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Immunology

Identification of endosialin, a cell surface glycoprotein of vascular endothelial cells in human cancer

(tumor stroma/O-glycosylation/sialic acid/monoclonal antibody/immunohistochemistry)

WOLFGANG J. RETTIG*, PILAR GARIN-CHESA†‡, JOHN H. HEALEY§, SAI L. SU*, ERIC A. JAFFE¶, AND LLOYD J. OLD†

*Immunology Program, †Ludwig Institute for Cancer Research, ‡Department of Pathology, and §Department of Surgery (Orthopedic Service), Memorial Sloan–Kettering Cancer Center, New York, NY 10021; and ¶Department of Medicine, Cornell University Medical College, New York, NY 10021

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ABSTRACT **Cell surface antigens of transformed cells are the traditional targets for antibody-guided detection and therapy of solid neoplasms. Alternative targets may be found in the tumor stroma, which contains newly formed blood vessels, reactive fibroblasts, and extracellular matrix proteins. The F19 cell surface glycoprotein of reactive fibroblasts is a prototypic stromal antigen since it is found in the stroma of >90% of common epithelial cancers but is absent or expressed at low levels in normal tissues and benign epithelial tumors. In the present study, we define an additional tumor stromal antigen, FB5, that is selectively expressed in vascular endothelial cells of malignant tumors. Immunohistochemical analysis of 128 tumors identified FB5 in endothelial cells in 67% of the samples (including 41 of 61 sarcomas, 26 of 37 carcinomas, and 18 of 25 neuroectodermal tumors) whereas normal blood vessels and other adult tissues tested lacked FB5 expression. *In vitro* studies showed that FB5 is a M_r 165,000 cell surface glycoprotein, comprised of a M_r 95,000 core polypeptide and highly sialylated O-linked oligosaccharides but few if any N-linked sugars, and that the FB5 gene is located on chromosome 11q13–qter. The restricted tissue distribution of the FB5 protein, which we refer to as endosialin, suggests strategies for tumor imaging and therapy that are aimed primarily at the tumor vasculature.**

Carcinogenesis involves a series of somatic genetic changes affecting the structure and/or expression of oncogenes and tumor suppressor genes (1). Secondary genetic changes and epigenetic mechanisms may also be necessary to allow small nests of malignant cells to form clinically apparent primary and metastatic tumors. For example, in solid neoplasms, growth beyond a diameter of 1–2 mm depends on the formation of a supporting stroma of newly formed blood vessels (2), commonly accompanied by reactive stromal fibroblasts, lymphoid and phagocytic infiltrates, and extracellular matrix proteins. Cells of the reactive tumor stroma, while not transformed, may differ from corresponding cells in normal tissues in proliferative activity and expression of regulatory peptides, proteolytic enzymes, extracellular matrix proteins, and cell surface antigens (3–7). Consequently, they may provide additional targets for pharmacological and immunological interventions in cancer. As one example, the F19 cell surface glycoprotein of reactive stromal fibroblasts is expressed in the stroma of >90% of common epithelial cancers, including carcinomas of the breast, colon, lung, bladder, and pancreas (3), with no or only limited expression in normal adult tissues (3, 8). In a phase I study, Welt *et al.* (9) have found that an ^{131}I-labeled monoclonal antibody (mAb) against the F19 antigen accumulates in tumor sites, allowing tumor imaging in patients with hepatic metastases from colorectal carcinomas.

Immunologic targeting of tumor vascular endothelial cells has not yet been accomplished but is an attractive approach for two reasons. (*i*) Endothelial surface antigens are highly accessible to antibodies or antibody conjugates circulating in the blood stream. (*ii*) Immunological destruction of tumor blood vessels could lead to widespread necrosis in solid tumors. For instance, the antitumor effect of tumor necrosis factor (TNF)α in mouse sarcomas (10) and the effects of isolated limb perfusion with high doses of TNF-α, γ-interferon, and melphalan in patients with melanomas and soft-tissue sarcomas (11, 12) may result from vascular endothelial cell damage rather than direct tumor cell killing. In the present study, we identify a tumor vascular endothelial antigen, endosialin, that may allow future immunological attack on tumor blood vessels.

MATERIALS AND METHODS

Antibodies and Serological Procedures. mAb FB5 (IgG2a) was derived from a (BALB/c × A)F_1 mouse immunized with human fibroblasts after fusion (13) with X63-Ag8.653 myeloma cells. Other mAbs (3, 8, 14, 15) were used as positive and negative controls. Mixed hemadsorption (MHA) rosetting assays were carried out as described (16, 17).

Cell Culture. GM05387 and GM01398 fibroblasts (National Institute of General Medical Sciences), human umbilical cord endothelial cells (HUVEC), somatic cell hybrids, and other cells taken from the cell line bank at Sloan–Kettering Institute were maintained in culture as described (16–21). Medium additives were TNF-α (Genentech), interleukin (IL) 1β, IL-4, IL-6 (Genzyme), basic fibroblast growth factor, transforming growth factor β1 (R & D Systems, Minneapolis), γ-interferon (Boehringer Mannheim), phorbol 12-myristate 13-acetate, and forskolin (Sigma).

Immunochemical Procedures. Cells labeled with a mixture of [^{35}S]methionine and [^{35}S]cysteine (Tran^{35}S-label, ICN; 40 μCi/ml; 1 Ci = 37 GBq) for 18–24 h were extracted in lysis buffer [0.01 M Tris·HCl/0.15 M NaCl/0.01 M $MgCl_2$/0.5% Nonidet P-40/aprotinin (20 μg/ml)/2 mM phenylmethylsulfonyl fluoride] and used for immunoprecipitation assays followed by $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography, as described (21). In some experiments, purified antigens or cell extracts were digested with neuraminidase (*Vibrio cholerae*, Calbiochem), endoglycosidase H (25 × 10^{-3} international unit/ml), N-Glycanase (10 units/ml), or O-Glycanase (0.1 unit/ml; Genzyme) (22). Inhibitors of protein glycosylation used were phenyl-*N*-acetyl-α-galactosaminide (Ph-α-GalNAc; 5 mM), monensin (10 μg/ml), and tunicamycin (5 μg/ml; Sigma). Immunoblot

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Abbreviations: mAb, monoclonal antibody; MHA, mixed hemadsorption; HUVEC, human umbilical cord endothelial cell; WGA, wheat germ agglutinin; IL, interleukin; TNF, tumor necrosis factor.

experiments were carried out with the alkaline phosphatase detection system (14). Lectin chromatography was performed with Con A-Sepharose (Pharmacia; elution with 250 mM methyl α-D-mannopyranoside) and *Triticum vulgaris* (wheat germ agglutinin, WGA)-Sepharose (Sigma; elution with 250 mM galactosamine).

Tissues and Immunohistochemistry. Tissues were obtained from autopsy or surgical specimens, frozen in isopentane precooled in liquid nitrogen, and stored at −70°C. Five-micrometer-thick sections were cut, mounted on poly-(L-lysine)-coated slides, air-dried, and fixed in acetone (4°C, 10 min). mAbs were used at 10–20 μg/ml and the avidin–biotin immunoperoxidase procedure was performed as described (3, 8). Bone marrow cells were centrifuged onto glass slides using a Cytospin 2 (Shandon, Pittsburgh) and tested with the streptavidin/alkaline phosphatase system (Dako, Carpinteria, CA). Substrate-adherent cultured cells grown in Micro-Well plates (Nunc) were fixed, permeabilized with ethanol (10 min, 4°C), and tested by the avidin–biotin immunoperoxidase method (15).

RESULTS

mAb FB5, raised against cultured fetal fibroblasts, was tested for cell surface reactivity with a panel of cell types *in vitro* (Table 1). Cultured fibroblasts and neuroblastoma cell lines were found to be FB5$^+$, whereas other neuroectodermal tumor lines, carcinomas, sarcomas and HUVEC cultures were FB5$^-$. HUVEC cultures were also activated with cytokines prior to testing for cell surface and intracellular FB5, but no antigen induction was observed (Table 1).

Table 1. Reactivity of mAb FB5 with cultured human cells

FB5$^+$	FB5$^-$
Fibroblast	Melanoma
WI-38, GM05387, F135-35-18, Hs27, Hs68, FA537, SKF-AH	SK-MEL-13, SK-MEL-19, SK-MEL-23, SK-MEL-178, SK-MEL-198
Neuroblastoma	Glioma
LA1-5s (control, boiled, NANase treated), IMR-32, SMS-SAN, SMS-KAN	U251MG, U343MG, U373MG, SK-MG-28
	Sarcoma
	SW872, 8387, Saos-2, HT-1080, RD
	Carcinoma
	MCF-7, BT20, SK-RC-9, SK-RC-28, Colo205, HCT15, HT-29, SK-OV6
	Leukemia
	U937, HL-60, Raji
	Endothelial cell
	HUVEC, activated HUVEC

Cell surface antigen expression was determined by MHA assays with 1:5 serial dilutions of mAb FB5 (starting concentration, 20 μg/ml). Fibroblasts and neuroblastoma cells showed titration end points in excess of 1:50,000. LA1-5s cells were tested by MHA assays untreated (control) or treated with neuraminidase (0.1 unit/ml for 1 h at 37°C) or with boiling phosphate-buffered saline for 5 min (15). For HUVECs, seven cultures (passages 2–4) derived from different individuals were tested by MHA assays to detect surface antigen and by immunoperoxidase staining of permeabilized cells and immunoprecipitation assays to detect intracellular antigen. Immunoprecipitation assays were also performed with neuraminidase-treated HUVECs and HUVECs labeled in the presence of tunicamycin or Ph-α-GalNAc to test for cryptic epitopes. For activated HUVECs, cells were pretreated for 6 or 24 h with TNF-α (50 ng/ml), IL-1β (0.5 ng/ml), transforming growth factor β1 (2 ng/ml), phorbol 12-myristate 13-acetate (5 μg/ml), forskolin (50 mM), γ-interferon (200 units/ml), basic fibroblast growth factor (5–25 ng/ml), IL-4 (1 ng/ml), or IL-6 (20 ng/ml) and tested by MHA assay, immunocytochemistry, and immunoprecipitation assays.

Table 2. Normal adult tissues tested with mAb FB5 by immunohistochemical methods

Organ system	FB5$^-$ normal tissues
Nervous	Cerebral cortex, cerebellum, spinal cord, peripheral nerves
Endocrine	Adrenal gland, thyroid gland, pancreas
Urinary	Kidney, urinary bladder, prostate
Reproductive	Testis, ovary, uterus
Digestive tract	Esophagus, stomach, small and large intestine, liver, pancreas
Pulmonary	Lung, bronchus, trachea
Cardiovascular	Heart, arteries, veins, capillaries, lymphatics
Lymphoid	Thymus, spleen, lymph node
Hematopoietic	Bone marrow
Skin	Epidermis, dermis, appendages
Breast	Mammary gland
Connective tissues	Skeletal muscle, visceral and vascular smooth muscle, adipose tissue, cartilage

Immunohistochemical methods were used to determine the distribution of FB5 *in vivo*. Among the normal tissues tested (Table 2), none showed FB5 immunoreactivity. In contrast, tests with tumor tissues identified FB5 expression in tumor vascular endothelial cells of a substantial proportion of samples (Table 3), including 41 of 61 sarcomas, 26 of 37 carcinomas, and 18 of 25 neuroectodermal tumors. Fig. 1 illustrates this pattern of endothelial FB5 expression. The malignant cells in the tumors included in this study were FB5$^-$; however, in an extended immunopathological study, we have identified a subset of sarcomas with FB5$^+$ malignant cells (unpublished data). Finally, a small proportion of epithelial cancers showed weak FB5 immunostaining in reactive stromal fibroblasts.

Table 3. Summary of FB5 antigen expression in vascular endothelial cells of human tumors

Tumor type	FB5$^+$ phenotype
Carcinoma	
Renal cancer	6/9
Breast cancer	8/12
Colon cancer	4/5
Pancreas cancer	3/5
Lung cancer	3/4
Mesothelioma	2/2
Sarcoma	
Leiomyosarcoma	5/9
Osteogenic sarcoma	7/12
Chondrosarcoma	5/8
Fibrosarcoma	4/6
ASPS	2/2
Rhabdomyosarcoma	6/8
Ewing sarcoma	6/7
Synovial sarcoma	6/9
Neuroectodermal tumor	
PNET	4/4
MPNT	8/12
Neuroblastoma	2/3
Melanoma	3/5
Glioma	1/1
Lymphoma	0/5

Numbers indicate proportion of cases for each tumor type listed showing FB5$^+$ vascular endothelial cells (no. FB5$^+$/total number tested) in avidin–biotin immunoperoxidase assays. ASPS, alveolar soft-part sarcoma; PNET, primitive neuroectodermal tumor; MPNT, malignant peripheral nerve sheath tumor.

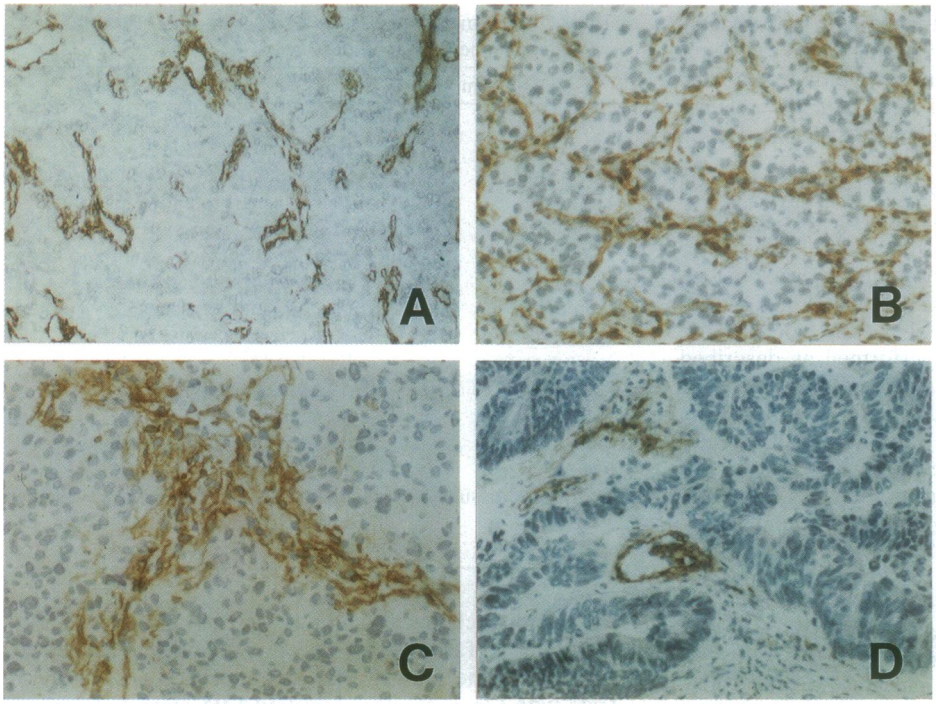

FIG. 1. Immunohistochemical detection of the FB5 antigen in tumor vascular endothelial cells of a leiomyosarcoma (*A*), renal cell carcinoma (*B*), osteogenic sarcoma (*C*), and colon carcinoma (*D*). (Avidin–biotin immunoperoxidase staining with hematoxylin counterstaining. *A*, ×10; *B–D*, ×20.)

FB5 expression in tumor vasculature was confined to small blood vessels, primarily capillaries, and not found in the endothelium of the larger tumor vessels. There was considerable variability in the number of $FB5^+$ vessels in various tumors, ranging from a small subset of capillaries to virtually the entire capillary bed. No discernible parameter distinguished tumors with a $FB5^+$ or $FB5^-$ phenotype or with different degrees of FB5 reactivity.

FB5 expression in neuroblastoma cell lines and cultured fibroblasts provided a readily available source of antigen for biochemical analysis. Immunoprecipitation (Fig. 2*A*) and immunoblot experiments (Fig. 2*B*) with these cells identified FB5 as a protein that migrates as a single M_r 165,000 species (p165) on $NaDodSO_4$/polyacrylamide gels under reducing and nonreducing conditions. Enzymatic digestions and metabolic inhibition studies (Fig. 2*C*) revealed that the M_r 165,000 protein is composed of a M_r 95,000 core polypeptide with abundant highly glycosylated O-linked oligosaccharides. For example, neuraminidase treatment caused a pronounced change in the electrophoretic mobility of FB5, resulting in a desialylated M_r 120,000 protein (p120). Combined treatment with neuraminidase and O-Glycanase generated a

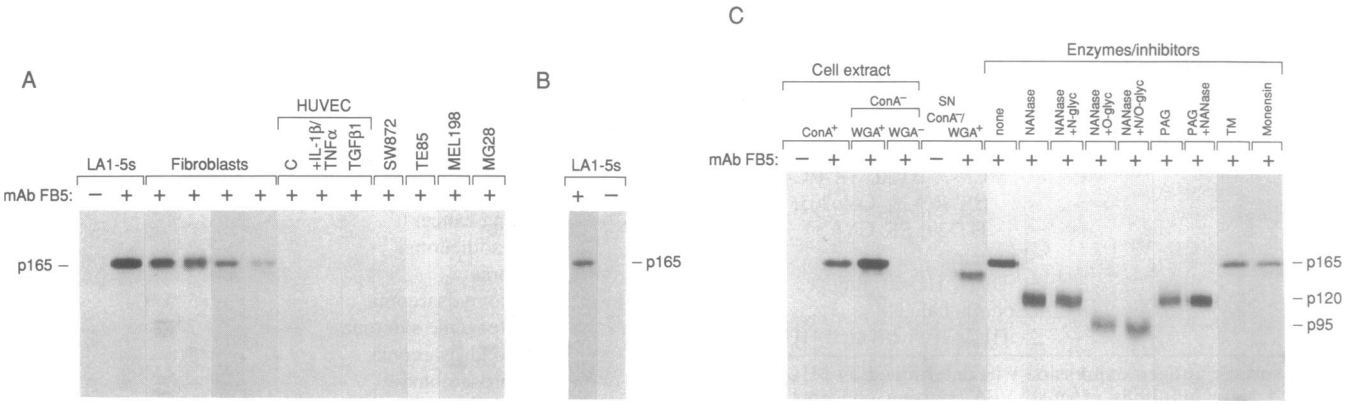

FIG. 2. Immunochemical analysis of the FB5 antigen. (*A*) Radioimmunoprecipitation analysis with mAb FB5 using cell extracts prepared from LA1-5s neuroblastoma cells; fibroblasts (F135-35-18, WI-38, FA-334, and GM01398); HUVECs; or tumor cell lines SW872 (leiomyosarcoma), TE85 (osteosarcomas), SK-MEL-198 (melanoma), and SK-MG-28 (glioma). For HUVECs, untreated controls (C) and cultures labeled in the presence of IL-1β (0.5 ng/ml) plus TNF-α (50 ng/ml) or of transforming growth factor β1 (2 ng/ml) were tested. Negative control experiments with unrelated mAbs were carried out with each extract to determine specificity of immunoprecipitates (data not shown). The size of the FB5 protein (p165) was determined with molecular weight markers and is indicated to the left. (*B*) Immunoblot analysis of LA1-5s cell extract with mAb FB5 (+) and negative control mAb (−). (*C*) Lectin binding and carbohydrate analysis of FB5 antigens. LA1-5s cell extracts and cell-free culture supernatants (SN) labeled with Tran^{35}S-label were separated into Con A and WGA bound (+) and unbound (−) fractions, respectively, and tested by immunoprecipitation. Note that WGA binds FB5 present in the Con A flow-through fraction, as shown, and FB5 from unfractionated LA1-5s extracts equally well (data not shown). Enzymatic digestions of purified FB5 glycoproteins derived from LA1-5s cell extracts were carried out prior to gel electrophoresis by using neuraminidase (NANase, 0.1 unit/ml), N-Glycanase (N-glyc; 10 units/ml), and O-Glycanase (O-glyc; 0.1 unit/ml) for 18 h at 37°C, in the presence of protease inhibitors (aprotinin at 10 μg/ml and 2 mM phenylmethylsulfonyl fluoride). For glycosylation inhibition studies, LA1-5s cells were radiolabeled in the presence of Ph-α-GalNAc (PAG, 2 mM; 24-h preincubation and 20-h incubation with Tran^{35}S-label), tunicamycin (TM; 5 μg/ml, 3-h preincubation and 18-h incubation with Tran^{35}S-label), or monensin (10 μg/ml; 3-h preincubation and 3-h incubation with Tran^{35}S-label), extracted, and used for immunoprecipitation assays. The size of the p165, p120, and p95 forms of FB5, as deduced from the positions of molecular weight standards, is indicated on the right.

M_r 95,000 protein (p95). Endoglycosidase H and N-Glycanase had no effect on the size of the FB5 protein. Tunicamycin, which blocks N-linked glycosylation, and monensin, which interferes with protein processing in the Golgi apparatus, also showed no effect on the size of the FB5 protein.

Radiolabeling of cells in the presence of 5 mM Ph-α-GalNAc, a putative inhibitor of O-linked glycosylation (23), resulted in the synthesis of a M_r 120,000 protein species. This M_r 120,000 protein appears to be an asialo form of FB5 since its mobility on gels is unaffected by neuraminidase treatment (Fig. 2*C*) but is reduced to M_r 95,000 by O-Glycanase.

Consistent with the high sialic acid content of the FB5 glycoprotein, we found that the native antigen binds to WGA-Sepharose, whereas desialylated FB5 does not bind this lectin. Only partial binding to Con A-Sepharose was observed. This pattern of lectin binding allowed enrichment and detection of low levels of FB5 protein in the Con A-unbound/WGA-bound fraction of LA1-5s culture supernatant. For as yet unknown reasons, the supernatant-derived FB5 proved to be slightly smaller than the cell-extract-derived protein (Fig. 2*C*).

The mAb FB5-defined epitope on cultured LA1-5s neuroblastoma cells is heat-stable and resistant to neuraminidase treatment (Table 1). Furthermore, immunoprecipitation and immunoblot experiments showed that the p120 and p95 species of FB5 generated by neuraminidase and neuraminidase/O-Glycanase digestion, respectively, retain immunoreactivity with mAb FB5. These findings suggest that mAb FB5 defines a peptide epitope on the FB5 core polypeptide.

The chromosomal location of the FB5 gene was determined through serologic analysis of a panel of rodent–human somatic cell hybrids (16, 18, 19), chiefly derived from FB5$^+$ human neuroblastoma cells and FB5$^-$ mouse neuroblastoma cells and containing different portions of the human chromosome complement (Table 4). Discordancy analysis showed that human chromosome 11 segregates with FB5 cell surface expression, with all other chromosomes being excluded by at least four discordant hybrids. Several of the FB5$^+$ hybrid lines carried human marker chromosomes comprising part of the long arm of human chromosome 11, but no complete copy of this chromosome. These hybrids allowed assignment of the FB5 gene to chromosomal region 11q13–qter.

DISCUSSION

Endosialin is a vascular endothelial antigen with several distinctive features. Most notably, endosialin is expressed in tumor blood vessels of a significant proportion of malignant neoplasms but is not found in blood vessels of normal tissues or in any of the other normal adult cell types tested *in vivo*. Unlike previously defined vascular endothelial antigens (24–26), endosialin is not expressed in cultured normal umbilical cord endothelial cells, even after activation of these cells with TNF-α, IL-1, several other regulatory peptides, or activators of protein kinase C or adenylate cyclase.

Table 4. Chromosomal assignment of the FB5 antigen-coding gene through serologic analysis of human–mouse somatic cell hybrids

Hybrid clone	FB5	Human Chromosomes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
NSK-1	+	○				●	●	●		●	●	◒	●		●	●				●				●	
NSK-4	+	○			●		●		●			●	●		●	●				●		●		●	
NSK-5	+	○		○			●	●			●	●	●		●	●		○			●	●			
NSK-9	+					●		●				◒			●	●	●				●	●		●	
NBE-C2	+	○										◒				●				○	●	●		●	
NBE-D4	+	○			●	●					●	◒	●												
NBE-E1	+	●			●		●				●	●	●		●	●	○			○	●	●		●	
NBE-G1	+	○					●	●			○	◒			●		●				●	●	○	●	
NBE-H1	+	●			●							◒			●	●		●		○	●	●		●	
NBE-J2	+	○	○									◒				●				●	●	●		●	
NBE-K1	+	○									●	◒	○		●	●					●			●	
NBE-M1	+	●	●		●		●				●	◒			●	●				○	●	●		●	
NBE-N1	+	○			○						○	◒			●	●				○	●			●	
LNK-1.6	+			●			●		●		●	●					●	●							
LNK-1.8	+			●	●				●		●	●					●	●			●		○		
LNK-1.15	+			●					●		●	●		●				●			●				
ANK-3.11	−					●															●		○	●	
ANK-4/4	−		●										●			●								●	
A9GM10-19c	−	●																							
LM29	−																	●							●
NSK-3	−	○					●	○	●				●		●	●		○		●	●	○			
NSK-6	−	○		○	●								●		○	○		○		●		○			
NSK-10	−	○									○				●	●				●		●		●	
No. discordant		5	15	12	10	14	10	12	13	15	6	0	13	15	8	8	11	12	16	11	7	8	15	8	17

FB5 expression was determined by MHA rosetting assays with serial dilutions of FB5 hybridoma supernatant. Titration end points were 1:1250 to 1:6250 for positive hybrids, with >90% of FB5$^+$ cells in each case. The antigen-negative hybrids showed no MHA reactivity at the highest concentration of mAb FB5 tested (1:2 dilution of hybridoma supernatant; immunoglobulin at 20 μg/ml). The presence of human chromosomes as determined through karyotype, isozyme, molecular genetic, and serologic assays (16, 18, 19) is indicated as follows: ●, chromosome present; ◒, chromosome fragment 11q13–qter present in the absence of a complete copy of chromosome 11; ○, other chromosome fragments present, as described (16, 18, 19); no entry, chromosome not present. The number of hybrid clones showing discordance between FB5 phenotype and presence/absence of a given human chromosome are listed at the bottom of the figure.

Tumor vascular endothelial cells are not readily purified and adapted to tissue culture, hampering biochemical and functional studies of vascular endosialin. However, endosialin expression in cultured fibroblasts and established neuroblastoma cell lines has permitted immunochemical characterization of the molecule and assignment of the endosialin gene to chromosome 11q13–qter, a region containing a number of cell surface antigen genes (27). Both cultured fibroblasts and neuroblastoma cells express endosialin as highly sialylated M_r 165,000 glycoproteins with predominantly O-linked oligosaccharides, and the mAb FB5-defined epitope appears to reside on the M_r 95,000 endosialin core polypeptide rather than the oligosaccharide side chains. The finding that Ph-α-GalNAc-treated cells synthesize p120, an asialo form of endosialin, is unexpected. Aryl N-acetyl-α-galactosaminides, such as Ph-α-GalNAc, are thought to inhibit O-linked glycosylation by competing with N-acetylgalactosamine, the first sugar linked to mucin core proteins, for binding to galactosyltransferase (23). Yet, we did not observe the suspected M_r 95,000 species of endosialin lacking O-linked oligosaccharides in our Ph-α-GalNAc-treated cells, suggesting that other effects may be mediated by this class of glycosylation inhibitor.

Glycoproteins with abundant O-glycans are found in secretions and on the surface of normal mucin-producing epithelial cells and in certain neoplasms, notably colorectal, breast, pancreatic, and ovarian carcinomas (28). However, several examples of nonepithelial cell surface glycoproteins with abundant O-linked glycosylation have been described, including the CD43 (leukosialin) and CD34 antigens of lymphoid cells (29, 30). CD43 and CD34 are readily distinguished from endosialin with regard to tissue specificity, size of the core polypeptides, and chromosomal location of the coding genes (29–31). CD34 and several other, biochemically still undefined antigens have been found on normal and tumor blood vessels (32, 33).

The function and regulation of endosialin expression in tumor vascular endothelium are currently unknown. An initial survey of peptide mediators known to activate HUVECs and to alter their surface antigenic phenotype (24–26) has not uncovered any factors that induce endosialin expression. However, transformed cells, tumor-infiltrating lymphocytes, phagocytes, and reactive tumor fibroblasts provide a rich source of additional peptide and lipid mediators that may be capable of inducing endosialin expression in HUVECs. Alternatively, umbilical vein endothelium may simply differ from tumor endothelial cells with regard to FB5 inducibility. Further clues about endosialin function and regulation may come from studies of FB5 expression in benign tumors, inflammatory lesions, and embryonic and fetal development.

Antibody targeting of tumor stromal components, such as F19 and endosialin, has several potential advantages. For example, tumor stroma is generally organized around the tumor vasculature and may be readily accessible to circulating antibody. In addition, tumors of diverse histologic types and with different patterns of antigen expression on malignant cells may induce similar stromal cells expressing a common set of activation antigens; such stromal antigens could serve as shared targets for diverse tumor types. Finally, the activation program of tumor stromal cells may be more uniform and more stable than the phenotypes of malignant cells, which are notorious for their antigenic heterogeneity and phenotypic instability. Some of these principles have already been tested for the F19 cell surface glycoprotein of reactive stromal fibroblasts in epithelial cancers (3), and Welt *et al.* (9) have shown that an ^{131}I-labeled mAb specific for the F19 antigen can be used to image hepatic metastases in patients with colorectal carcinomas. Endosialin represents another potential target for stroma-directed tumor imaging and immunotherapy. Since the target antigen is presumed to be located on the luminal surface of tumor endothelial cells, it would represent the most accessible site in a solid tumor and a range of strategies are available to take therapeutic advantage of such a target. For example, humanized mAb FB5 or recombinant FB5 conjugates could be constructed that would induce endothelial cell damage and hemorrhagic necrosis of solid tumors. Alternatively, FB5 conjugates could be designed to initiate intratumoral blood clotting. Finally, radiolabeled mAb FB5 is rapidly internalized into endosialin-expressing cells (unpublished observations), opening up the possibility of selective delivery of cytotoxic agents with intracellular modes of action to tumor vascular endothelium.

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