ABSTRACT

At least 20 different isoforms of the human CD44 lymphocyte-homing receptor/hyaluronan receptor have been described to date that arise from the differential splicing of up to 10 alternative exons (termed v1-v10) encoding the membrane-proximal extracellular domain. Although numerous analyses at the mRNA level have indicated tissue-specific expression of CD44 variants, few analyses have been performed at the protein level because of limited availability of suitable monoclonal antibodies. Recently, however, exon-specific monoclonal antibodies have been generated using bacterial fusion proteins, and these have been reported to detect high levels of vCD44 containing the v6 exon on human tumors. Together with earlier evidence linking this particular exon with tumor metastasis in the rat, these latter experiments have led to the interpretation that v6 splice variants play a causative role in tumor dissemination. In this paper we describe the use of a new and comprehensive panel of CD44 exon-specific monoclonal antibodies generated against a recombinant CD44(v3-10)-immunoglobulin chimera to study vCD44 expression in a large number of normal and neoplastic tissues. We show that the expression of vCD44 varies greatly among different human tumors and that some express either very low levels of vCD44 or no CD44 at all. Furthermore, we demonstrate that expression is not limited to isoforms containing the v6 exon but includes variants carrying v3, v4/v5, and v8/9. Additionally, normal epithelial tissues are shown to express considerable levels of these same vCD44 isoforms. Such results argue against a ubiquitous role for vCD44 isoforms in promoting tumor growth and metastasis.

INTRODUCTION

CD44 is the major cell surface receptor for hyaluronan (1-4) and exists as multiple isoforms generated by the alternative splicing of up to 10 exons (v1-v10) encoding parts of the extracellular domain (5-7). An abundant M, 120,000 isoform termed the hemopoietic variant (CD44H) lacks all 10 variable exons and is ubiquitously expressed on cells of mesodermal and hemopoietic origin. In contrast, the CD44 isoforms, which contain multiple alternatively spliced exons, range in size from M, 120,000 (CD44 v8-10) to 250,000 (CD44 v3-10) and are predominantly expressed on cells and tumors of epithelial origin (2, 6, 7).

While CD44H appears to function in such diverse processes as lymphocyte homing, lymphocyte activation, and extracellular matrix adhesion (8, 9), the precise functions of each of the alternatively spliced CD44 isoforms are less clear. Interestingly, a rat isoform containing exons v4-v7 has been shown to promote metastasis after transfection of the cDNA into nonmetastatic rat carcinoma and sarcoma cell lines (10-13). In a different set of experiments, human CD44H has also been shown to enhance metastasis when transfected into a human B-cell lymphoma (14). Clearly, these results indicate an important role for CD44 in regulating tumor progression. However, the relative contribution of each individual CD44 variant in different types of tumor is not yet clear.

In a recent series of studies, a number of polyclonal and monoclonal antisera directed to human CD44 alternatively spliced exons have been used to detect vCD44 expression in different human tumors by immunohistochemical staining. In one group of studies, high level expression of CD44 isoforms carrying the v6 exon was reported in colorectal carcinomas, non-Hodgkin lymphomas, and certain types of gastric adenocarcinomas (15-18). Indeed, the level of CD44v6 expression appeared to indicate the stage of tumor progression in cases of colorectal carcinoma. However, other workers have shown a down-regulation of CD44v6 expression in tumors of squamocellular origin and an up-regulation of CD44v9 expression in primary gastric tumors (17, 18).

In order to gain a clearer picture of the role and expression of CD44 variants in different tumors, we have generated a panel of mouse monoclonal antibodies to the human CD44 exons v3, v4/v5, v6, and v8/9 using soluble CD44-immunoglobulin Fc fusion proteins as the immunogen. We have used these reagents in a comprehensive analysis of human vCD44 expression which has revealed that expression of CD44 isoforms in tumors is not restricted to those carrying the v6 exon but also includes those carrying v3, v4, and v8/9. Furthermore, we have shown that vCD44 expression by human tumors is extremely heterogeneous and that some malignant tumors appear to express no CD44 at all. In agreement with a recent study in which v4, v6, and v9 expression profiles in normal tissues were analyzed (19), we have found that the expression of vCD44 by normal human tissues is largely restricted to the epithelia, indicating that in most normal cells the process of CD44 alternative splicing is tightly regulated.

MATERIALS AND METHODS

Construction of pCD44H-Fc, pCD44(v8-10)-Fc, and pCD44(v3-10)-Fc. Two strategies were used to clone vCD44 cDNAs. In the first strategy, the CD44 mAb F.10.44.2 (20) was used to isolate full-length CD44 clones from a human cos 7 cell line, and cDNA libraries constructed from murine spleen and thymus RNA were screened using CD44 mAb F.10.44.2. In the second strategy, cDNAs representing the extracellular domains of vCD44 cDNAs were generated by reverse transcriptase-PCR from the breast carcinoma line ZR75 and cloned into the CD33-immunoglobulin Fc fusion vector (see below). The strategy was amplified primer 5'-TGAAGATCTCGCCG CAGATC- GATTGGAATAACCC'-3' was located at position 175 of the published CD44H sequence and incorporated a Bgl II site. The reverse amplification primer 5'-TGAAGATCTCCTACCTCCG TCACTTTGAATATTGGGT-GTCCCTAT-3' was located at position 5' of the transmembrane domain at position 920 and incorporated an Bgl II site and a splice donor consensus site. The PCR conditions used were: 95°C, 30 s; 55°C, 30 s; 72°C, 30 s, cycled 30
times. PCR products were end filled with Escherichia coli DNA polymerase (Klenow fragment), blunted with T4 polynucleotide kinase, and cloned into EcoRV-cut, phosphatase-treated pBluescript. Individual transformants containing CD44H, CD44(v8-10), and CD44(v3-10) inserts were identified by double-stranded sequencing and cloned into the HindIII/BglII site of pCD33 signal immunoglobulin, a derivative of the original plgG1 expression vector (21, 22).

Expression of Chimeric Fusion Proteins. pCD44H-Fc, pCD44(v8-10)-Fc, and pCD44(v3-10)-Fc constructs were transfected into COS cells (10 μg/100 COS cells) using DEAE-dextran as a facilitator. The medium was changed at 24 h to 1% fetal calf serum, and supernatants were harvested at 7 days. Fusion proteins were affinity isolated on protein A-Sepharose, eluted at pH 3.0, neutralized immediately in 10% (v/v) 1 M Tris base buffer exchanged into 100 mM Tris (pH 7.5), and concentrated by centrifugation (Centricon 10 filtration units, Amicon). For production of labeled proteins, COS transfectedants were grown in methionine-free medium containing 5% dialyzed fetal calf serum and 50 μCi/ml [15S]methionine/cysteine (Translabel, ICN), for 18 h. Supernatants were harvested, and labeled secreted proteins were isolated by affinity purification on columns of protein A-Sepharose. Bound proteins were eluted by boiling in Laemmli sample buffer under reducing conditions by affinity purification on columns of protein A-Sepharose. Bound proteins were eluted by boiling in Laemmli sample buffer under reducing conditions and resolved at 10% SDS-PAGE. The gel was fixed, impregnated with Amplify (Amersham), dried, and exposed to X-ray film for 12 h at −80°C.

Preparation of Normal and Neoplastic Tissues and Immunohistochemical Staining. Fresh tissue samples were obtained from the histopathology department at the John Radcliffe Hospital and immediately snap frozen in liquid nitrogen prior to storage at −70°C. Cryostat sections (8 μm) were cut onto multiwell slides, dried overnight at room temperature, fixed in acetone for 10 min, and then air dried. Slides were then stained or wrapped in aluminum foil and stored at −20°C until stained. Tissue samples were also available as routinely fixed specimens in paraffin blocks. Tissues studied included skin, bladder, thyrum, spleen, tonsil, lymph node, thyroid, adrenal, pancreas, salivary gland, liver, kidney, heart, esophagus, stomach, duodenum, colon, lung, ovary, testes, uterus, cervix, placenta, brain, spinal cord, prostate, breast, and muscle. Sections from a series of breast, colorectal, and bronchogenic carcinomas either snap frozen or formalin-fixed and paraffin-embedded were also examined.

For immunohistochemical staining each of the anti-CD44 monoclonal antibodies listed in Table 1 was applied to tissue sections for detection by the alkaline phosphatase/anti-alkaline phosphatase or streptavidin-biotin-peroxidase (Dako Duet Kit; Dako, United Kingdom) techniques. For tissues with endogenous biotin a two-stage immunoperoxidase procedure was used (25). Omission of the primary antibody was used as a negative control. A microwave retrieval technique was performed with all antibodies on a selection of routinely processed normal and neoplastic tissues (26, 27).

Generation of CD44 mAbs. Mice were immunized with one of three injections of 10 μg CD44(v8-10)-Fc or CD44(v3-10)-Fc proteins, the first in complete Freund's adjuvant, followed by the remaining two injections in Freund's incomplete adjuvant. Spleens were isolated and hybridomas produced by standard methods (24). Hybridoma supernatants were screened by solid phase ELISA using the fusion protein immunogens, pCD44H-Fc, pCD44(v8-10)-Fc, and pCD44(v3-10)-Fc, and pCD14-Fc as a control for Fc reactivity. Reactivity profiles were established for all positive wells against these 4 proteins. All hybridomas were cloned 3 times by limiting dilution and then isotyped. Determination of Antibody Specificity. The exon specificity of the vCD44 monoclonal antibodies was determined by indirect immunofluorescent antibody staining of a panel of transfected COS 1 cells each expressing different full-length human vCD44 cDNAs including CD44(v10), CD44(v8–10), CD44(v7–10), CD44(v6–10), CD44(v3, 8–10), and CD44(v3–10). Full details concerning the construction of the full-length vCD44 cDNAs and the derivation of the CD44*pRcCMV "cassette cloning vector" used for expression will be published elsewhere. Briefly, PCR products containing the appropriate alternative exon combinations amplified from human leukocyte or tumor cell cDNAs were ligated into a unique BglII/NarI-cloning site within a full-length CD44(v10) cDNA in the expression vector pRcCMV. Individual constructs (1 mg DNA) were introduced into recipient COS cells plated in 3.5-cm tissue culture dishes by incubation (2 h, 37°C) with DEAE-dextran and chloroquine in serum-free RPMI, and 10% Nu-Serum (Collaborative Research), followed by a 10% dimethyl sulfoxide shock (21). After 48 h in culture the transfected cells were incubated with samples of undiluted hybridoma culture supernatants or purified immunoglobulin (10 μg/ml) for 20 min at 5°C, followed by staining with fluorescein isothiocyanate-conjugated goat anti-mouse IgG or IgM antibodies (Sigma Chemical Co., Poole, United Kingdom), as appropriate.

Cell Surface Protein Labeling and Immunoprecipitations. Samples (108 cells) of the ZR75 breast carcinoma were surface labeled with Na-¹²⁵I (1 mCi; Amersham, United Kingdom), using immobilized glucose oxidase/lactoperoxidase (Enzymobeads, Bio-Rad) according to the protocols of the manufacturers, and extracted in 100 mM Tris (pH 7.4)-2 mM EDTA-2 mM phenylmethylsulfonyl fluoride-1% Nonidet P-40, and immunoprecipitations were carried out using 10 μg purified antibodies or, in the case of mAb 1E8 (IgM), as a 10-fold concentrate of tissue culture supernatant. Antigen-mAb complexes were recovered with affinity-purified goat anti-mouse IgG, IgA, and IgM antibody, coupled to agarose (Sigma), and following elution, proteins were resolved on 7.5% SDS-PAGE gels. Control mAbs included F.10.44.2 (a framework CD44 antibody; 20), 9G11 (anti-CD31, R&D Systems Europe), and mAb 38 (anti-CD11a; Nancy Hogg, Imperial Cancer Research Fund).

Table 1 ELISA profiles of CD44 mAbs on CD44Fc fusion proteins

<table>
<thead>
<tr>
<th>CD44 Fc</th>
<th>CD44 (v8-10)</th>
<th>CD44 (v3-10)</th>
<th>CD14-Fc</th>
<th>CD50-Fc</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD44</td>
<td>2C5</td>
<td>3G5</td>
<td>3D2</td>
<td>2F10</td>
</tr>
<tr>
<td>H-Fc</td>
<td>1.936</td>
<td>0.038</td>
<td>0.032</td>
<td>0.031</td>
</tr>
<tr>
<td>(v8-10)Fc</td>
<td>1.731</td>
<td>1.420</td>
<td>1.012</td>
<td>1.29</td>
</tr>
<tr>
<td>(v3-10)Fc</td>
<td>0.981</td>
<td>0.034</td>
<td>0.039</td>
<td>0.034</td>
</tr>
<tr>
<td>CD14-Fc</td>
<td>0.024</td>
<td>0.032</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CD50-Fc</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
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</table>

* n.d., not determined.

Fig. 1. CD44 isofom PCR products generated from first-strand cDNA made from RNA extracted from cell lines; lane 1, BsIlE II size markers; lane 2, ZR75, breast carcinoma; lane 2, MCF7 breast carcinoma; lane 3, U937, promonoctyic leukemia. Products were resolved by electrophoresis on a 1% agarose gel.

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RESULTS

Generation of Soluble CD44-Fc Fusion Proteins. Two different approaches were used to generate CD44 isoforms for the production of CD44 exon-specific mAbs. The first approach, which involved antibody panning of COS cells transfected with a pool of cDNA libraries in the pCDM8 expression vector, yielded CD44H and CD44(v8–10). However, despite repeated attempts, this method failed to yield cDNAs encoding other larger CD44 isoforms because of the low levels of expression found in most cell types. Consequently, two breast tumor cell lines, MCF7 and ZR75, that were known to express high molecular weight CD44 isoforms (data not shown) were screened for vCD44 mRNA levels by reverse transcriptase-PCR using primers that encompass the entire coding region for the extracellular domain. Analysis of the products (Fig. 1) confirmed the presence of multiple CD44 splice variant RNAs in the ZR75 and MCF7 cell lines and their absence from the hemopoietic cell line U937. The largest PCR product generated from ZR75 cells was subsequently cloned, sequenced, and identified as CD44(v3–10).

Next, all three CD44 isoforms were expressed as soluble immunoglobulin Fc chimeras by subcloning the region encoding the extracellular domain in each case into the expression vector pCD33 signal immunoglobulin (Fig. 2) for expression and secretion from COS 1 cells. High levels of soluble CD44 secretion (approximately 10 µg/ml) were confirmed for each construct by SDS-PAGE analysis of tissue culture supernatants after biosynthetic labeling with [35S]methionine/cysteine (Fig. 3).
Generation of a New Panel of CD44 Exon-specific mAbs. Purified CD44(v8–10)-Fc and CD44(v3–10)-Fc fusion proteins were used as immunogens to generate CD44 alternative exon-specific monoclonal antibodies. As an initial screen, hybridoma supernatants were tested by solid phase ELISA with CD44H-Fc, CD44(v8–10)-Fc, and CD44(v3–10)-Fc and a control CD14-Fc, to define reactivity with either the v8–10 or v3–7 regions of the CD44 molecule (Table 1). Each mAb was then screened for reactivity against a panel of COS cell transfectants expressing cloned CD44 cDNAs carrying different combinations of the v3–v10 alternative exons. As a result of this analysis (Table 2) five different CD44 exon-specific mAbs were identified that recognized specifically v3, v4/5, v6, and v8/9. In addition, all five mAbs reacted with unfixed ZR75 breast carcinoma cells and revealed strong expression of v3, v4/5, and v6 but smaller amounts of v8/9 (data not shown). Finally, all five antibodies, in addition to the framework mAbs F10.44.2 and 2C9, immunoprecipitated the same wide spectrum of protein bands ranging in size from Mr 100,000–200,000 from ZR75 cells, consistent with the presence of high levels of CD44 isoforms carrying multiple alternative exon combinations (Fig. 4).

Normal Tissues Express a Diverse Repertoire of CD44 Isoforms. Immunohistochemical staining of normal tissues revealed considerable expression of vCD44 isoforms that was largely confined to the epithelia (Table 3). In particular, respiratory epithelium, tran...
Fig. 5. CD44H (2C5) expression in tonsil demonstrating intense immunoreactivity of lymphoid tissue (A, magnification × 40) and overlying crypt epithelium (B, magnification × 400). C, CD44H in placenta localized to cytotrophoblast and endothelium with weaker matrix staining. D, CD44H in thymus strongly expressed by most medullary lymphocytes and occasional cortical thymocytes (magnification × 40). E, CD44H (2C5) positivity in cerebral cortex (magnification × 40) and endothelium. F, Nuclear stippling with 2C5 in testes (magnification × 400). G, Intense CD44E (1E8) membrane staining of cells in salivary gland (magnification × 400).

Fig. 6. A, CD44E (1E8) staining of macrophages and lymphocytes in a reactive lymph node (magnification × 40). B, v3 (3G5) immunoreactivity in stratified squamous epithelium of skin (magnification × 40). C, v3 (3G5) immunoreactivity in transitional epithelium of bladder (magnification × 400). D, v4/5 (2G9) expression in glands of secretory phase endometrium (magnification × 400). E, intense v6 (2F10) immunoreactivity of cervical squamous epithelium (magnification, × 400). F, strong v6 (2F10) staining of respiratory epithelium (magnification × 400). G, strong v6 (2F10) staining of prostatic epithelium (magnification × 400) with basal layer accentuation (arrows).
Fig. 7. A, intense v6 (2F10) positivity of esophageal squamous epithelium (magnification × 40). B, strong but focal v6 (2F10) expression in duodenal epithelium (magnification × 40, arrows). C, normal breast stained for v6 (2F10) demonstrating strong staining of myoepithelial cells of acini and ducts (magnification × 40). D, v6 (4B3) of reactive lymph node showing moderate staining of the germinal centers (magnification × 40). E, pan v8/9 (1E8) tumor staining of lung carcinoma (magnification × 40).

Fig. 8. A, colorectal carcinoma stained for v3 (3G5; magnification × 40). B, colorectal carcinoma stained for v4/5 (2G9; magnification × 40) showing intense cell membrane immunoreactivity with accentuation of the infiltrating margin (arrow). C and D, breast carcinoma negative for v6 (2F10) in both the intraduct (×) and invasive element (*) (magnification × 40) but with positive staining of the residual myoepithelial layer of the duct (arrow).
CD44 Variant Expression in Tumors. The expression of vCD44 isoforms detected in a comprehensive panel of tumors including breast, lung, and colorectal carcinomas is summarized in Table 4. The majority of tumors expressed CD44H together with vCD44 isoforms carrying the v3 and v4/5 exons and the so-called metastatic v6 exon. Although many tumor cells were strongly immunoreactive with different exon-specific antibodies, there was considerable heterogeneity in staining intensity among individual tumors (Fig. 8). For example, staining for CD44 v8/9 was weak and focal in many tumors, and yet some tumors were intensely positive (Fig. 8). Interestingly, strong expression of CD44(v8/9) was often seen at the invading edges of carcinomas and around the periphery of intraductal elements of breast carcinomas (Fig. 8), suggesting a possible role for CD44 molecules in the regulation of tumor cell adhesion.

A surprinsingly complex pattern of CD44v expression in different tumors, compared to the CD44 expression of the normal cell of origin, was seen. A striking result was the high levels of expression of many of the variant exons by breast carcinomas that arise from breast ductal epithelium, which do not normally express CD44. Conversely, normal gastrointestinal epithelium expressed low levels of many variants, but the cognate colon tumors expressed high and variable levels of the variants. A third pattern was demonstrated by respiratory epithelium, in which variants were expressed at high levels in normal cells and continued to be expressed at those levels in lung carcinomas. Significantly, wherever variants were expressed at high levels, the whole range of isoforms was usually found (v3, v4/5, v6, and v8/9), and not just v6.

These results indicate that differential RNA splicing of CD44 transcripts may be deregulated in tumor cells, leading to the inclusion of alternative exons in some tumor types and their exclusion in others. Such complex patterns underline the need for caution in using vCD44 expression as a diagnostic indicator for malignancy.

DISCUSSION

In this paper we have described the production of a panel of CD44 alternative exon-specific mAbs using recombinant CD44-immunglobulin Fc fusion proteins as immunogens. A total of five antibodies specific for the alternative exons v3, v4/5, v6, and v8/v9 have been characterized, all of which recognize both native and formalin-fixed cells. These have allowed us to characterize the expression patterns of CD44 variants, and they have revealed a surprisingly complex pattern of vCD44 expression in both normal and neoplastic tissues.

Epithelial cells express a wide range of variants at high levels. Many types of epithelium in many different organs express v3, v4/5, v6, and v8/v9. In contrast, activated leukocytes express a more restricted repertoire of variants. The predominant isoforms contain only v4/5 and v6. Only occasionally was v3 seen in normal activated lymphocytes.

In contrast to numerous reports describing CD44H expression, there have been only three immunohistochemical studies of CD44v expression in normal and neoplastic human tissues (15–19). We have generated monoclonal antibodies specific for v3, v4/5, v6, and v8/v9 and have described their distribution in an extensive survey of human tissues. The findings among these studies are generally concordant, where it is possible to directly compare. All detected CD44H in hemopoietic cells and v6 in epithelia. A minor disagreement is the pattern of v6 staining in squamous epithelium; we observed panepithelial expression rather than preferential staining of the superficial portion of squamous epithelium noted by Heider et al. (18). These discrepancies are probably due to minor epitope variations as recognized by the different antibodies used. Although gastrointestinal glandular epithelium was generally vCD44 negative, endometrial glands were positive during all phases of the menstrual cycle and during decidualization of the stroma.

The tissue distribution of CD44(v8–10) is not known. This particular CD44 isoform was originally designated as the epithelial variant (CD44E) since it was initially identified in epithelial and carcinomas. We did observe strong expression of CD44E(v8/9) in many epithelia, but it was not present in all types examined (Table 3). A recent study using PCR (28) detected CD44(v8–10) in 15.4% (2 of 13 specimens) and 33% (2 of 6 specimens) of normal colon and liver, respectively, whereas we could demonstrate no CD44(v8/9) expression in these tissues. This is unlikely to be due to the binding affinity of mAb 1E8 since a range of staining intensities was observed with this antibody in both normal and neoplastic tissues. A more likely explanation is the rare expression of this variant together with the sensitivity of PCR.

Recently, there have been many reports concerning the role of CD44 variants in primary tumors and their metastases (10–14). In model systems, expression of isoforms carrying the v6 exon confers a
metastatic phenotype, and antibodies directed against this particular exon prevent tumor spread. Overexpression of vCD44 is also reported to be a feature of human neoplastic cell lines and tumors. Indeed, recent studies using PCR have suggested that the expression of particular CD44 splice variants is restricted to tumors (29).

However, in contrast to these reports and in accordance with other immunohistochemical and PCR-based techniques, we identified a number of different CD44 variants in the three tumor types examined but could not demonstrate expression of vCD44 in all tumors. The finding of vCD44 in tumors might be due either to up-regulation or to aberrant expression by neoplastic cells. Thus, bronchogenic carcinomas, which arise from respiratory epithelium which normally expresses CD44H, CD44(v8-10), and all vCD44s examined, also express these CD44 isoforms. In contrast, breast carcinomas which arise from ductal epithelium, which does not normally express any CD44 isoform, express vCD44 (Table 3). Furthermore, whereas expression of vCD44 in normal tissues was predominantly membranous, in neoplastic cells, expression was both membranous and cytoplasmic with accentuation of immunoreactivity at the infiltrating tumor margin. The biological significance of this altered cellular distribution and its preferential location at the invading tumor edge conforms to the role of CD44 as a candidate adhesion molecule involved in tumor invasion and metastasis.

In conclusion, we have generated a panel of monoclonal antibodies which specifically recognize variants encoded by the alternatively spliced exons of CD44 by using recombinant CD44(v3-10)-Fc as an immunogen. mAbs have been produced which recognize v3, v4/v5, v6, and v8/9. These have allowed us to characterize the expression patterns of CD44 variants in normal and neoplastic tissues and have revealed a surprisingly complex pattern of vCD44 expression. We are currently testing whether any of the variant-specific mAbs inhibit CD44 isoform function in a range of adhesion and cell proliferation assays.

REFERENCES

Normal Human Tissues, in Addition to Some Tumors, Express Multiple Different CD44 Isoforms

Stephen B. Fox, Jonathan Fawcett, David G. Jackson, et al.


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