Distinct Effects of Two CD44 Isoforms on Tumor Growth In Vivo

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Summary

Tumor growth is dependent in part on interactions between tumor cells and the extracellular matrix of host tissues. Expression of the cell surface glycoprotein CD44/Pgp-1, which mediates cell-substrate interactions is increased in many types of malignancies, but the role of CD44 in tumor growth is largely undefined. Recently, two isoforms of CD44 have been identified: an 80–90 kD form, which has high affinity for cell bound hyaluronate and a 150 kD form which does not mediate attachment to hyaluronate-coated surfaces. In this work, human B cell lymphoma cells stably transfected with cDNA clones encoding either of the two CD44 isoforms were compared for tumorigenicity and metastatic potential in nude mice. Expression of the 80–90 kD form but not the 150 kD form of CD44 greatly enhanced both local tumor formation and metastatic proclivity of the lymphoma cells. Our results suggest that CD44 polypeptides may play an important role in regulating primary and metastatic tumor development in vivo.

The cell surface proteoglycan CD44 has been shown to play a role in lymphocyte activation (1, 2), cell-cell adhesion (3), and cell-substrate interactions (4). Recently, two isoforms of CD44 have been identified which differ both in molecular mass and affinity for substrate. The first, CD44H, is an 80–90 kD glycoprotein, expressed in cells of both mesodermal and neuroectodermal origin (5, 6), and has been proposed to be the principal cell surface receptor for hyaluronate (7, 8). The second, CD44E, is a 150-kD species, expressed in a subpopulation of epithelial cells, and does not appear to display affinity for hyaluronate (5, 9). Isolation and characterization of cDNA clones encoding both isoforms have shown that the predicted proteins are identical with the exception that the CD44E polypeptide contains an additional stretch of 134 amino acids intercalated between 220 and 224 of the extracellular domain of CD44H (9, 10).

The difference in affinity for surface-bound hyaluronate between the two CD44 isoforms is likely to reflect different functional roles. Hyaluronate belongs to the glycosaminoglycan class of molecules which participate in the assembly of the extracellular matrix (ECM)[1], and is believed to play an important role in embryogenesis (11), wound healing (11, 12), and inflammation (13). Increased production of hyaluronate is associated with tumor growth, possibly as a result of tumor-stromal cell interaction (14), and has been proposed to enhance tumor invasiveness (15). Expression of CD44 in malignant cells may therefore help regulate primary tumor growth, local invasiveness and metastatic proclivity.

At least four lines of evidence support CD44 involvement in tumor development: immunochemical and RNA blot data show that malignant cells express higher levels of CD44 than their nonmalignant counterparts (5); CD44/pgp-1 is one of a small number of cell surface molecules expressed in tumorigenic but not nontumorigenic variants of the murine thymoma SL12 (16); invasiveness of human bladder carcinoma cells has been shown to correlate with the level of expression of CD44 (17); and recent studies suggest that high expression of CD44 is associated with aggressive behavior and poor prognosis of human non-Hodgkin's lymphomas (18). This observation is highlighted by the absence of correlation between expression of the adhesion molecules LFA-1 and ICAM-1, which have been implicated in tumor metastasis, and clinical evolution of non-Hodgkin's lymphomas (18). Because the currently available anti–human CD44 mAbs do not distinguish between the two isoforms, immunohistochemical approaches cannot easily determine whether expression of either or both polypeptides correlates with tumor behavior. However, the observation that expression of RNA transcripts specific for both forms is increased in carcinomas (5, 9) suggests that both CD44H and CD44E may be associated with tumorigenesis.

Materials and Methods

Creation of Stable Transfectants. Namalwa cells obtained from ATCC. The Nawalwa cell line was established from a human Burkitt (B cell) lymphoma and secretes small amounts of a mAb (IgM, λ light chain) of unknown specificity (19). Stable transfectants were created as described (9). Briefly, CD44H and CD44E-containing

[1] *Abbreviation used in this paper:* ECM, extracellular matrix.

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CDM8 vectors were linearized with SpeI and ligated to a p205 vector digested with XbaI. The resulting constructs were introduced into Namalwa cells by electroporation (400 V/960 μF). Electroporated cells were cultured in RPMI supplemented with 20% fetal bovine serum for 48 h following which stable transfectants were selected in the presence of 500 μg/ml hygromycin (Sigma Chemical Co., St. Louis, MO). Outgrowth of resistant cells was observed 4 wk following transfection. Transfectant were subcloned by limiting dilution and tested for surface expression of the CD44 isoforms by indirect immunofluorescence and immunoprecipitation.

Immunoprecipitations. Cells were metabolically labeled with ^{35}S cysteine-methionine (Trans-label; ICN, Costa Mesa, CA), in cysteine/methionine-free RPMI supplemented in 5% dialyzed fetal bovine serum, overnight and lysed in a Tris-buffered saline solution containing 1% NP-40, 20 mM iodoacetamide and 1 mM phenylmethylsulfonylfluoride (PMSF). After preclearing with murine isotype matched immunoglobulin (Cappel, Malvern, PA) and protein A sepharose CL-4B (Sigma Chemical Co.) for 4 h, the lysates were treated with I-173 mAb and protein A sepharose beads overnight. The beads were washed, and the precipitates eluted and electrophoresed on an 8% acrylamide gel under reducing conditions.

Immunofluorescence. Transfectants and parental Namalwa cells were incubated with mAb I-173, specific for human CD44 (5), or an irrelevant isotype-matched control antibody for 45 min at 4°C, washed in PBS, incubated with affinity purified fluorescein-conjugated rabbit anti-mouse antibody (Cappel) for 30 min at 4°C, washed and analyzed on a FCS analyzer (Becton-Dickinson and Co., Mountain View, CA).

For staining tissue sections with soluble CD44-Ig chimeras, murine frozen tissue sections were incubated with CD44-Ig in PBS diluted 1:10 (approximately 100 μg/ml) for 1 h at RT, washed in PBS, incubated with an affinity-purified goat anti-human fluorescein-conjugated antibody (Cappel) for 30 min at RT, washed, and analyzed under a fluorescence microscope, as described (7).

Injection of Transfectants. To test for local tumorigenicity, nude (Balb/c *nu/nu*) mice were injected subcutaneously with CD44H, CD44E transfectants or parental Namalwa cells. In the initial experiment, ten animals each were injected subcutaneously with 10^6 Namalwa cells, or either of the two transfectants. These experiments were then repeated by injecting 5 mice each with 10^6 cells of different isolates of each transfectant; two independent CD44H transfectant isolates, CD44.5 and CD44.51, and three independent CD44E transfectant isolates, CD44HT3, HT4 or HT6 were injected. Animals were monitored weekly for visible tumor growth. On day 42, some animals were sacrificed to examine tumors histologically. Other animals were observed for a total of 90 d.

To test for metastatic potential, parental cells or each of the two transfectants were injected into the tail vein of nude mice. In the first set of experiments, nude mice were subdivided into three groups of 10 mice. Each group was injected with either CD44H transfectants, CD44E transfectants or parental Namalwa cells, and each animal received 10^6 cells. In the second set of experiments, each animal received 5×10^6 cells; 11 mice received CD44H transfectants, 8 mice received parental Namalwa cells and 15 mice received CD44E transfectants.

Monitoring of Tumor Growth. Sera were obtained from each mouse on a weekly basis and human IgM level determined using an ELISA assay specific for λ chain bearing human IgM. Briefly, 96-well ELISA plates were coated with 0.5 mg/ml purified goat anti-human IgM antibodies (Southern Biotechnology Associates, Birmingham, AL) and serum samples were serially diluted. The second antibody was an affinity purified alkaline phosphatase-conjugated goat anti-human lambda chain-specific antibody (Southern Biotechnology Associates). An affinity-purified human IgM was used as a control.

Histology. Tissues were fixed in formalin, embedded in paraffin and 4–8 μm sections were mounted onto slides and stained with hematoxylin-eosin according to standard procedures.

Adhesion Assays. 96-well U bottom plates (Microtest III, Falcon 3911; Becton Dickinson & Co., Lincoln Park, NJ) were coated with 100 μg/ml hyaluronate (Sigma Chemical Co.) chondroitin-6-sulphate (Sigma Chemical Co.) or chondroitin-4-sulphate (Sigma Chemical Co.) in PBS overnight at room temperature. Transfectants and parental cells were radiolabeled with 50 μCi ^{51}Cr (New England Nuclear, Boston, MA) for 1 h. 10^4 cells were added to each well and incubated at room temperature. Plates were washed 4 times with PBS, adherent cells lysed with 100 μl of 1% NP-40, and bound cpm determined. All samples were measured in triplicate.

Comparison of Cellular Growth Rate In Vitro. 5×10^4 cells were cultured per well in RPMI medium supplemented with 10% FBS in 96-well flat bottomed microtiter plates (Falcon, Lincoln Park, NJ). After 24 h, cells were pulsed with 1 μCi of [^3H]thymidine for 4 and 24 h. Cells were harvested with an automatic pH.D. harvester (Cambridge Bioscience, Cambridge, MA) and [^3H]thymidine incorporation was determined. All cultures were done in triplicate.

Production of Soluble CD44-Ig Chimeras. Creation of soluble CD44-immunoglobulin chimeras were described previously (7). Briefly, PCR-amplified cDNA sequences encoding the extracellular domain of CD44H were ligated to expression plasmids containing genomic sequences encoding human immunoglobulin constant regions. Expression plasmids bearing the chimeras were introduced into COS cells and supernatants harvested 7–10 d after transfection. Typically 1–3 μg/ml of soluble protein were obtained. For use in tissue staining, the soluble protein was purified on Trisacryl protein A columns (Pierce Chemical Co., Rockford, IL) as described (7).

Results

Creation of Stable Transfectants and Assays for Affinity to Substrate. To determine whether expression of either isoform of CD44 may influence tumor growth, cDNA clones encoding CD44H and CD44E were stably introduced into Burkitt lymphoma Namalwa cells (19), which do not constitutively express CD44. The CDM8 expression vector (5, 9) containing either CD44H or CD44E cDNAs was linearized with the restriction endonuclease SpeI and ligated to a p205 plasmid (9) digested with XbaI. p205 contains the Epstein-Barr virus EBNA-1 gene, allowing the plasmid to be stably maintained in episomal form, and a hygromycin resistance gene as a selectable marker (9). CD44Hp205 and CD44Ep205 were introduced into Namalwa cells by electroporation and stable transfectants were selected in the presence of hygromycin, subcloned and tested for surface expression of CD44 by indirect immunofluorescence and immunoprecipitation (Fig. 1).

Previous observations suggested that the two CD44 isoforms display different affinities for cell-surface bound hyaluronate (9). To determine whether the two polypeptides differ in their affinity for cell-free substrate, transfectants were tested for adherence to plastic microtiter wells coated with hyaluronate, chondroitin-6-sulphate and chondroitin-4-sulphate. Only transfectants expressing CD44H adhered to hyaluronate-coated wells and, less avidly, to chondroitin-6-

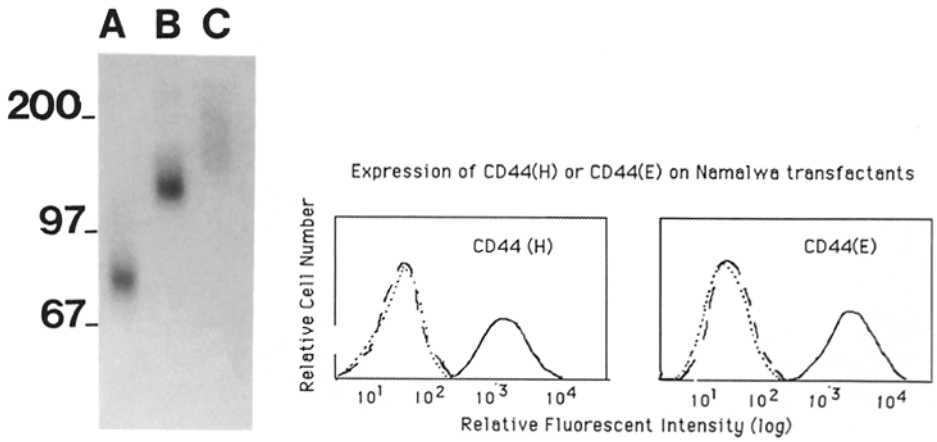

Figure 1. Expression of CD44H and CD44E in stable transfectants as assessed by immunoprecipitation (*A–C*), and indirect immunofluorescence (*D* and *E*). Immunoprecipitation of CD44 from: lane *A*: CD44H transfectants; lane *B*: CD44E transfectants; lane *C*: parental Namalwa cells. Mol wt are indicated on the left. (*D*): reactivity with CD44 mAb I-173 (5) of CD44H transfectants (*solid line*), compared to parental cells (*broken line*); (*E*): reactivity of CD44E transfectants (*solid line*) compared to parental cells (*broken line*). Dotted lines indicate reactivity of transfectants with an unrelated isotype-matched control antibody.

sulphate coated wells (Table 1). CD44E transfectants failed to display specific adhesion to any of the glycosaminoglycans tested. These observations are consistent with earlier findings that CD44H but not CD44E promotes adhesion to lymph node stromal cells and that the adhesion is mediated by hyaluronate (9).

CD44H Transfectants Promote While CD44E Transfectants Inhibit Lymphoma Growth in Nude Mice. To determine whether CD44 expression in Namalwa cells modifies their behaviour in vivo, 10^6 transfectants expressing either CD44H or CD44E, or parental Namalwa cells were injected s.c. into three groups of 10 nude mice each. The animals were monitored weekly for visible tumor growth. Within 7 d, the first tumors in animals injected with CD44H transfectants were visible. By 42 d, 90% of animals injected with CD44H transfectants had grossly visible tumors ranging from 0.5 to 2 cm in diameter (Fig. 2). In animals injected with parental Namalwa cells, visible tumors appeared only on day 28, and only 30% of the animals developed tumors after 70 d of observation (Fig. 3). However, animals which had received CD44E transfectants displayed no tumor growth even 90 d following injection.

Tumor-bearing animals were sacrified between days 42 and 70 and the tumors excised. Tumors were typically encapsulated and displayed no infiltration of underlying muscle tissue. Histologic examination revealed large cell lymphomas, but only CD44 transfectant-derived tumors reacted with anti-CD44 antibodies (data not shown), suggesting that parental cell-derived tumor growth was not associated with induction of CD44 expression.

Because transfectant-associated tumor development may have been due to intrinsic properties of the particular Namalwa cell clones rather than CD44 expression, the initial experiments were repeated with two independent transfectant isolates expressing CD44H (CD44.5 and CD44.51), three independent isolates expressing CD44E (HT3, HT4 and HT6) and parental cells. The clones were selected on the basis of a comparable level of expression of the CD44 polypeptides and five mice were injected with 10^6 cells derived from each isolate or the parental cell line. None of the CD44E transfectants developed tumors while both CD44H transfectants formed tumors in 80% of animals confirming the initial observations. Because Namalwa cells secrete detectable amounts of IgM (19), tumor growth could be conveniently monitored by weekly measurements of human IgM levels in the murine sera. Human IgM levels were found to correlate with tumor size in both transfectant- and parental cell-derived tumors, and IgM production by Namalwa cells and transfectants, in vitro, was found to be comparable (data not shown). Results obtained by monitoring human IgM levels in mice injected

Table 1. *Binding of Namalwa CD44(H) but not Namalwa CD44(E) to Hyaluronate and Chondroitin Sulfate A*

	Namalwa CD44(E)	Namalwa CD44(H)	Namalwa
		cpm bound	
Background	2,333 ± 176	4,500 ± 290	4,000 ± 120
Hyaluronate	2,500 ± 200	17,000 ± 198	5,200 ± 456
Chon. Sulf. A	3,000 ± 78	11,000 ± 389	5,500 ± 349
Chon. Sulf. C	3,000 ± 34	5,590 ± 467	5,900 ± 400

Comparison of adhesion of transfectants and parental Namalwa cells to substrate. The numbers represent the average of the triplicate and the standard error is <5%. The total ^{51}Cr incorporation for the three cell lines was comparable.

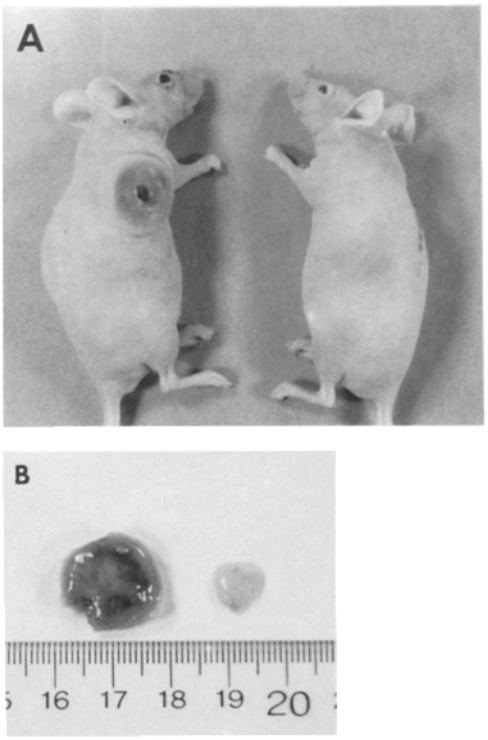

Figure 2. Tumors obtained in nude mice 42 d post s.c. injection. (*A*): representative mice injected with CD44H transfectants (*left*) (9 out of 10 mice developed tumors of similar size), and parental Namalwa cells (*right*) (3 out of 10 mice developed similar tumors) are shown. (*B*): Excised tumors from a CD44H recipient (*left*) and a parental cell recipient (*right*).

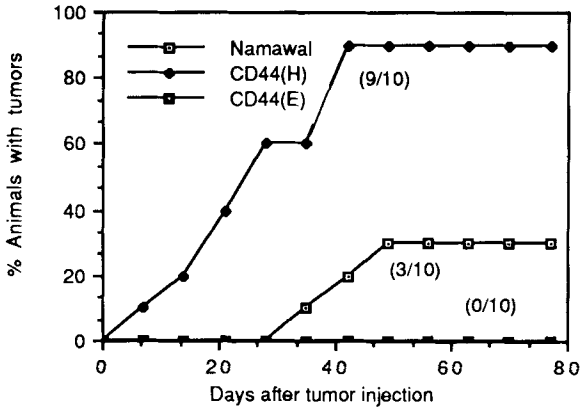

Figure 3. Rate of tumor development in animals injected subcutaneously with CD44H, CD44E transfectants and parental Namalwa cells. The number of animals injected with each cell type and the fraction of animals developing tumors in each group are indicated.

subcutaneously with transfectants derived from clone CD44.51 and parental Namalwa cells are shown in Table 2.

CD44H and CD44E Transfectants and Parental Cells Have Comparable Growth Characteristics In Vitro. To establish tumor-derived CD44H cell lines, tumors were excised and cells teased from the tumor mass and grown in in vitro culture in the presence of hygromycin. Cultured tumor-derived cells reacted with mAbs directed against human MHC class I and class II molecules, confirming that the tumors were of human origin, and surface expression of CD44 in tumor-derived cells was comparable to that in CD44 transfectants (data not shown). Proliferation assays revealed that parental Namalwa cells, CD44H and CD44E transfectants as well as CD44 tumor-derived cells had the same proliferation rate in vitro (Table 3). This finding eliminated the possibility that the difference in in vivo growth between CD44 transfectant and parental cell tumors was a reflection of different baseline proliferation rates between the cell lines.

Glycosaminoglycan-coated Surfaces Do not Promote CD44H Transfectant Growth In Vitro. To determine whether the enhanced growth of CD44H tumors was the result of CD44-hyaluronate or CD44-chondroitin sulfate interaction, CD44H transfectants and parental cells were cultured in the presence of various concentrations of hyaluronate or grown on hyaluronate or chondroitin-6-sulphate-coated plates. However, neither presence of hyaluronate in the cell cultures nor the growth of cells on hyaluronate or chondroitin sulphate coated-plates revealed any significant modification in prolifer-

Table 2. *Concentration of Human IgM in the Serum of Nude Mice Injected with Namalwa or Namalwa CD44 (H).51*

	Namalwa					Namalwa CD44 (H).51				
	d after injection					d after injection				
Animals	14	28	35	42	49	14	28	35	42	49
					mg/ml					
1	-	-	-	-	-	-	0.6	5	12	(S)
2	-	-	-	-	-	-	0.5	10	18	(S)
3	-	-	-	0.2	8	-	0.2	0.5	6	18
4	-	-	-	-	-	-	-	0.3	3	11
5	-	-	-	-	-	-	-	-	-	-

Comparison of human IgM levels in nude mice injected with Namalwa cells or CD44.51 (CD44H) transfectants. (-: not detectable; s: sacrificed).

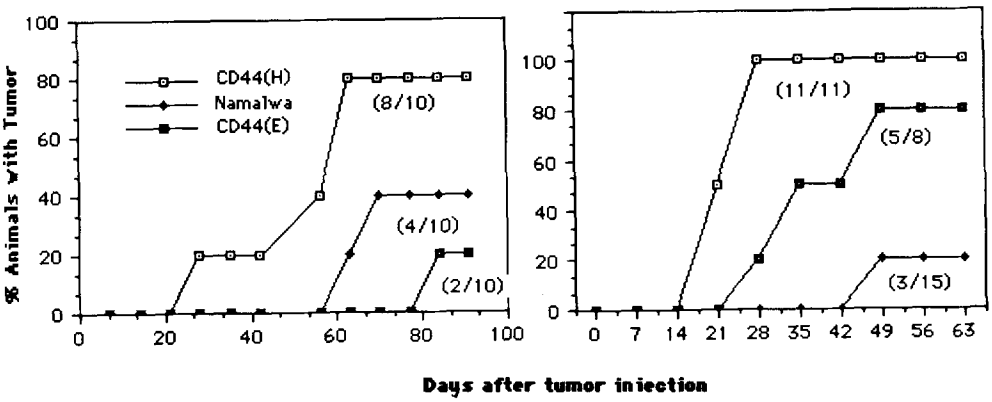

Figure 4. Rate of tumor development in animals having received CD44 transfectants or parental cells intravenously. Results obtained in animals injected with 10^6 and 5×10^6 cells are shown on left and right, respectively. The number of animals in each group is shown in brackets. Tumor growth was assessed by human IgM concentration in the serum of each individual mouse and verified by histologic examination.

ation of either transfectant or parental cells (data not shown). This observation suggests that the presence of hyaluronate or chondroitin sulphate alone is not sufficient to trigger proliferation of CD44H expressing cells in vitro.

CD44H-transfectant Tumor Growth In Vivo Is not Due to Greater Resistance to NK Cytotoxicity. Several tumor cell lines which express the hyaluronate receptor form a pericellular coat of hyaluronate which may confer protection from T cell or NK cell-mediated cytotoxicity (11). To rule out the possibility that growth of CD44 tumors was due to protection against NK-mediated cytotoxicity, transfectants and parental cells were cocultured with nude mouse spleen cells but no significant cytolysis of either target was observed (data not shown).

CD44H Induces a Higher Metastatic Potential in Lymphoma Cells, While CD44E Reduces Metastatic Proclivity. The observation that CD44H promotes lymphoma growth in s.c. tissues raised the possibility that CD44 polypeptides may also play a role in determining the tissue distribution and growth rate of metastatic tumors. The frequency of tumor development in intravenously injected animals was consistent with that observed as a result of s.c. injections and correlated with the number of cells injected (Fig. 4). Tumors developed in 80% and 100% of animals receiving CD44H transfectants in experiments 1 (10^6 cells) and 2 (5×10^6 cells), respectively. Animals injected with parental Namalwa cells and CD44E transfectants developed detectable tumors respectively in 40% and 20% of cases in experiment 1, and 60% and 33% of cases in experiment 2 (Fig. 4). The rapidity of detectable tumor appearance, as assessed by measurement of human IgM levels in murine serum, was consistent with tumor development in s.c. tissues. The first CD44H-expressing tumors were detected at days 20 and 14 in experiments 1 and 2, respectively, while the first parental cell tumors appeared at days 55 and 21, respectively. In the few cases of CD44E-derived tumors, the first detectable growth occurred only 75 and 42 d post injection, respectively (Fig. 4).

However, with the exception of renal tissue, where only CD44H transfectants formed tumors, tissue distribution of

Table 3. *In Vitro Proliferation of Namalwa, Namalwa CD44(H), Namalwa CD44(E) and Three Different Tumor-derived Namalwa-CD-44(H) Cell Lines*

	[^{3}H]Thymidine incorporation	
	4 h	24 h
Namalwa	62,350 ± 4,100	295,300 ± 12,000
Namalwa CD44 (H)	57,000 ± 3,400	210,000 ± 5,700
Namalwa CD44 (E)	80,100 ± 5,100	340,000 ± 3,140
CD44.(H)N 2	82,000 ± 320	280,000 ± 1,100
CD44.(H)N 3	73,000 ± 4,200	276,000 ± 987
CD44.(H)N 4	97,000 ± 543	273,400 ± 1,300

Comparison of in vitro growth rates, expressed as [^{3}H]thymidine incorporation, between parental Namalwa cells and transfectants. All cultures were done in triplicate and the numbers presented are the average of the radioactive thymidine incorporation in each triplicate. Standard errors are indicated (+/−). CD44.HN2, CD44H.N3 and CD44H.N4 are cell lines derived from tumors excised from three different mice injected with CD44H transfectants.

Table 4. *Tissue Distribution of Metastases Derived from CD44H and E Transfectants and Parental Namalwa Cells*

	Number of animals with metastases		
Tissue	CD44H (19/21)	Namalwa (9/18)	CD44E (5/25)
marrow	17	5	2
gut	7	5	3
kidney	8	0	0
pancreas	3	4	2
liver	1	0	0
spleen	1	0	0
muscle	5	3	0
adrenal	3	0	0
peritoneum	5	3	0

Tissue distribution of metastases derived from CD44H and E transfectants and parental Namalwa cells. Cumulative results from experiments 1 (10^6 cells) and 2 (5×10^6 cells) are shown. The fraction of injected animals which developed metastases in each group is indicated in brackets.

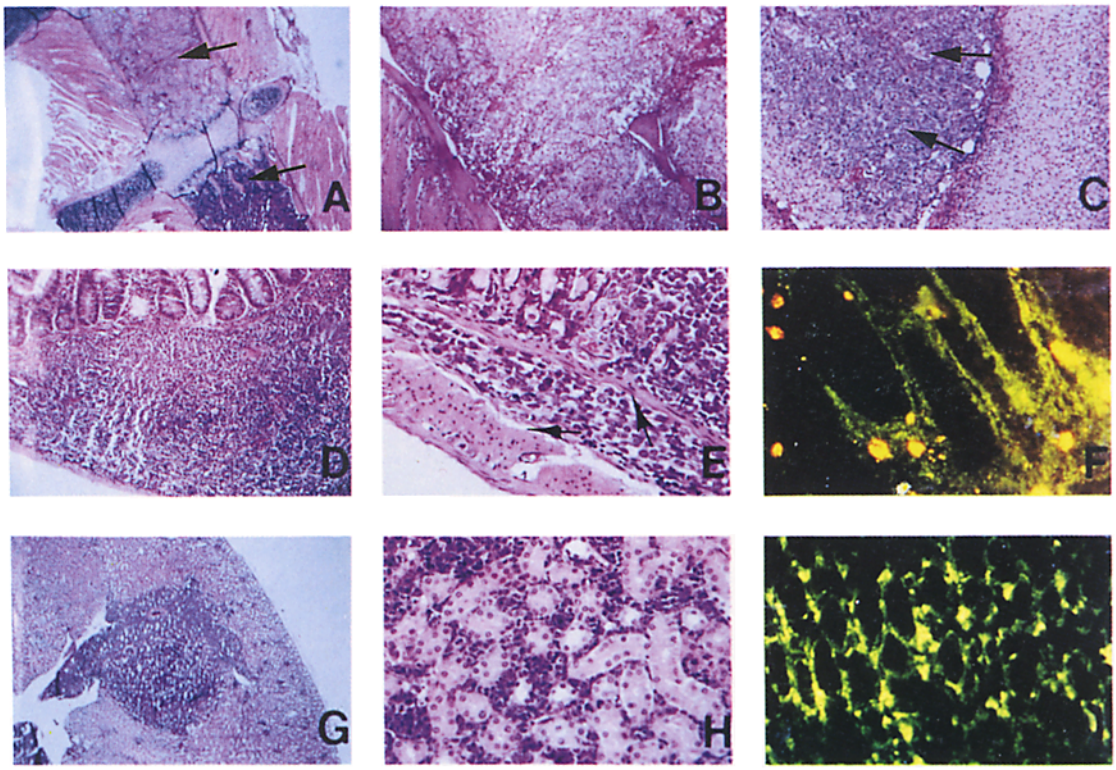

Figure 5. Histology of CD44H tumor metastases; all tissue examined by light microscopy are stained with hematoxylin and eosin. (*A*) low power (×64) magnification of bone marrow metastases showing normal marrow (*bottom arrow*) and marrow replaced by tumor cells (*top arrow*); (*B*) Higher power magnification of tumor cells in (*A*) showing monomorphic tumor appearance (×128); (*C*) Metastatic growth in adrenal medulla (*arrows*), while the cortex is spared (×128); (*D*) tumor infiltration of intestinal submucosa (×64); (*E*) Higher power (×128) magnification of submucosal tumor infiltration showing sparing of muscle layers (*arrows*); (*F*) Fluorescent micrograph showing intestinal submucosal reactivity with CD44-Ig (×128); (*G*) Low power magnification of renal medulla metastasis (×64); (*H*) Higher power magnification (×128) of metastasis in (*G*) showing tumor cell infiltration in the interstitium between renal tubules; (*I*) CD44-Ig reactivity with renal medulla interstitium, showing a pattern reminiscent of tumor cell infiltration (×128).

metastases was not significantly different between CD44H and parental cell-derived tumors (Table 4). Both cell types preferentially formed metastases in the bone marrow, with local invasion and destruction of muscle, bone, and neural tissue (Fig. 5). Metastases were also commonly found in the gut submucosa (Fig. 5) and pancreas (data not shown). Interestingly, in renal tissue, tumor growth was observed in the medullary and papillary interstitium where concentrations of hyaluronate, and consequently reactivity with soluble CD44, appear to be the highest (Fig. 5) (7, 20). Similarly, metastases in the gastrointestinal tract developed within the submucosa (Fig. 5) which is strongly reactive with soluble CD44 (Fig. 5) (7). In the only case of liver metastasis in a CD44H-transfectant-injected animal, tumor developed around centrilobular veins and beneath the capsule, the only two regions which react with soluble CD44 (7) (data not shown).

Discussion

Our results demonstrate that expression of CD44H in a B cell lymphoma enhances both primary and secondary tumor growth in nude mice. However, the observation that 30–60% of parental cell recipients also developed primary and metastatic tumors after a longer latency, suggests that CD44H expression in lymphoma cells may promote the initiation of tumor growth but is not essential for tumor development. Animals injected subcutaneously or intravenously with cells expressing the epithelial isoform of CD44, on the other hand, rarely developed tumors, suggesting that expression of CD44E may interfere with lymphoma growth in vivo.

With the exception of renal metastases, which were caused exclusively by CD44H transfectants, the tissue distribution of transfectant and parental cell-derived metastases was similar. These observations are consistent with earlier suggestions that expression of CD44H does not correlate with the metastatic pattern of human lymphomas (21, 22) implying that CD44H may not distinguish specific endothelial cell receptors in different organs. Rather, CD44H may enhance secondary tumor growth by facilitating interactions between tumor cells and host tissues. The pronounced tropism of CD44H transfectants to the bone marrow may be explained by observations that CD44H expression in hematopoietic cells may play an important role in their interaction with the medullary microenvironment (23). It is noteworthy that each of the tissues in which rapid CD44H tumor development occurred has an elevated content of hyaluronate and reacts strongly with soluble CD44 (7, 20), suggesting that CD44-hyaluronate

interaction may be directly responsible for enhancement of tumor growth in vivo.

The difference in tumor growth promoting effects between the two CD44 isoforms correlates with the difference in their affinity for surface-bound hyaluronate. The failure of hyaluronate to enhance CD44 transfectant proliferation in vitro may therefore not reflect its in vivo role. Hyaluronate is generally thought to promote cell motility by creating a low resistance, highly hydrated matrix. However, a substantial portion of hyaluronate is bound to stromal cells and various ECM molecules, including link proteins and proteoglycans (24), and may therefore provide a molecular bridge which facilitates adhesion of CD44H expressing malignant cells to host tissue stromal cells and ECM. The resulting interactions may stimulate tumor development in several ways. First, immobilization of tumor cells can provide foci for subsequent colony formation; second, tumor cell-stromal cell interaction has been shown to generate the production of growth and angiogenic factors which promote tumor growth (25); and third, malignant cells may gain better access to growth factors sequestered by ECM proteoglycans (26). However, CD44H may recognize additional ligands in host tissues which may directly stimulate tumor cell proliferation. The cytoplasmic domain of CD44H has been shown to interact with cytoskeletal proteins (27) and may provide a pathway for intracellular signal transmission. This suggestion is supported by recent evidence that crosslinking of CD44 is comitogenic for T lymphocytes (2) and may induce monocytes to secrete cytokines (28).

Because both forms of CD44 have an identical cytoplasmic domain, the observed differences in their tumor promoting properties in lymphoma cells are most likely due to the presence of the additional stretch of 134 residues in the extracellular domain of CD44E. In normal epithelial cells, expression of CD44H or CD44E may reflect the requirement for different types of interaction between a given cell and its neighbors. One example may be provided by pluristratified epithelia, where apical, intermediate and basal cells each interact with a different milieu. Tumors derived from epithelial cells expressing one or the other of the isoforms are therefore likely to display differences in affinity for different types of microenvironment which may condition their growth and development.

While this work was in progress, Gunthert et al. (29) showed that rat carcinoma cell lines which do not metastasize can acquire metastatic properties upon transfection with the rat homologue of CD44E. One possible explanation for the apparent discrepancy between these observations and ours may be that CD44E confers different tumorigenic properties to epithelial and lymphoid cells. However, in the experiments of Gunthert et al. (29) the transfected tumor cells constitutively expressed the rat homologue of CD44H. The enhanced metastatic potential of transfected cells may therefore have been related in part to coexpression of both CD44 isoforms.

The present observations suggest that CD44H participates in determination of the rate of both primary and metastatic lymphoid tumor growth. Expression of CD44H may therefore constitute a major prognostic factor in human hematopoietic malignancies.

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