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Cell Surface Molecules of Human Melanoma

Immunohistochemical Analysis of the gp57, G_{D3}, and mel-CSPG Antigenic Systems

Pilar Garin-Chesa, H. Richard Beresford, Alfredo Carrato-Mena, Herbert F. Oettgen, Lloyd J. Old, Myron R. Melamed, and Wolfgang J. Rettig

From the Department of Pathology and the Laboratory of Human Cancer Immunology, Memorial Sloan-Kettering Cancer Center, New York, New York

The rapidly expanding list of monoclonal antibodies (MAbs) to human cell surface antigens provides reagents to probe the biology of malignant melanoma and to develop new diagnostic and therapeutic approaches to this disease. The criteria used to select MAb-defined antigens as targets for passive immunotherapy or immunolocalization of melanoma include: 1) consistent antigen expression in melanomas, 2) restricted antigen distribution in normal tissues and nonmelanocytic tumors, and 3) cytotoxic activity of the MAb or MAb conjugates. The present study examined the tissue distribution of three prototype melanoma cell surface antigens, the M_r 57,000 glycoprotein (gp57) recognized by MAb A42, the G_{D3} ganglioside, and the mel-CSPG chondroitin sulfate proteoglycan. The avidin-biotin immunoperoxidase method was used to examine a large panel of normal tissues and over 150 malignant tumors. It was found that A42 has a highly restricted distribution in normal tissues and is expressed in subsets of melanomas and nonmelanocytic tumors. It was also found that G_{D3} and mel-CSPG are more widely distributed in normal tissues and among tumors than was thought previously. These immunohistochemical patterns provide an essential data base to evaluate the ongoing clinical trials employing MAbs to G_{D3} and mel-CSPG for the therapy and immunolocalization of melanomas, and they identify gp57 as a potential marker for subsets of normal and transformed melanocytic cells. (Am J Pathol 1989, 134:295–303)

Definition of the surface antigenic phenotypes of normal melanocytes, nevus cells and malignant melanomas has advanced rapidly through the use of monoclonal antibodies (MAbs) and the recent success in culturing normal and transformed melanocytic cells *in vitro*.[1–3] Three well-characterized cell surface antigens of cultured melanocytes and melanomas that belong to biochemically distinct classes of cell surface molecules are the ganglioside G_{D3} (detected by MAbs R24[4,5] and 4.2[6,7]), the chondroitin sulfate proteoglycan mel-CSPG (detected by MAbs B5,[8,9] 9.2.27,[10,11] 225.28S,[12] Mel-14,[13] 0-95-45,[14] AO122, and AO92,[9,15] and the M_r 57,000 glycoprotein (gp57) detected by MAb A42.[16] Based on previous immunohistochemical studies suggesting that G_{D3} and mel-CSPG have a highly restricted distribution in normal tissues and are specific markers for malignant melanoma and a few other tumor types,[8,12,17–20] unmodified or radiolabeled MAbs to these antigens have been used by a number of investigators for phase I clinical trials and radiolocalization studies in melanoma patients.[21–24] To date, however, no rigorous immunopathologic studies have been performed to evaluate the usefulness of G_{D3} and mel-CSPG, or of the recently described gp57 melanoma antigen[16] as immunologic tumor markers or as markers for normal cells derived from specific cell lineages. In this report, we describe the results of an extended immunohistochemical analysis using a wide range of normal and malignant tissues. We found that G_{D3} and mel-CSPG are consistently expressed in melanomas, but that they are much less restricted in normal tissues and in a diversified screening panel of nonmelanocytic tumors than previously thought; neither antigen is specifically a marker for neuroectoderm-derived cells or the transformed phenotype. In contrast, we found that the A42 antigen (gp57) has a highly restricted distribution in normal tissues. Consistent with our previous finding that A42 expression distinguishes subsets of cultured melanomas,[16] we observed that uncultured melanomas can also be divided into $A42^+$ and $A42^-$ subsets.

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Address reprint requests to Dr. Pilar Garin-Chesa, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

Table 1. *Monoclonal Antibodies to Cell Surface Antigens of Human Melanoma*

Monoclonal antibody				
Name	Isotype	Antigen	Gene/chromosome assignment	Reference
A42	(IgG2a)	M, 57,000 glycoprotein	*MSK29*/6q12-q15	16
R24	(IgG3)	Ganglioside G_{D3}		4, 5
B5	(IgG2a)	mel-CSPG*	*MSK16*/15	8, 9
A0122	(IgG1)	mel-CSPG	*MSK16*/15q13-qter	9, 15
A092	(IgG1)	mel-CSPG	*MSK16*/15	9, 15
9.2.27	(IgG2a)	mel-CSPG	*MSK16*/15	10, 11
225.28S	(IgG2a)	mel-CSPG	*MSK16*/15	12

* mel-CSPG, serologically defined chondroitin sulfate proteoglycan of human melanoma cells;[8,9,11] MAbs B5 and 9.2.27 recognize the core glycoprotein of mel-CSPG.[9,11] Gene designations and chromosomal assignments have been described previously.[9,16,36]

Because A42 expression *in vitro* is correlated with specific cell-matrix interactions and the adhesive properties of neuroectodermal tumor cells,[16] it is tempting to speculate that the $A42^+$ and $A42^-$ tumor phenotypes *in vivo* also indicate differences in the expression of cell-matrix interaction molecules.

Materials and Methods

Monoclonal Antibodies

MAbs R24, B5, AO122, AO92, and A42 were produced in the Laboratory of Human Cancer Immunology at Memorial Sloan-Kettering Cancer Center; MAb 9.2.27 was kindly provided by Dr. R. A. Reisfeld, Scripps Clinic, La Jolla, CA, and MAb 225.28S was kindly provided by Dr. S. Ferrone, New York Medical College, Valhalla, NY (Table 1). MAbs were routinely used at an Ig concentration of 10 μg/mL, but identical results were obtained at 5 and 20 μg/mL.

Tissues and Immunohistochemical Procedures

Normal and tumor tissues were obtained at autopsy or from surgical specimens received in the Department of Pathology at Memorial Hospital. For normal tissues, specimens from several different individuals were tested in parallel to define specific antigenic patterns. For all tumor specimens, diagnoses were established by routine pathologic evaluation in the Department of Pathology, Memorial Hospital. Fresh tissues were embedded in OCT compound (Miles, Naperville, IL), frozen in isopentane precooled in liquid nitrogen, and stored at −70 C until used. Sections of 5 μ thickness were cut, mounted on gelatin-coated slides, air-dried, and used unfixed or fixed in cold acetone (4 C, 10 minutes) or formaldehyde (3.7% in phosphate-buffered saline [PBS], 10 minutes at RT). The avidin-biotin immunoperoxidase procedure was performed as described.[25] Negative control experiments were performed with unrelated, isotype-matched mouse Ig (IgG1 and IgG2a, Becton-Dickinson, Mountain View, CA; IgG3, Coulter, Hialeah, FL) and did not produce any staining. Positive controls included MAbs to several human cell surface and intracellular antigens with known tissue distribution: MAbs MC25, AE1, 142-24E05, and K117.[25-28] In preliminary experiments, MAbs A42, R24, and B5 were tested in parallel on unfixed, formaldehyde-fixed, and acetone-fixed frozen sections of an antigen-positive melanoma, normal skin, lymph node, and brain, and identical staining patterns (see Results) were observed in the unfixed and fixed sections. Acetone fixation was used routinely for the extended analysis of normal and neoplastic tissues because it produced the best preservation of tissue morphology, as illustrated in Figs. 1–3.

Cytofluorometric Analysis

Peripheral blood lymphocytes and spleen cells were isolated on a Ficoll-Hypaque gradient, washed with PBS/BSA, incubated with MAb for 1 hour at 4 C, washed twice with PBS/BSA, incubated with FITC-conjugated rabbit anti-mouse Ig (Vector Laboratories, Burlingame, CA), and washed twice with PBS/BSA. After fixation in 50% ethanol, cells were analyzed for immunofluorescence staining on a System 30-L cytofluorograph (Ortho Diagnostics, Westwood, MA).[29]

Results

The MAbs used in this study and some of the pertinent biochemical and genetic data that define the respective cell surface antigenic systems are presented in Table 1. The following is a summary of the most important immunohistochemical results obtained with three prototype MAbs, A42 (anti-gp57), R24 (anti-G_{D3}), and B5 (anti-mel-CSPG).

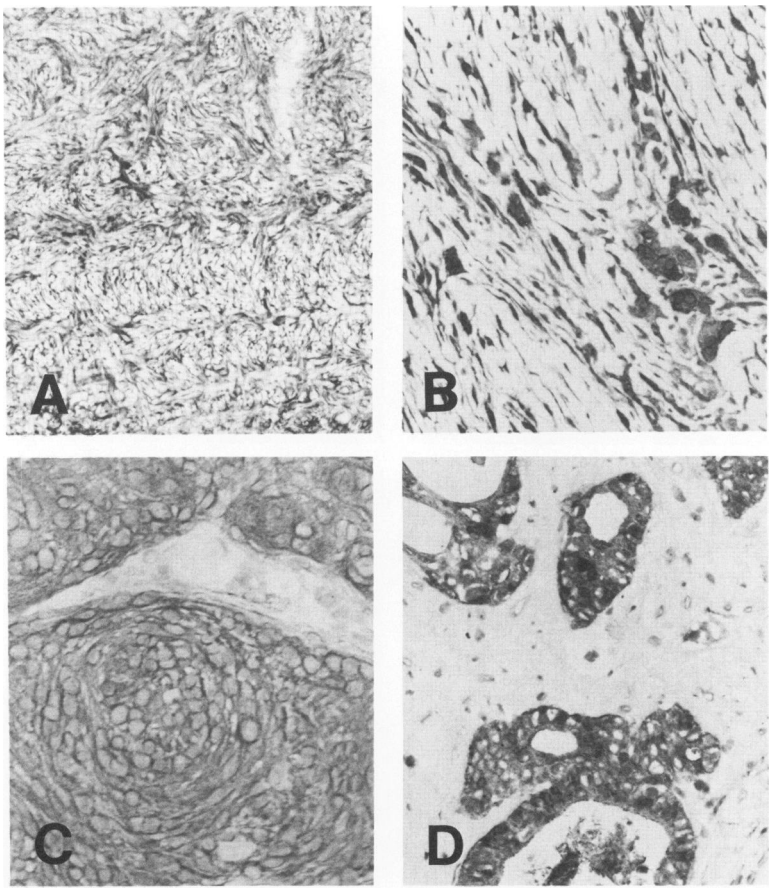

Figure 1. *Immunoperoxidase staining of normal and neoplastic human tissues with MAb A42.* **A:** *Ovary. Note strong reactivity of connective tissue cells.* **B:** *Malignant melanoma.* **C:** *Meningioma.* **D:** *Colonic adenocarcinoma; tumor cells strongly labeled but stroma unreactive. Acetone-fixed frozen tissue sections were tested by the avidin-biotin immunoperoxidase procedure and counterstained with hematoxylin. Original magnification, ×250.*

A42 Antigenic System

In avidin-biotin immunoperoxidase assays with normal tissues, MAb A42 showed strong reactivity only with capillary endothelial cells and with stromal fibroblasts in the uterus, testis, and ovary (Figure 1A). In contrast, endothelial cells of veins and arteries, fibroblasts in organs other than ovary, testis, and uterus, and most of the other normal tissues tested (Table 2) did not show any A42 staining; in the skin, no A42 staining was detected in either keratinocytes or melanocytes. Visceral smooth muscle and germinal centers in the white pulp of the spleen and in lymph node follicles showed weak and inconsistent A42 staining, suggesting low levels of antigen expression. Peripheral blood lymphocytes and cell suspensions prepared from normal spleen were tested by the indirect immunofluorescence procedure and cytofluorometry and reproducible but weak labeling was observed for 2 to 8% of the cells.

In the tumor panel (Table 3), A42 expression was found in 5 of 15 metastatic melanomas (Figure 1B), in meningiomas (Figure 1C), astrocytomas, and certain sarcomas, epithelial cancers (Figure 1D), and Hodgkin's lymphomas. In some $A42^+$ tumors, antigen expression was restricted to a subset of tumor cells (25–75% of cells), indicating antigenic heterogeneity within these neoplasms. The five nevi included in this study were $A42^-$ (Table 3).

R24 Antigenic System

Avidin-biotin immunoperoxidase assays with MAb R24 identied antigen-positive cells in most normal tissues tested (Table 2). R24 reactivity was seen with diverse cell types, ranging from central nervous system neurons and astrocytes to adrenal medullary cells, keratinocytes, T lymphocytes (Figure 2A), mammary gland epithelium, visceral smooth muscle, fibroblasts, chondrocytes, bronchial epithelium, renal tubules (Figure 2B), pancreatic acinar cells, thyroid epithelium, prostate epithelium, and Leydig cells in the testis. The strong R24 staining seen with keratinocytes in normal skin precluded the assessment of G_{D3} expression in skin melanocytes. Despite its widespread distribution, G_{D3} is not ubiquitously expressed in normal tissues, and skeletal muscle, vascular smooth muscle, hepatocytes, and peripheral nerves are examples of $R24^-$ tissues.

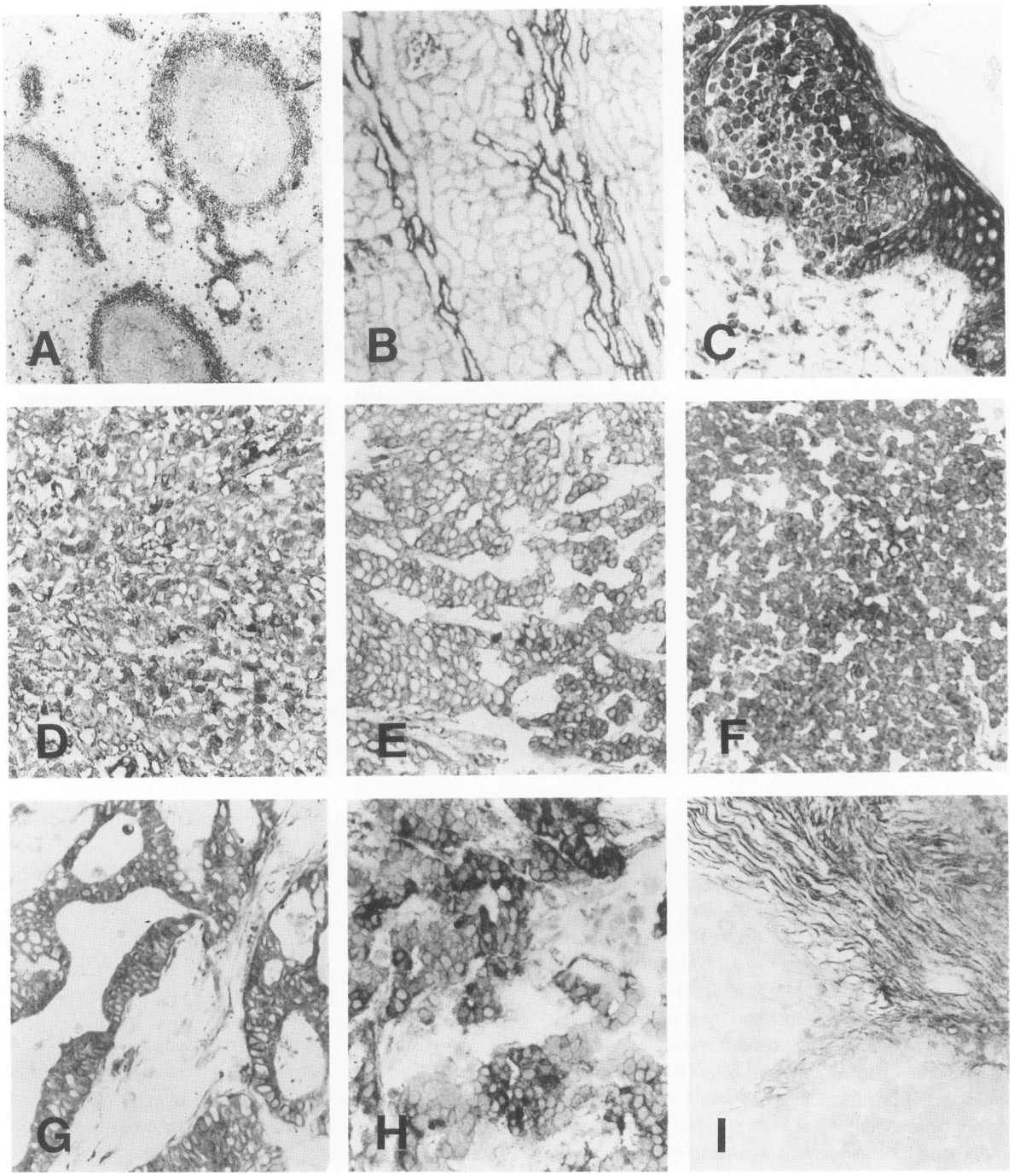

Figure 2. *Immunoperoxidase staining of normal and neoplastic human tissues with MAb R24.* **A:** *Spleen, reactivity of parafollicular cells in white pulp and scattered cells in red pulp.* **B:** *Kidney, reactivity of straight portions of renal tubules and mesangium in glomeruli.* **C:** *Compound nevus, reactivity of nevus cells, keratinocytes (all epidermal layers) and dermal fibroblasts.* **D:** *Malignant melanoma.* **E:** *Basal cell carcinoma, reactivity of tumor cells but not stroma.* **F:** *Merkel cell tumor.* **G:** *Colonic adenocarcinoma.* **H:** *Infiltrating duct carcinoma of the breast, heterogeneity in antigen expression among tumor cells, stroma unreactive.* **I:** *Ovarian carcinoma, tumor cells (lower left) unreactive but adjacent stroma (upper right) reactive for R24. Acetone-fixed frozen tissue sections were tested by the avidin-biotin immunoperoxidase procedure and counterstained with hematoxylin. Original magnification, ×40 (A, B), ×250 (C–I).*

In the tumor panel, MAb R24 reacts with a large proportion of melanomas and nonmelanocytic neoplasms (Table 3). Fourteen of 15 melanomas and the 5 nevi tested showed strong antigen expression (Figure 2C, D). A large number of nonmelanocytic tumors, including astrocytomas, neuroblastomas, basal and squamous cell

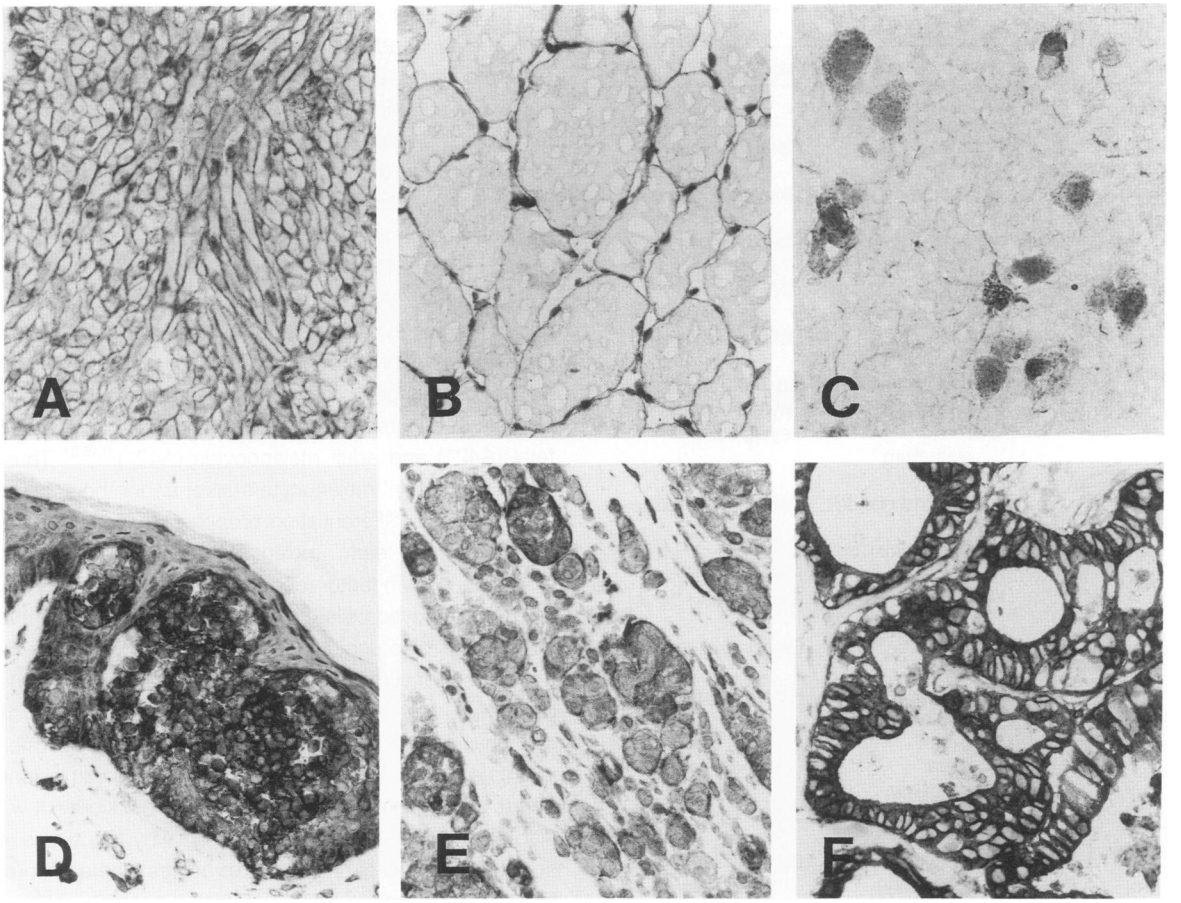

Figure 3. *Immunoperoxidase staining of normal and neoplastic human tissues with MAb B5.* **A:** *Visceral smooth muscle of urinary bladder, strong membrane staining.* **B:** *Skeletal muscle, strong membrane staining.* **C:** *Cerebral cortex, reactivity of neurons and astrocytes.* **D:** *Compound nevus, strong B5 reactivity of nevus cells and weak staining of keratinocytes.* **E:** *Malignant melanoma.* **F:** *Colonic adenocarcinoma. Acetone-fixed frozen tissue sections were tested by the avidin-biotin immunoperoxidase procedure and counterstained with hematoxylin. Original magnification, ×250 (A, B, D, E), ×400 (C).*

carcinomas of the skin (Figure 2E), a Merkel cell tumor (Figure 2F), sarcomas, teratocarcinomas, colon and breast carcinomas (Figure 2G, H), and Hodgkin's lymphomas were also strongly $R24^+$. In some $R24^+$ tumors, antigen expression was restricted to a subset of tumor cells, but in contrast to one previous report,[8] we found that antigenic heterogeneity is not a common feature in melanomas. Finally, in some $R24^-$ tumors, strong R24 staining was seen in the stromal fibroblasts surrounding $R24^-$ tumor cells (Figure 2I).

Mel-CSPG Antigenic System

Avidin-biotin immunoperoxidase assays with MAb B5, which recognizes the core glycoprotein of the mel-CSPG proteoglycan antigen,[8,9] showed antigen expression in a wide range of normal cell types (Table 2), including vascular and visceral smooth muscle (Figure 3A), skeletal muscle (Figure 3B), central nervous system neurons and astrocytes (Figure 3C), Schwann cells, perineural cells, intestinal mucosa, and bronchial epithelial cells. In the tumor panel (Table 3), MAb B5 reacted with 14 of 17 melanomas and with all 4 nevi tested (Figure 3D, E), with meningiomas, astrocytomas, Schwannomas, neuroblastomas, and a proportion of sarcomas and epithelial cancers (Figure 3F).

We and other investigators have shown in studies with cultured tumor cells that MAbs, B5, 9.2.27, 225.28S, AO122, and AO92 define several distinct epitopes on the mel-CSPG proteoglycan.[9,30] To examine the possibility that these epitopes are differentially expressed *in vivo*, we tested several normal and tumor tissues with four anti-mel-CSPG MAbs directed against unique epitopes,[9] namely MAbs B5, 9.2.27, 225.28S, and AO122. Identical staining patterns were seen for these MAbs in all the tissues tested, including melanomas, sarcomas, skeletal muscle, vascular and visceral smooth muscle; among the four MAbs tested, AO122 consistently gave the weakest staining results whereas the remaining MAbs gave similar

Table 2. *Normal Tissue Distribution of MAb-Defined Antigens A42, R24, and B5* *

Antigen-expressing cells or tissues		
A42	R24	B5
Capillary endothelium	Keratinocytes	Keratinocytes
Fibroblasts (subset)	Skin appendages	Skin appendages
Smooth muscle (weak)	Mammary gland acini, ducts	Mammary gland acini, ducts
Lymphocytes (weak)	CNS neurons, astrocytes	CNS neurons, astrocytes
	Adrenal cortex, medulla	Schwann cells
	Thyroid epithelium	Adrenal cortex
	Leydig cells	Thyroid epithelium
	Coelomic epithelium (ovary)	Leydig cells
	Endometrial glands	Seminiferous tubules (weak)
	Prostate epithelium	Bile duct epithelium
	Kidney tubules †	Pancreatic acini
	Mesangium	Parotid gland ducts, acini
	Pancreatic acini, islets	Bronchial epithelium
	Esophageal epithelium	Kidney tubules †
	Lung septi	Intestinal mucosa
	Bronchial epithelium	Pneumocytes
	Lymphocytes (subset)	Skeletal muscle
	Thymocytes (medullary)	Capillary endothelium
	Smooth muscle ‡	Smooth muscle
	Chondrocytes	Chondrocytes
	Fibroblasts §	Myoepithelial cells
		Fibroblasts (subset)

* Adult tissues tested: Skin (palm, face, back, breast, leg), mammary gland, brain (cerebral cortex, mesencephalon, cerebellum), peripheral nerves, adrenal gland, thyroid gland, testis, ovary, uterus, prc state, kidney, bladder, liver, pancreas, parotid gland, esophagus, stomach, small and large intestine, lung, bronchus, spleen, lymph node, thymus (newborn), skeletal muscle.

† MAb R24 reacts with straight portions of renal tubules, macula densa, mesangium, and connective tissue of the renal medulla; MAb B5 reacts with collecting tubules.

‡ MAb R24 reacts with visceral smooth muscle in bladder, uterus, esophagus, stomach, and small and large intestine (muscularis mucosae).

§ MAb R24 reacts strongly with fibroblasts in several organs, including dermis, liver (portal spaces), spleen, lymph node, small and large intestine (lamina propria), bladder, kidney (medulla), and prostate.

results. Thus, there is no evidence that the distinct MAb-defined epitopes of the mel-CSPG proteoglycan are differentially expressed in human tissues.

Discussion

We have used the avidin-biotin immunoperoxidase procedure in frozen sections of normal and neoplastic human tissues to define the distribution of gp57 (defined by MAb A42), a novel cell surface differentiation marker of cultured melanocytes and melanomas.[16] In addition, we have compared the tissue distribution of gp57 with the distribution of G_{D3} and mel-CSPG, two biochemically well-characterized cell surface antigens of melanomas.[4–15] Our results show that the A42 antigen has a highly restricted distribution in normal tissues, being detected only in capillary endothelial cells and certain fibroblasts, and, at low levels, in a small subset of lymphocytes and in visceral smooth muscle. A42 is not expressed in normal skin melanocytes or in nevus cells, but 5 of 15 melanomas tested showed A42 expression. We have shown previously that A42 is one of a group of cell surface antigens that define subsets of cultured melanomas and distinguish cultured fetal ($A42^+$) and adult melanocytes ($A42^-$).[2,16,31] Thus, A42 is an "early" melanocyte marker under the scheme of melanocyte differentiation proposed by Houghton et al.[2] While other "early" melanocyte markers such as the epidermal growth factor receptor[31] define subsets of cultured melanocytic cells only,[8,31] our results suggest that MAb A42 is also useful for studying uncultured melanomas and normal melanocyte differentiation *in vivo*. In the present study, no obvious differences in biologic or clinical behavior were found between the $A42^+$ and $A42^-$ melanoma subsets, but a larger series of melanocytic lesions needs to be examined to evaluated this possibility. It will also be of great interest to determine why fibroblasts in some organs are strong A42 expressors whereas fibroblasts in other organs completely lack detectable antigen. Similar antigenic differences in subsets of normal fibroblasts (although with different organ patterns) were described recently for the F19 and G171 antigens,[32] indicating phenotypic and possibly functional heterogeneity among various types of normal fibroblasts.

Aside from melanomas, certain neural, epithelial, and mesenchymal tumors express A42. Because many of the corresponding normal tissues are $A42^-$, antigen expression in these cases may relate to the specific growth properties of the tumor rather than tissue derivation. In support of this notion, we have shown that A42 is newly expressed on substrate-adherent variants derived from several nonadherent $A42^-$ neuroblastoma and Ewing's sarcoma cell lines.[16,33] Furthermore, some $A42^-$ neural cells are induced to express the antigen when grown on fibronectin or endothelial cell-derived matrix instead of tissue culture plastic.[16] Because altered interactions between tumor cells and extracellular matrix are important determinants of tumor invasiveness and metastatic potential,[34] it is tempting to speculate that A42 is one of the cell-matrix interaction molecules involved in these processes.[16]

Our immunohistochemical findings with MAbs R24 and B5 differ from those reported previously for the same MAbs,[8,17] or for other MAbs recognizing the same antigens.[12,18–20] For example, with respect to G_{D3}, we found

Table 3. *Distribution of MAb-Defined Cell Surface Antigens in Human Tumors**

Tumor type	Antigen-expressing tumors		
	A42	R24	B5
Neuroectodermal tumors			
Melanoma	●●●◒◒○○○○○ ○○○○○	●●●●●●●●●● ●●●●○	●●●●●●●●●● ●●●●○○○
Nevus†	○○○○○	●●●●	●●●●◒
Meningioma	●●◒○	◒○	●●
Astrocytoma	●●●●◍○○○	●●●●●●●●	○○○
Neuroblastoma‡	○○	●●◒◒	●●○
Neuroendocrine carcinoma§	●○○○○○○	●◒○○○○	●○○○○○○○
Schwannoma	●○	●○	●●
Mesenchymal tumors			
Synovial sarcoma	◒◍◍○	◍○	●●●○
Leiomyosarcoma	○○	○○	●●○
Embryonal rhabdomyosarcoma	○	●●	●◒
Ewing's sarcoma	○○○	●●	○○○
Osteogenic sarcoma	◍○	●○	●●
Rhabdomyosarcoma	●○	●●	●○
Chondrosarcoma	○	●	●
Other sarcomas‖	●◍○○○○○	●●◒	●●●◒◍○
Epithelial cancers			
Breast carcinoma	○○○○○○○○○	●◒◒◒○○○○○	●●●◒◍◍○○○○
Squamous cell carcinoma	○○	●◍	●
Basal cell carcinoma	○○○	●●●	●
Esophageal carcinoma	○	NT	○
Gastric carcinoma	●○○○○	◍○○○	●○○○○
Colon carcinoma	●◒◒◒◍◍○○○○ ○○○○○○○○	●●◒◒◒◒○○○○ ○○○○○○	●●●◒○
Ovarian carcinoma	◒◍◍○○○○	◒○○○○	●●◍◍◍○○○
Endometrial carcinoma	●◒◍○	◒○	◍◍○
Teratocarcinoma	◒○○○	●●	●●○○
Prostate carcinoma	◒○○	◒○○	◒○○
Bladder carcinoma	●◍○○○○	○○○	●◍◍◍◍○
Renal carcinoma	●○○○○	◒○○○	○○○○
Lung carcinoma	○○	○○	●○
Mesothelioma	○	●	●○
Thyroid carcinoma	●	NT	●
Adrenal cortical carcinoma	NT	●	●
Lymphoid malignancies			
Hodgkin's lymphoma	◍◍◍○○○	●●●◒◒◒	○○○○○
Non-Hodgkin's lymphoma	○○○○○○○○	○○○○○	○○○○○○

* Acetone-fixed frozen tissue sections of over 150 tumors were tested by the avidin-biotin immunoperoxidase procedure. Staining results are indicated as follows: ●, strong reactivity; ◍, weak reactivity; ○, antigen-negative; ◒, heterogeneity in antigen expression with 25–75% of tumor cells showing immunostaining. Each symbol in the body of the table represents typing results with a single tumor specimen; symbols in corresponding positions under different MAbs do not necessarily represent typing results with the same tumor specimen. NT, not tested.

† Two compound, one junctional, one intradermal, one giant cell nevus.

‡ One neuroblastoma and three ganglioneuroblastomas; MAb R24 showed preferential reactivity with ganglion cells and MAb B5 with Schwannlike cells.

§ Two pheochromocytomas, two insulinomas, one gastrinoma, one glucagonoma, one Merkel cell tumor, one small cell lung cancer.

‖ Two alveolar soft part sarcomas, two undifferentiated sarcomas, one angiosarcoma, one spindle cell sarcoma, and one hemangiosarcoma.

that MAb R24 reacts strongly with normal skin keratinocytes (rather than melanocytes[8]), connective tissue in many normal organs (eg, dermis, portal spaces of the liver, lung, intestinal wall, kidney), smooth muscle, chondrocytes, a range of epithelial cell types, medullary thymocytes and T lymphocytes; many nonmelanocytic tumors also typed R24$^+$. Similarly, we found that MAbs to mel-CSPG react with many normal and malignant tissues of neural, mesenchymal, and epithelial origin previously thought to be mel-CSPG$^-$.[8,12,18–20] Among the mel-CSPG$^+$ tissues identified in this study are normal vascular and visceral smooth muscle, skeletal muscle, and several muscle-derived tumors. Thus, it is of interest that we have found in a parallel study (Rettig WJ, Garin-Chesa P, Triche TJ, in preparation) that five of five rhabdomyosarcoma cell lines tested also express cell surface mel-CSPG, at levels similar to those seen with melanoma cell lines. In addition, the mel-CSPG antigens expressed in these rhabdomyosarcoma cells are indistinguishable from the corresponding melanoma antigens when tested by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Most likely, the differences between our findings and those of previous reports on G_{D3} and mel-CSPG tissue distribution reflect the use of improved and more consistent immunohistochemical techniques in the present study. In our experience, three factors are important to obtain optimal immunohistochemical results: good tissue

preservation (ie, rapid processing of tissues and use of adequate freezing procedures to avoid freezing artifacts), selection of an appropriate fixative, and use of a sensitive immunodetection system (eg, avidin-biotin immunoperoxidase procedure). In addition to permitting more sensitive detection of antigen, the immunohistochemical procedures used here also yielded more consistent typing results than were obtained in some earlier studies. This fact is illustrated by our finding of uniform G_{D3} expression in a majority of melanomas, which contrasts with the high degree of antigenic heterogeneity reported previously.[8]

Application of MAbs to cell surface antigens for immunolocalization or passive immunotherapy of human tumors is still at an early stage of clinical experimentation, and no general criteria have been established that identify suitable target antigens or biologically effective MAbs. Thus, it is of obvious interest to correlate the biodistribution and biologic activity of those MAbs that have been selected for use in melanoma patients[21-24] with the corresponding patterns of antigen expression established by immunohistochemical analysis of normal and tumor tissues. Although restricted patterns of expression in normal tissues, such as that defined here for the A42 antigen, or cytotoxic activity *in vitro* may help to identify potential targets for *in vivo* application of MAbs, it is not yet known whether widespread distribution of an antigen in normal tissues (as described here for G_{D3} and mel-CSPG) implies that the antigen cannot be used clinically. In this respect, it is intriguing that the anti-G_{D3} MAb R24, which triggers complement-mediated cytotoxicity and antibody-dependent cellular cytotoxicity *in vitro,* is one of the few examples of a MAb producing antitumor effects in melanoma patients.[22,24] It is also noteworthy, however, that we have detected G_{D3} immunohistochemically in T lymphocytes and that Hersey et al have shown that MAbs to G_{D3} can potentiate T lymphocyte responses to mitogenic stimuli,[35] raising the possibility of an immunodulatory action rather than specific immunotoxic effect of MAb R24 in melanoma patients.

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