, 9.4 ± 3.7, 10.1 ± 2.5, 11.4 ± 2.6 μm/h for MC-C, MC-H_{high}, MC-H_{low}, and E, respectively). The two CD44H-expressing cell lines, MC-44H_{high} (Figs. 3 and 4) and MC-44H_{low} (Fig. 4) displayed a significantly increased migration rate on hyaluronate-coated substrates, and the observed increase in motility correlated with the level of CD44H expression (Fig. 4). The migration rate of MC-44H_{high} was 19 ± 2.2 and 31.9 ± 5.1 μm/h on 0.5 and 5 mg/ml hyaluronate-coated substrates, respectively, compared to 9.4 ± 3.7 ($p < 0.001$) on heat-denatured BSA, while that of MC-44H_{low} was 16 ± 2.6 and 22.3 ± 4.3 μm/h, respectively, compared to 10.1 ± 2.5 μm/h ($p < 0.001$) on heat-denatured BSA (Fig. 4). CD44E-expressing transfectants failed to show any significant increase in migration rate on hyaluronate-coated surfaces when compared to BSA-coated substrates, and none of the transfectants displayed substantial acceleration on chondroitin-6-sulfate–coated coverslips (Figs. 3 and 4).

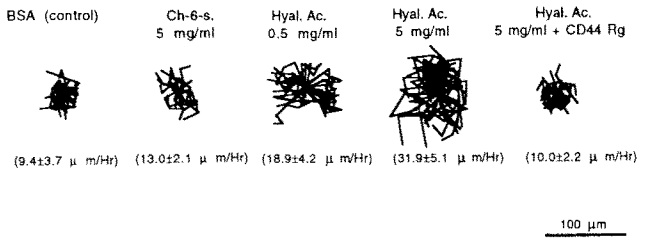

Figure 3. "Spider" diagram representation of migration pathways for 3 h of the MC-44H_{high} transfectant on different substrates and in the presence of soluble CD44. Coating concentrations of substrate, concentration of soluble CD44 and migration rate are indicated. *Hyal Ac*, Hyaluronate; *Ch-6-s*, chondroitin-6-sulfate.

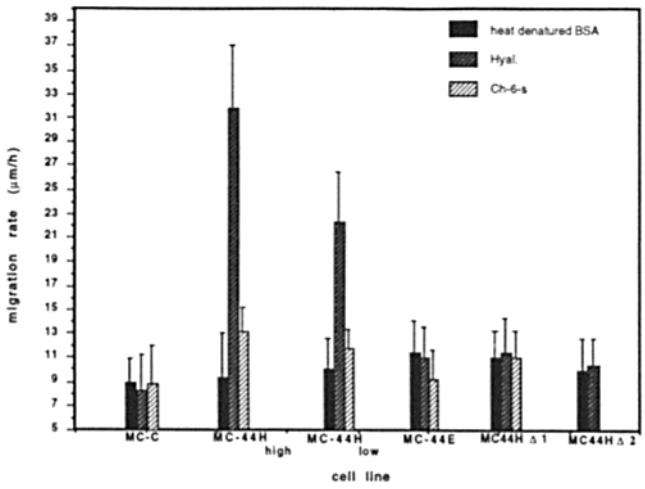

Figure 4. Migration rate of CD44 transfectants on different substrates (5 mg/ml coating). Substrates are denoted by shading. Hyal: hyaluronate; *Ch-6-s*, chondroitin-6-sulfate. All experiments were done in triplicate and standard errors are shown.

Migration of CD44H Melanoma Transfectants Is Blocked by Soluble CD44Rg and Anti-CD44 Antibody

To further characterize the role of CD44 in cell migration, we conducted migration blocking assays using a soluble form of CD44. The construction of chimeric CD44-immunoglobulin cDNA fusions and subsequent fusion protein production have been described previously (Aruffo, et al., 1990). CD44-Ig fusion expression vectors were introduced into COS cells, and 5–8 d after transfection, COS cell supernatants containing soluble CD44 (CD44Rg for CD44-receptoglobulin) were harvested. CD44Rg was purified on protein A columns as previously described (Aruffo et al., 1990). CD44Rg has been shown to bind hyaluronate in vitro and in vivo (Aruffo et al., 1990). In our blocking experiments, transfectants were tested for migration on 5 mg/ml hyaluronate-coated substrates in the presence of CD44Rg at a concentration of 25 μg/ml. Transfectants were first allowed to adhere to substrates, which were then subjected to incubation with CD44Rg. CD44H-expression–dependent migration on hyaluronate was found to be completely abrogated by the presence of CD44Rg (Figs. 3 and 5). The observed blocking effect was most likely the result of CD44Rg interaction with surface-bound hyaluronate, rendering it inaccessible to cell surface CD44H. GMP-140Rg controls (Aruffo et al., 1991) failed to display blocking (Fig. 5). Similarly, incubation of adherent MC-44H cells with anti-CD44 mAb KM201 (Miyake et all, 1990) resulted in inhibition of migration while incubation with an unrelated antibody had no effect (Fig. 5). These observations support the notion that CD44H plays a direct role in regulating cell migration on hyaluronate-associated substrates.

Migration on but Not Attachment to Hyaluronate-associated Substrate Is Abrogated in Melanomas-expressing CD44H Cytoplasmic Mutants

The observation that CD44 colocalizes with cytoskeletal proteins suggests that the presence of the cytoplasmic domain is likely to be required for attachment to and motility

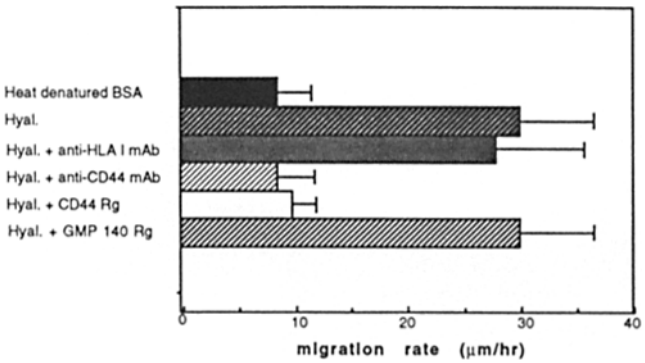

Figure 5. Blocking of migration of CD44H transfectants on hyaluronate-coated surfaces. Uninhibited migration of $CD44H_{high}$ on hyaluronate-coated glass slides compared to migration in the presence of CD44Rg (25 μg/ml) or anti-CD44 mAb KM201 (undiluted hybridoma supernatant). GMP140Rg and anti-HLA I mAb were used as controls. Baseline migration on heat-denatured BSA is shown. All experiments were done in triplicate and standard errors are shown.

on hyaluronate substrates. To test these hypotheses, melanoma cell lines transfected with cytoplasmic deletion mutants, MC-44HΔ1 and MC-44HΔ2, were assayed for attachment to and migration on hyaluronate-coated surfaces. Both cell lines expressed high levels of truncated CD44, comparable to those expressed by $CD44H_{high}$ cells (Fig. 2). The MC-44HΔ1 cell line displayed significant attachment to hyaluronate substrates when compared to MC-C and MC-44E cells (Fig. 2), although the attachment was lower than that of $CD44H_{high}$ cells, consistent with the observations of Lesley et al. (1992), suggesting that the presence of the cytoplasmic domain plays a role in stabilizing CD44 interactions with substrate. However, neither of the cytoplasmic deletion mutants displayed any significant increase in motility on hyaluronate substrates with respect to controls (Fig. 4).

Discussion

Earlier work had demonstrated that CD44H is the principal cell surface receptor for hyaluronate (Aruffo et al., 1990; Culty et al., 1990; Miyake et al., 1990), and that CD44H-expressing cells adhere to hyaluronate-coated cells and substrate in vivo and in vitro (Stamenkovic et al., 1991; Aruffo et al., 1990; Culty et al, 1990; Miyake et al., 1990; Sy et al., 1991; St. John et al., 1990). The notion that hyaluronate creates a low resistance, highly hydrated matrix, and the observations that hyaluronate production is increased at inflammatory sites (Weigel et al., 1989, Hallgren et al., 1990) and in areas of tumor invasion (Knudson et al., 1984; Knudson, et al., 1989; Knudson and Knudson, 1990; Toole et al., 1979) suggest that CD44–hyaluronate interaction may also play a role in regulating cell motility. The present study provides direct evidence that expression of CD44H in melanoma cells confers capacity not only for attachment but also for motility on hyaluronate substrates. The dual function of promoting cell attachment to and motility on substrate may help explain the physiologic role of CD44 in the regulation of cell trafficking in tissues as well as its role in tumor growth. CD44H has been suggested to mediate lymphocyte homing to lymphoid tissues (Jalkanen et al., 1986, 1987). Unlike L-selectin (previously called Mel-14/Leu-8/LAM-1), which recognizes specific ligands on lymph node high endothelial venules (Watson et al., 1990), CD44H displays only weak reactivity with high endothelial venules in vivo (Aruffo et al., 1990) and does not appear to be necessary for lymphocyte binding to vascular endothelium. However, expression of CD44H by leukocytes is likely to be required for events following endothelial adhesion, including endothelial transmigration, penetration into the extracellular matrix of lymphoid tissue, and subsequent migration to sites of antigen presentation. Similarly, leukocytes may depend on CD44H expression for efficient penetration of inflammatory sites and migration to areas where their effector functions are required.

Expression of CD44H is augmented in various types of malignancies (Stamenkovic et al., 1989; Stamenkovic et al., 1991). Melanomas that react with anti-CD44 mAb have been observed to express the CD44H isoform (Stamenkovic et al., 1989; Stamenkovic et al., 1991) and display higher attachment to and motility on hyaluronate-coated substrate than CD44 negative counterparts in vitro (Thomas, L., and H. R. Byers, unpublished observations). Human lymphoma cells, stably transfected with CD44H, display higher tumorigenicity and metastatic proclivity than CD44-negative parental cells or CD44E-expressing transfectants in vivo. CD44H expression may therefore provide tumor cells of different origin with a common mechanism for invasion of host tissues and formation of foci at secondary sites. Because hyaluronate is not present in free form in the ECM, but is principally bound to proteoglycans and link proteins (Lindahl and Hook, 1978), it may promote development of CD44H-expressing tumors in two ways: by facilitating tumor cell migration and tissue penetration and by providing a molecular bridge that mediates adhesion of tumor cells to host tissue stromal cells and ECM proteoglycans. Tumor cell interaction with stromal cells has been shown to stimulate secretion of angiogenic factors (West and Kumar, 1989) and hyaluronate production (Knudson et al., 1984), while attachment to proteoglycans may allow tumor cells to gain access to growth factors sequestered within the ECM (Ruoslahti, 1989; Ruoslahti and Yamaguchi, 1991). The combined effects on tumor cell motility and adhesion of CD44H-hyaluronate interaction may help explain the high degree of aggressiveness (Sy et al., 1991, Knudson et al., 1989, Horst et al., 1990) displayed by tumors expressing elevated levels of CD44H.

Anti-CD44 mAbs have been shown to block attachment of CD44H-expressing cells to hyaluronate (Lesley et al., 1990; Miyake et al., 1990). In the present study, we used anti-CD44 mAbs and soluble CD44 Rg in an attempt to inhibit migration of CD44H-expressing melanoma cells. Migration of CD44H transfectants was observed to be inhibited by the anti-CD44 mAb KM201 which prevents CD44-hyaluronate interaction (Miyake et al., 1990). In addition, our results show that soluble CD44, which reacts with hyaluronate in vitro as well as in vivo (Aruffo et al., 1990), blocks CD44H-transfectant migration on hyaluronate-coated surfaces, probably by disrupting cell surface CD44-hyaluronate interaction. Taken together, these observations add further support to the notion that migration on hyaluronate is promoted by the expression of CD44H and suggest that soluble CD44 may provide a valuable tool to study tumor cell motility and invasiveness.

Antibodies to CD44 have been shown to trigger a variety

of cellular responses, including T cell activation (Shimizu et al., 1989; Denning et al., 1990) and monocyte cytokine secretion (Webb et al., 1990). These effects may be explained by signal transduction pathways involving cytoskeletal proteins with which the cytoplasmic domain of CD44 has been suggested to be associated (Jacobson et al., 1984*a*,*b*; Kalomiris and Bourguignon, 1989; Lacy and Underhill, 1987; Tarone et al., 1984). Association with the cytoskeleton also suggests that the cytoplasmic domain of CD44 may be primarily required for promotion of cell motility. This hypothesis was verified by observing that melanoma cells transfected with CD44H lacking the cytoplasmic domain fail to display enhanced motility on hyaluronate substrates. However, attachment of these cells to hyaluronate remained significant, although slightly lower than that of transfectants expressing wild-type CD44H, consistent with recent findings of Lesley et al. (1992). These observations suggest that the cytoplasmic domain of CD44H is required for cell motility but not for attachment to substrate and may have important physiologic implications. A recent study has shown that activation of macrophages results in dissociation of the cytoplasmic tail of CD44 from the cytoskeleton (Camp et al., 1991). Macrophages provide a good example of cells which may be motile or sessile depending on the function they are required to perform. Dissociation of CD44 from the cytoskeleton may result in a reduction or loss of cellular motility while allowing retention of significant substrate-binding capacity. It is attractive to speculate that invading tumor cells having penetrated host tissue, may take advantage of a similar mechanism to become immobilized and form new colonies. By selectively providing cells with adhesion and migration capability, CD44H may play a pivotal role in regulating cell trafficking in tissues in both physiologic and pathologic settings.

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