EXTRACELLULAR MATRIX-MODULATED EXPRESSION OF HUMAN CELL SURFACE GLYCOPROTEINS A42 AND J143

Intrinsic and Extrinsic Signals Determine Antigenic Phenotype

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Extracellular matrix (ECM)[1] plays an important regulatory role in cellular growth, migration, and differentiation (1–4). Pathologic processes such as tumor cell invasion and metastasis are also determined by cellular interactions with ECM (5, 6). Biochemical studies have identified collagens, fibronectin, laminin, proteoglycans, and several other proteins as major ECM components (1–3), and have shown that ECM composition varies between different normal and tumor tissues. The complexity and heterogeneity of ECM composition have hampered the molecular analysis of ECM–cell interactions. However, a range of phenotypic changes has been described for cultured cells after transfer from plastic surfaces to substrates coated with native ECM (7, 8) or with purified ECM components; ECM-induced phenotypic changes include enhanced substrate adhesiveness, cell spreading and migration, changes in cell morphology and proliferative activity, and expression of differentiated cellular functions (1–4). Some of these effects, e.g., increased substrate adhesion, may result directly from the binding of specialized cell surface structures to ECM molecules. Others are likely mediated by an active cellular response triggered by the interaction of ECM with cell surface receptors. Thus, ECM-derived signals (9) may activate a cascade of molecular changes within the cell and on the cell surface that account for the pleiotropic effects observed with ECM.

To investigate molecular changes on the cell surface that are controlled by ECM–cell interactions, we have compared patterns of cell surface antigen expression on cells cultured on ECM-coated substrates with those of cells cultured on plastic surfaces. In a previous study (10), we described the first example of a human cell surface antigen, a chondroitin sulfate proteoglycan designated as mel-CSPG, that is specifically induced by ECM. These earlier observations were made through analysis of a series of mouse–human neuroblastoma hybrids that are uniquely responsive to ECM. They show a rapid change in substrate adhesiveness, cell spreading, and cell morphology when transferred from plastic

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[1] *Abbreviations used in this paper:* ECM, extracellular matrix; BCE-ECM, ECM produced by bovine corneal endothelial cells; EGF-R, epidermal growth factor receptor; EHS-BM, reconstituted basement membrane gel from the Engelbrecht-Holm-Swarm tumor; FN, fibronectin; mel-CSPG, serologically defined chondroitin sulfate proteolgycan.

surfaces to substrates coated with ECM secreted by bovine corneal endothelial cells (BCE-ECM). Concomitantly, hybrids that have retained the mel-CSPG-encoding human chromosome 15 show induction of mel-CSPG surface expression (10). Other cell types, which do not show the characteristic changes in cell morphology and growth behavior when cultured on ECM, also lack ECM-inducible mel-CSPG expression (10). In the present study, we have exploited the neuroblastoma hybrid system to identify two additional human cell surface glycoproteins modulated by BCE-ECM and purified ECM components. Our findings indicate that ECM-derived signals play a regulatory role analogous to that played by cytokines and other soluble factors in determining differentiated cellular phenotypes and patterns of surface antigen expression.

Materials and Methods

Cell Lines. Human tumor cell lines were taken from the cell line collection maintained at Sloan-Kettering Institute; neuroblastoma cell lines were obtained from Dr. J. L. Biedler (Sloan-Kettering Institute), IARC-EW1 cells from Dr. G. Lenoir (International Association for Research on Cancer, Lyon, France), and RD-ES and IMR-32 from the American Type Culture Collection (Rockville, MD). Cell lines were grown in DME or DME/Ham's F12 (1:1) supplemented with 2 mM glutamine, 1% nonessential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, and 7.5% FCS; 15% FCS was supplemented for neuroblastoma cell lines. Normal skin melanocyte cultures (11) were provided by Dr. A. N. Houghton, and cultures of normal kidney epithelial cells by Dr. N. Bander (Sloan-Kettering Institute). Rodent–human somatic cell hybrids were generated as described (10, 12–15) and human chromosomes retained in hybrid cells were determined by karyotype analysis and typing for human isozymes and cell surface antigens (10, 12–16).

Monoclonal Antibodies. mAb A42 (IgG2b) was derived from a (BALB/c × C57BL/6)F_1 mouse immunized with SK-OV-3 cells following standard fusion, hybrid selection, and cloning procedures. mAb J143, AO10, and AO122 have been described (13, 17) and the W6/32 clone was obtained from the American Type Culture Collection. A single batch of each mAb was used throughout this study.

Serologic Procedures. mAb-mediated erythrocyte rosetting assays for detection of cell surface antigens on cultured cells have been described (17–19). Briefly, cells grown in 60-well MicroWell plates (Nunc, Roskilde, Denmark) for 24–48 h were incubated with serial dilutions of mAb for 1 h. After repeated washes, cells were incubated for 45 min with indicator cells, prepared by conjugating human type O erythrocytes with purified rabbit anti–mouse Ig (Dako Corp., Santa Barbara, CA) using chromium chloride. Plates were then washed and scored microscopically by determining the proportion of target cells showing attachment of indicator cells (erythrocyte rosetting). All cell lines were tested with serial dilutions of mAb (starting dilution 1:1,000 *nu/nu* serum or ascites) to determine highest dilutions giving rosette formation (titration endpoints).

Immunochemical Procedures. Cells were labeled with [^{35}S]methionine or [^{3}H]glucosamine (New England Nuclear, Boston, MA) and extracted with NP-40 buffer. For some experiments, lysates were separated after labeling on a Con A–Sepharose column. Alternatively, cell membranes were ^{125}I-labeled by the chloramine-T method (17). Immunoprecipitation experiments, SDS-PAGE, and fluorography were carried out as described (17).

Cell Culture on ECM-coated Substrates. Plastic dishes and 60-well MicroWell plates coated with BCE-ECM (7), were purchased from International Biotechnologies (Jerusalem, Israel). Fibronectin (FN)-coated substrates were prepared by incubating plastic dishes and 60-well MicroWell plates (Nunc) with purified human plasma FN (20) at 50 μg/ml for 1 h at 37°C; FN was kindly provided by Dr. J. Sorvillo (Sloan-Kettering Institute). Reconstituted basement membrane gel from the Engelbrecht-Holm-Swarm tumor (EHS-BM; 8) was purchased from Collaborative Research (Matrigel, Lexington, MA) and used according to the manufacturer's instructions for cell culture on a thin layer of Matrigel. For

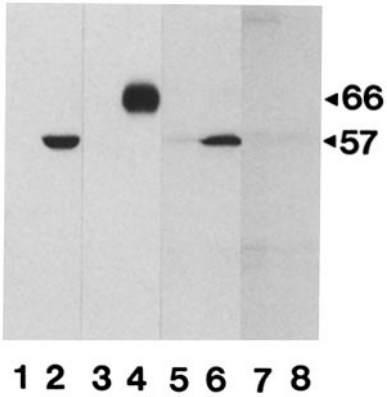

FIGURE 1. Fluorogram of immunoprecipitates obtained with mAb A42 from NP-40 lysates of metabolically labeled astrocytoma cells and separated by SDS-PAGE. SK-MG-10 (lanes *1–4*), U251MG (lanes *5* and *6*) and SK-GS-1 cells (lanes *7* and *8*) were labeled with [^{3}H]-glucosamine, extracted with NP-40-containing buffer, and aliquots with equal ^{3}H counts per minute, as determined after precipitation of cellular proteins from cell lysates with 10% TCA, were used in experiments with mAb A42 (lanes *1*, *3*, *5*, and *7*) or unrelated control mAb (lanes *2*, *4*, *6*, and *8*). Protein separation by SDS-PAGE was carried out under reducing conditions (extraction buffer containing 12 mg/ml DTT) in all experiments except for experiment shown in lanes *3* and *4* (extraction buffer containing 14 mg/ml iodoacetamide). Film exposure times for fluorography were 7 d for experiments shown in lanes 1–6 and 21 d for experiment shown in lanes *7* and *8*. M_r of immunoprecipitated components ($\times 10^{-3}$) are indicated on the right.

serologic assays in 60-well MicroWell plates coated with BCE-ECM, FN, or EHS-BM, target cells were seeded at 300 cells/well and cultured for 24–48 h at 37°C in a 5% CO_2 humidified air atmosphere before erythrocyte rosetting assays.

Results

A42 Surface Expression on Human Cells. mAb A42 recognizes a cell surface glycoprotein that migrates with an M_r of 57,000 on SDS-polyacrylamide gels in the presence of reducing agents, and at M_r 66,000 under nonreducing conditions (Figs. 1 and 2*A*). The 57,000 A42 glycoprotein was detected in immunoprecipitation experiments with a number of cell types belonging to different cell lineages, including astrocytomas (SK-MG-1, SK-MG-2, SK-MG-10, SK-MG-15, U251MG), melanomas (SK-MEL-28, SK-MEL-37), simian virus 40 (SV40)-transformed WI-38 fibroblasts, A10 epithelial cancer cells, and normal kidney epithelial cells. Identical components were precipitated with mAb A42 using three different labeling procedures: metabolic incorporation of [^{3}H]glucosamine or [^{35}S]methionine, and ^{125}I-labeling of Con A–bound cell membrane fractions.

Serial dilutions of mAb A42 were tested by erythrocyte rosetting assays for surface reactivity with a large panel of cultured human cell types (Table I), and titration endpoints were determined in triplicate for all antigen-positive cell lines, with variation between individual test results not exceeding a single fivefold titration step. Differences in titration endpoints between A42$^+$ cell lines closely paralleled the results of immunoprecipitation experiments as illustrated in Fig. 1 for three astrocytoma cell lines. Consistent with previous findings (21, 22) that had shown that erythrocyte rosetting assays are highly sensitive for detection of small numbers of surface antigen molecules per cell, some cell lines were found

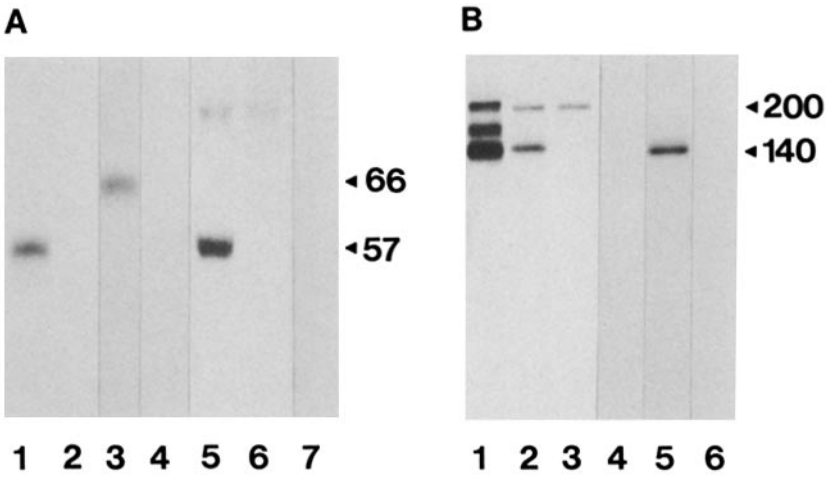

FIGURE 2. Fluorogram of immunoprecipitates obtained with mAbs to cell surface antigens A42 and J143 and separated by SDS-PAGE. (*A*) Extracts of [^{3}H]glucosamine-labeled SK-MEL-37 (lanes *1–3*), SK-MEL-23 (lane *4*), LA1–5s (lanes *5* and *6*) and LA-N-1n cells (lane *7*) were tested with mAb A42 (lanes *1*, *3–5*, and *7*) or with unrelated control mAb (lanes *2* and *6*). (*B*) Extracts of [^{3}H]glucosamine-labeled LA1–5s (lanes *1–3*), LA-N-1n (*lane 4*), and EW1s cells (lanes *5* and *6*) were tested with mAb AJ2 (lane *1*), mAb J143 (lanes *2*, *4*, and *5*), or unrelated control mAb (lanes *3* and *6*); all immunoprecipitates shown were separated under reducing conditions except for experiment shown in *A*, lane *3*, which was carried out under nonreducing conditions.

to type A42$^+$ in rosetting assays but lack detectable antigen by immunoprecipitation tests (compare results with SK-GS-1 cells in Table I and Fig. 1). A highly characteristic pattern of antigen expression was observed with cells that share a common neuroectodermal origin. Thus, A42 is strongly expressed on fetal and newborn skin melanocytes but is not detected on adult skin melanocytes. Similarly, a subset of melanoma cell lines (6 of 13 lines tested) are strongly reactive with mAb A42, whereas the remaining seven melanomas are antigen-negative (Table I). These results for cell surface expression were confirmed by immunoprecipitation tests (Fig. 2*A*) showing that the A42 glycoprotein is detectable in SK-MEL-28 and SK-MEL-37 but not SK-MEL-23 melanoma cell extracts. Comparison of the A42$^+$ and A42$^-$ melanoma subsets for expression of known differentiation markers of melanocytic cells (Table II) revealed a close correlation between A42 phenotype and stage of cellular differentiation, with A42$^+$ melanomas resembling fetal and newborn skin melanocytes and A42$^-$ melanomas resembling adult skin melanocytes. Thus, A42$^+$ melanomas typically express HLA-DR and epidermal growth factor receptors (EGF-R), but not the M144 and TA99 differentiation antigens of mature melanocytic cells, have an epithelial morphology and lack pigmentation. In contrast, A42$^-$ melanomas are typically pigmented, have a spindle-shaped or polydendritic morphology, and express M144 and TA99 but not HLA-DR and EGF-R (Table II).

Cell surface expression of A42 also distinguishes other cell types that share a common neuroectodermal origin with melanocytic cells. Astrocytomas are A42$^+$ whereas neuroblastomas and retinoblastomas are A42$^-$ (Table I). Ewing's sarcoma cells and normal and transformed cells of lymphoid or myeloid origin are

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TABLE I

Reactivity of mAb A42 with Cell Surface Antigens of Cultured Human Cells

Target cells		A42 reactivity (titer × 10^{-3})
Cell type	Designation	
Normal cells		
Skin melanocytes		
Adult	Short-term culture	—
Fetal, newborn	Short-term culture	625
Fibroblasts*	Short-term culture	625
Kidney epithelial cells	Short-term culture	3,125
Lymphocytes‡		—
Transformed cells		
Melanoma	SK-MEL-13, -28, -37, -131	3,125
	SK-MEL-31, -173	625
	SK-MEL-19, -23, -26, -29	—
	SK-MEL-127, -153, MeWo	—
Neuroblastoma	SK-N-BE(1), -BE(2), NAP	—
	CHP234, IMR-32, LA-N-1	—
	SMS-KAN, -SAN, -KCN	—
Retinoblastoma	Y79, Weri	—
Astrocytoma	SK-MG-10, -15	15,625
	SK-MG-1, -2, -3, -17, U251MG	3,125
	SK-GS-1	25
Ewing's sarcoma	IARC-EW1, RD-ES	—
T cell leukemia	MOLT-4, T45, CCRF-CEM, -HSB2	—
B cell leukemia	SK-LY-16, -18, Daudi, Raji	—
Myeloid leukemia	HL-60, K562	—
EBV⁺ B cells	DX-B, FD-B, AH-B, BD-B	—

Numbers indicate reciprocal of highest mAb dilution (× 10^{-3}) giving rosette formation with target cells in erythrocyte rosetting assays. Dash indicates no rosette formation at starting dilution of mAb.

* Fibroblasts derived from adult lung, skin, and kidney, and from fetal lung and skin.

‡ Peripheral blood lymphocytes separated by Ficoll gradient centrifugation.

also A42⁻. However, typing results with mesenchymal and epithelial cells show that A42 expression is not restricted to neuroectoderm-derived cells because normal skin and lung fibroblasts and kidney epithelial cells are strongly reactive with mAb A42 (Table I). In addition to the A42⁻ neuroblastoma and Ewing's sarcoma lines listed in Table I, we have tested morphologic variants derived from several of these cell lines for A42 expression. The predominant cell type in neuroblastoma cultures is characterized by a small, round cell body that may extend neuritic processes, contain dense-core vesicles, and show enzyme activities for neurotransmitter synthesis (23, 24). This neuronal phenotype (n-variants) can be distinguished from the epithelial or fibroblast-like morphology of large, flattened, substrate-adherent cells (s-variants) that arise spontaneously in a proportion of cell lines (24, 25), but which remain a minor cell population in unselected cultures. The s-variant cells do not extend neuritic processes and show no or little neurotransmitter synthetic activity, thus lacking neuronal properties. Pure n-variant and s-variant cultures have been obtained, and cytogenetic evidence has indicated a common clonal origin for variants in a given cell line (23, 26). Paired variants derived from three neuroblastomas, SK-N-

TABLE II

Comparative Analysis of Human Melanoma Cell Lines for Antigen Expression, Cell Morphology, and Degree of Pigmentation

Melanoma cell line	Antigenic system					Morphology*	Pigmentation‡
	A42	HLA-DR	EGF-R	M144	TA99		
SK-MEL-37	+	+	+	−	−	E	−
SK-MEL-173	+	+	+	−	−	E-S	−
SK-MEL-31	+	−	+	−	−	E	+/−
SK-MEL-131	+	+	+	−	−	S	−
SK-MEL-13	+	+	+	−	−	S	+/−
SK-MEL-28	+	+	+	−	+	E-S	+/−
SK-MEL-153	−	+	NT	−	NT	S	+
SK-MEL-26	−	−	NT	+	NT	E-S	−
SK-MEL-29	−	+	+	+	−	S	+/−
SK-MEL-127	−	+	NT	+	+	S-D	+
MeWo	−	−	−	+	+	S	+
SK-MEL-19	−	−	−	+	+	S-D	++
SK-MEL-23	−	−	−	−	+	S	+++

For typing results with mAb A42, see Table I; for morphology, pigmentation, and expression of HLA-DR, M114, EGF-R, and TA99, see Houghton et al. (11), Real et al. (22), and Thomson et al. (36). NT, not tested.

* Cell morphology indicated as follows: E, epithelioid; S, spindle-shaped; D, polydendritic.

‡ Pigmentation assessed as described (11): −, nonpigmented; +/−, weak pigmentation, generally limited to cultures at high cell densities; + to +++, moderate to very strong pigmentation.

BE(2), SK-N-SH, and LA-N-1, were available for this study and were tested for A42 expression. All three n-variants, BE(2)-88n, SH-EP15, and LA-N-1n, were found to be $A42^-$. In contrast, the three s-variants, BE(2)-88s, SH-EP1, and LA1-5s, show strong reactivity with mAb A42 (Fig. 3), and the 57,000 M_r A42 glycoprotein is detected in immunoprecipitation experiments with s-variant cells but not n-variant cells (Fig. 2*A*). Spontaneous morphologic variant formation, similar to that seen in the neuroblastoma cultures, is also observed in two cell lines derived from human Ewing's sarcomas, namely RD-ES and IARC-EW1. When grown on plastic culture dishes, RD-ES and IARC-EW1 cells are predominantly small and round or polygonal, and either grow in tightly packed clusters of floating cells or are loosely substrate-adherent, piling up into aggregates of viable cells at high cell density (Fig. 4). We have now identified strongly substrate-adherent variant cells in cultures of these two cell lines that are characterized by an epithelial or fibroblast morphology, and purified variant cell cultures, designated as EW1s, have been obtained from the IARC-EW1 line (Fig. 4). When tested for changes in cell surface antigen expression, EW1s cells were found to newly express the A42 antigen (Fig. 3).

J143 Surface Expression on Human Cells. mAb J143 recognizes a cell surface glycoprotein complex, composed of disulfide-linked subunits of 140,000 and 30,000 M_r, that is noncovalently associated on the surface of $J143^+$ cells with an additional 140,000 M_r surface glycoprotein, detected by mAb AJ2 (13). The J143 cell surface antigen is strongly expressed on most cultured human cell types growing as substrate-adherent monolayers, but is not or only weakly expressed on cells growing in suspension culture or loosely substrate-adherent (13). In the

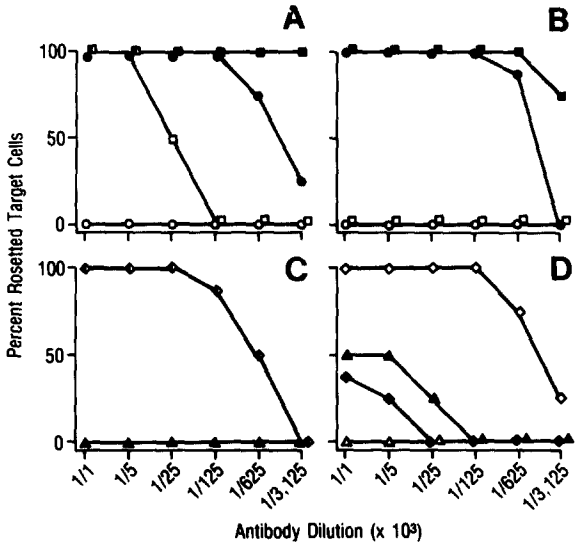

FIGURE 3. Differential expression of cell surface antigens A42, J143, AO10, and mel-CSPG on human neuroblastoma and Ewing's sarcoma variants determined by erythrocyte rosetting assays. (*A*) mAb A42 (○, ●) and J143 (□, ■) tested with IACR-EW1 Ewing's sarcoma cells (○, □) and the derived substrate-adherent EW1s variant cells (●, ■). (*B*) mAb A42 (○, ●) and J143 (□, ■) tested with n-variant BE(2)-88n neuroblastoma cells (○, □) and substrate-adherent BE(2)-88s variants (●, ■). (*C*) mAb AO10 (◇, ◆) and AO122, detecting mel-CSPG (△, ▲) tested with IARC-EW1 (◇, ◆) and Ew1s cells (◆, ▲). (*D*) mAb AO10 (◇, ◆) and (AO122 (△, ▲) tested with BE(2)-88n (◇, △) and BE(2)-88s cells (◆, ▲).

present study, the BE(2)-88n and BE(2)-88s neuroblastoma variants and the IARC-EW1 and EW1s sarcoma variants, respectively, were compared for J143 cell surface expression. Fig. 3 shows that in both systems the substrate-adherent variants are strongly reactive with mAb J143, whereas the n-variant or parental cells are unreactive or only weakly reactive with this mAb. Consistent with these findings for cell surface expression, mAb J143 was found to precipitate the characteristic 140,000 M_r glycoprotein from the substrate-adherent variant cells but not from the loosely substrate-adherent cell types (Fig. 2*B*).

A42 and J143 Surface Expression on Rodent–Human Hybrid Cells. mAb A42 and J143 do not react with the rodent cells used for construction of somatic cell hybrids but they do react with distinct subsets of the panel of 57 hybrid clones included in this study. We have determined through immunoprecipitation experiments that mAb A42 recognizes the characteristic 57,000 M_r glycoprotein and mAb J143 recognizes the 140,000 M_r glycoprotein in antigen-expressing hybrid cells (clones A9/DXB6 and NSK-3, respectively). Three types of A42$^+$ or J143$^+$ hybrids were distinguished: (*a*) hybrids derived from fusions between antigen-expressing human cells and permissive[2] rodent cell types (Table III, group I); (*b*) hybrids derived from antigen-negative human cells and inducing rodent cell types (Table III, group II); and (*c*) human neuroblastoma–derived hybrids that are conditionally antigen expressing; they do not express A42 or

[2] Rodent cells are termed permissive for expression of a human antigen when hybrids constructed with antigen-positive human cells are found to express the antigen, and inducing when hybrids constructed with antigen-negative human cells express the antigen; for a previous discussion see reference 13.

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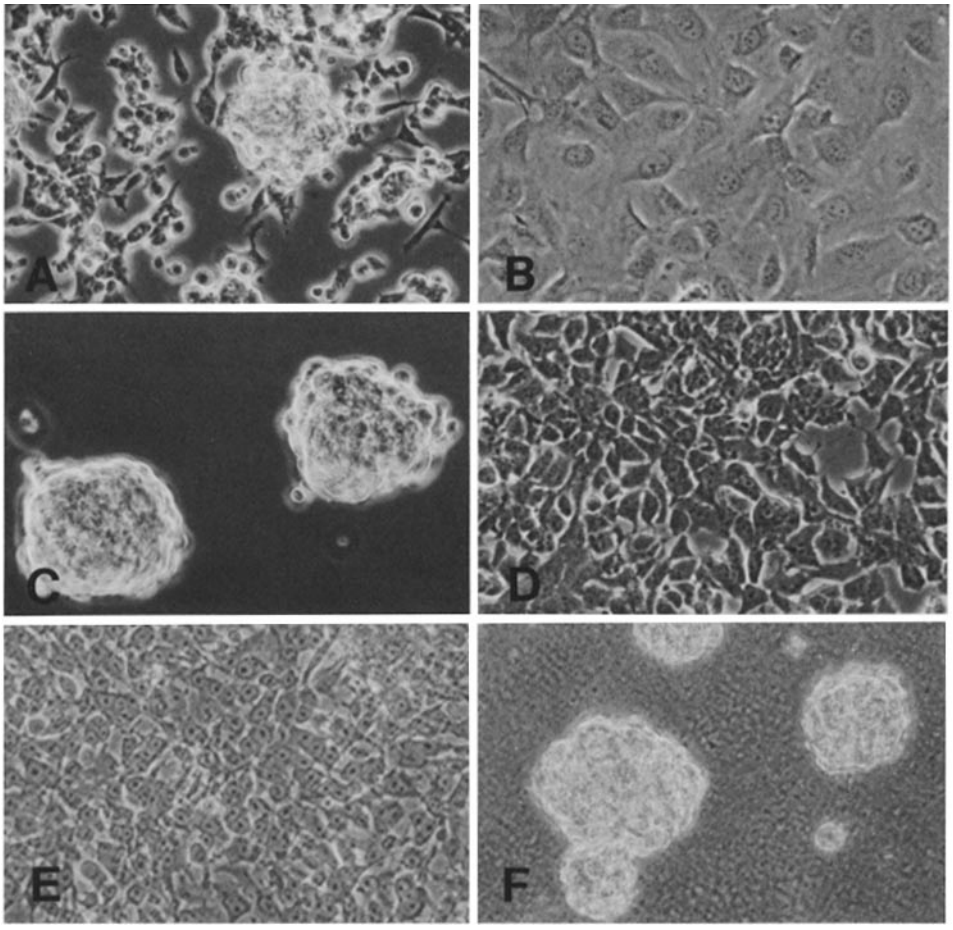

FIGURE 4. Morphology of Ewing's sarcoma cells in culture. (*A*) IACR-EW1 cells grown on plastic; (*B*) EW1s variant cells grown on plastic; (*C*) RD-ES cells grown on plastic, showing floating cell aggregates; (*D*) RD-ES cells grown on FN-coated substrate; (*E*) IARC-EW1 cells grown on BCE-ECM; and (*F*) IARC-EW1 cells grown on EHS-BM, showing substrate-attached cell aggregates. Photomicrographs were taken 24 h after transfer of cells from plastic flasks to new culture vessels.

J143 when grown on plastic substrates but they are induced to express the antigens when grown on BCE-ECM or FN (see below).

A comparison between the serologic typing results and the distribution of individual human chromosomes among the hybrid clones permitted the chromosomal assignment of gene loci controlling A42 and J143 expression. The J143 gene has previously been assigned to human chromosome 17q22-qter (13) and the results of this study confirm this assignment. Discordancy analysis for the A42 antigen (Table IV) shows that the presence of no single human chromosome is both necessary and sufficient for antigen expression in hybrid cells (column marked total number of discordancies). Only two human chromosomes, 6 and 14, are present in all $A42^+$ hybrid clones; the presence of no other human chromosome is required for antigen expression as indicated by ≥4 (and mostly

TABLE III

A42 and J143 Cell Surface Expression on a Panel of Rodent–Human Somatic Cell Hybrids Derived from Fusions between Different Human and Rodent Cell Lines Tested by Erythrocyte Rosetting Assays

Human parent			Rodent parent	Hybrid clone		
Cell type	Phenotype			Name	Phenotype*	
	A42	J143			A42	J143
Group I‡						
Kidney epithelial cell	+	+	RAG kidney cancer	RC2/11	+	
Fibroblast	+	+	A9 fibroblast	AFib3	+	
Fibroblast	+	+	RAG kidney cancer	RFib1	+	
SK-MEL-28 melanoma	+	+	YH21 fibroblast	CE12	+	
SK-MEL-131 melanoma	+	+	YH21 fibroblast	CC4/1	+	+
Kidney epithelial cell	+	+	RAG kidney cancer	RC5		+
Kidney epithelial cell	+	+	LTK fibroblast	LNK1/6		+
Group II‡						
EBV⁺ B cell	−	−	A9 fibroblast	A9/DXB6	+	
Lymphocyte	−	−	RAG kidney cancer	G1711K	+	
Lymphocyte	−	−	E36 fibroblast	G35K	+	
SK-MEL-29 melanoma	−	+	YH21 fibroblast	CW29	+	
Lymphocyte	−	−	V79 fibroblast	VLym-5		+
SK-N-MC neuroblastoma	−	−	LTK fibroblast	LMC2		+

Cell lines RAG, A9, and LTK are of mouse origin and cell lines YH21, E36, and V79 are of hamster origin.

* Results for A42 and J143 typing only shown for hybrids containing relevant human chromosomes, namely chromosomes 6 and 14 for A42 and chromosome 17 for J143 (see Results).

‡ Group I hybrid combinations define permissive rodent phenotype and group II hybrid combinations define inducing rodent phenotype (13).

>10) discordant hybrid clones (column labeled A42⁺/chromosome-negative). However, two independently derived hybrid clones retaining human chromosome 6 and eight clones retaining human chromosome 14 lack A42 expression (column marked A42⁻/chromosome-positive), indicating that neither chromosome by itself is sufficient for antigen expression. Therefore, we have examined the possibility that concomitant presence of two (or more) different human chromosomes is required for A42 expression. A single combination of human chromosomes, namely, 6 and 14, was found to show cosegregation with A42 cell surface expression; all other combinations of human chromosomes showed at least five discordancies with A42 expression in the hybrid panel.

ECM-modulated Cell Surface Expression of A42 and J143. In our previous work (10) we showed that a number of NBE and NSK neuroblastoma hybrids show coordinate changes in substrate adhesiveness, cell spreading, cell morphology, and surface antigen expression when cultured on BCE-ECM instead of plastic surfaces. To examine the effects of specific culture substrates on A42 and J143 expression, we have compared the antigenic phenotypes of 11 neuroblastoma hybrid clones (NBE-C2, -D4, -E1, -G1, -H1, -J2, -K1, -M1, -N1, NSK-4, and NSK-6), grown either on plastic dishes or on dishes coated with BCE-ECM or purified plasma FN. All 11 hybrid clones showed a rapid change in cell morphology and substrate adhesiveness after transfer from plastic dishes to BCE-

TABLE IV

Discordancy Analysis for Chromosomal Assignment of Human Gene Loci Determining A42 Cell Surface Expression in a Panel of 54 Hybrid Clones

Human chromosome	Number of discordant hybrid clones		
	$A42^+$/chromosome-negative	$A42^-$/chromosome-positive	Total
1	11	6	17
2	25	3	28
3	16	8	24
4	15	6	21
5	13	5	18
6	0	2	2
7	17	3	20
8	17	5	22
9	22	3	25
10	17	7	24
11	18	6	24
12	14	6	20
13	19	6	25
14	0	8	8
15	10	12	22
16	18	6	24
17	15	8	23
18	20	5	25
19	21	4	25
20	11	11	22
21	4	14	18
22	20	7	27
X	7	14	21
Y	25	3	28

Numbers in body of table refer to numbers of hybrid clones expressing A42 but lacking human chromosome listed on the left ($A42^+$/chromosome-negative), numbers of hybrid clones containing respective human chromosome but lacking A42 ($A42^-$/chromosome-positive), or total numbers of discordant hybrid clones.

ECM or FN, and Fig. 5 illustrates these changes for NBE-G1 and NSK-6 hybrid cells cultured on FN-coated substrates. When tested for changes in cell surface antigen expression, clones NBE-E1, NBE-G1, NBE-M1, and NSK-4, which contain human chromosomes 6 and 14 (but not chromosome 17), show induction of A42 expression, while clones NBE-H1 and NSK-6, which contain human chromosome 17 (but not chromosomes 6 and 14), show induction of J143 expression (Fig. 6).

A range of $A42^-$, $J143^-$ human cell types were cultured on BCE-ECM and FN to search for human cell lines that are similarly responsive to ECM-derived signals. Neuroblastomas, retinoblastomas, and a number of hematopoietic cell types tested did not show any changes in cell morphology or induction of A42 or J143 surface expression. However, two Ewing's sarcoma lines, IARC-EW1 and RD-ES responded to culture on both BCE-ECM and FN with greatly increased substrate adhesiveness and cell spreading, forming confluent well-

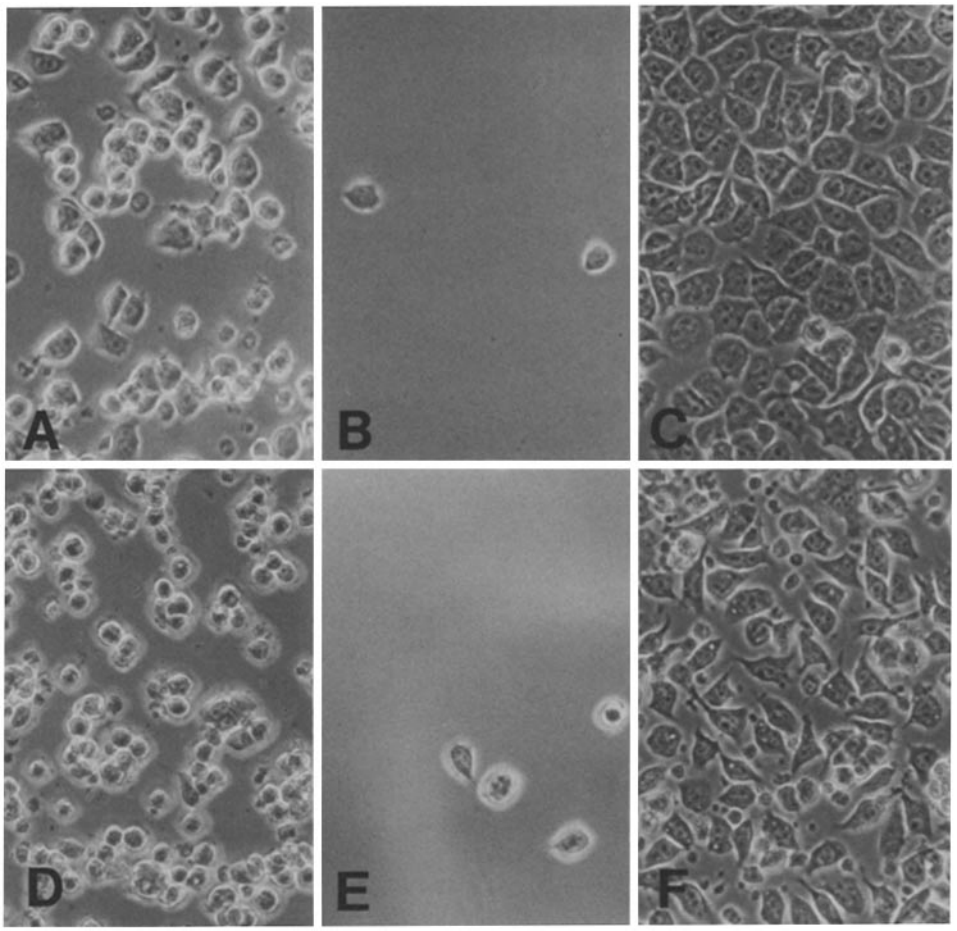

FIGURE 5. Comparison of cell morphology, cell spreading, and substrate adherence of mouse–human neuroblastoma hybrid cell lines, NSK-6 and NBE-G1, cultured on plastic surfaces or FN-coated substrates. (*A*) NSK-6 cells grown on plastic for 90 min after transfer, showing round cell morphology and no cell spreading. (*B*) Same cell culture as shown in *A*; culture was washed with PBS and cells remaining in flask were incubated with PBS for 15 min at 37°C, and subsequently gently washed twice with PBS to remove detached cells. After addition of culture media photomicrograph was taken, showing loss of ≥95% of cells. (*C*) NSK-6 cells grown on FN-coated substrate; photomicrograph taken after 90 min of culture and subsequent washes with PBS exactly as described above for *B*. No significant loss of cells (<5%) and no change in epithelial-like morphology of hybrid cells. (*C*) NBE-G1 cells grown on plastic for 90 min; round cells showing no cell spreading. (*E*) NBE-G1 cells grown on plastic for 90 min and subsequently washed with PBS exactly as described for *B*; loss of >95% of cells. (*F*) NBE-G1 cells grown on FN-coated substrate for 90 min followed by washes with PBS as above; no significant loss of cells (<5%) or change in epithelial-like morphology.

attached monolayers of epithelial-like cells instead of the loosely substrate-adherent or floating cell aggregates seen on plastic culture surfaces (Fig. 4). In contrast, when IARC-EW1 and RD-ES cells were transferred to substrates coated with EHS-BM they formed large cellular aggregates that were strongly adherent to the culture substrate but did not form monolayers (Fig. 4). Comparison of IARC-EW1 cells cultured on plastic or on FN, BCE-ECM, or EHS-BM showed

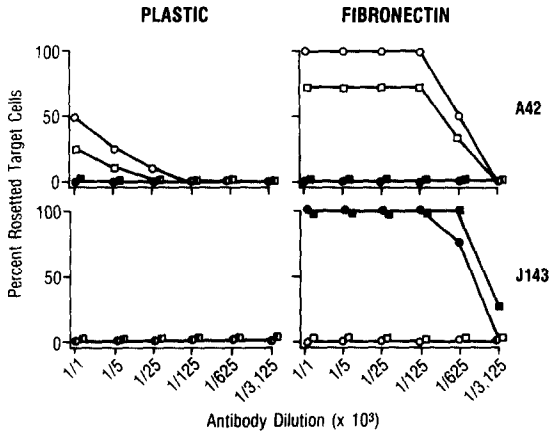

FIGURE 6. Induction of A42 and J143 cell surface expression in mouse–human neuroblastoma hybrids cultured on plastic surfaces versus substrates coated with FN. mAb A42 (*top panels*) and J143 (*bottom panels*) were tested by erythrocyte rosetting assays with hybrid clones NBE-E1 (○), NBE-G1 (□), NBE-H1 (●), and NSK-6 (■); clones NBE-E1 and NBE-G1 contain human chromosomes 6 and 14, clones NBE-H1 and NSK-6 contain human chromosome 17.

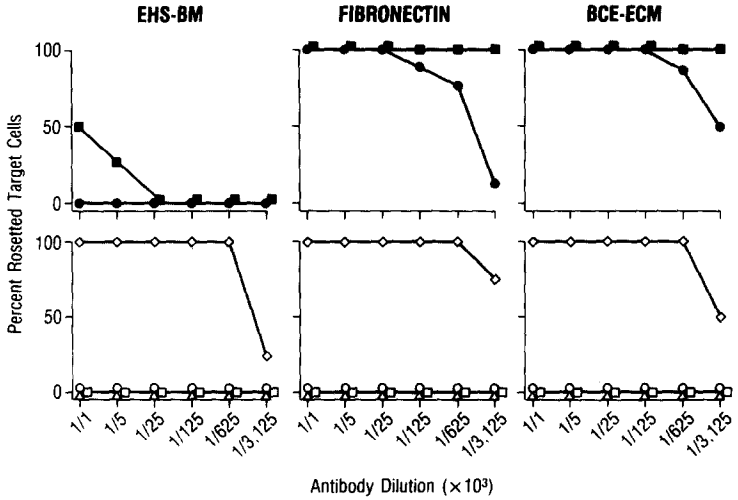

FIGURE 7. Expression of cell surface antigens A42 (●), J143 (■), HLA-A,B,C (◇), mel-CSPG (○), S4 (□), and F23 (△) on IARC-EW1 cells grown for 24 h on substrates coated with EHS-BM, FN, or BCE-ECM, respectively, and determined by erythrocyte rosetting assays. Serotyping results for IARC-EW1 cells grown on plastic surfaces were identical to those shown for cells grown on EHS-BM.

that A42 and J143 are specifically induced by FN and BCE-ECM (Fig. 7) but not by EHS-BM. To further show that A42 and J143 are specifically induced by BCE-ECM and FN, we examined IARC-EW1 cells grown on different substrates for changes in the expression of several unrelated cell surface antigens, including mel-CSPG (detected by mAb AO122 [10]), HLA-A,B,C (detected by mAb W6/32), S4 (18), and F23 (13). We found no changes in the expression of these

antigens when IARC-EW1 cells were transferred from plastic surfaces to BCE-ECM, FN, or EHS-BM–coated substrates (Fig. 7).

Discussion

Cell surface antigens with restricted tissue distribution have been used as markers for (*a*) cells derived from a common cell lineage (27, 28), (*b*) cells at specific stages of differentiation within a cell lineage (11, 28), and (*c*) cells activated by mitogens or differentiating factors (29–34). A large proportion of antigens that show a differentiation stage–specific pattern of expression or are modulated by differentiating factors and mitogens in one cell lineage have also been found in unrelated cell lineages with apparently different patterns of regulation and expression (28–32, 35). In the present study, we describe two cell surface molecules, A42 and J143, that are expressed in a stage-specific pattern on neural and melanocytic cells, but are not restricted to these two neuroectoderm-derived cell lineages. Furthermore, we report that cell surface expression of both molecules is modulated by extrinsic differentiation factors, and that A42 and J143, like the previously described cell surface proteoglycan, mel-CSPG (10), belong to a novel class of cell–ECM interaction molecules, namely ECM-modulated cell surface glycoconjugates. To determine the function of the A42 and J143 glycoproteins, it will be of interest to study their possible role in cell spreading or morphological differentiation. A42 and J143 may also function as receptors for ECM components, with cell surface expression being upregulated by ligand binding. Because multiple receptor/ligand systems are known to be involved in cell adhesion, such a role for A42 and J143 is not ruled out by our finding that neither mAb A42 nor mAb J143 blocks substrate attachment of antigen-expressing cells (unpublished results).

Houghton et al. (11) have shown that cultured fetal and newborn skin melanocytes differ from adult melanocytes in cell morphology, degree of pigmentation, and pattern of cell surface antigen expression, and that these distinct melanocyte phenotypes are conserved or reexpressed in subsets of established melanoma cell lines. Thus, fetal and newborn melanocytes (and a putative melanocyte precursor), as well as poorly differentiated melanoma cells, show an epithelial or spindle-shaped morphology, no or little pigmentation, low tyrosinase levels, and an HLA-DR$^+$, EGF-R$^+$, M144$^-$, TA99$^-$ antigenic phenotype, whereas adult melanocytes and well-differentiated melanomas show dendritic morphology, strong pigmentation, high tyrosinase levels, and HLA-DR$^-$, EGF-R$^-$, M144$^+$, TA99$^+$ phenotype (11, 22, 36). The results of the present study show that A42 belongs to the group of early melanocyte differentiation markers, and that A42 expression on normal and transformed melanocytic cells closely follows the pattern predicted by the scheme of melanocyte differentiation proposed by Houghton et al. (11). In contrast, J143 is equally expressed on fetal and adult melanocytes and on all melanoma cell lines. However, differential expression of A42 and J143 on neuroblastoma variants, retinoblastomas, and astrocytomas, all of which share a common neuroectodermal origin with melanocytic cells, suggests that both A42 and J143 are neural differentiation antigens. A more definitive placement of these findings in a scheme of neuronal and glial differentiation awaits the analysis of normal antigenic phenotypes of cultured human neurons

and astrocytes. Interestingly, the close linkage observed between variant formation and A42 and J143 expression in neuroblastoma and Ewing's sarcoma cell lines (but not other cell types) is in agreement with recent evidence suggesting a common neuroectodermal origin for both types of tumor cell lines (37).

Somatic cell genetic analysis has provided several types of information concerning the organization of genes encoding cell surface molecules within the human genome and regulatory signals determining the expression of antigens with restricted tissue distribution. First, a large number of gene loci coding for human cell surface antigens has been chromosomally mapped (38) and, in all previous instances (including HLA antigens, see below), a single human gene locus has been found to determine antigen expression in hybrid cells. A42 antigen appears to follow a novel pattern, being determined by two separate human gene loci on chromosomes 6 and 14. Most likely, one gene encodes the A42 polypeptide and the second gene may encode an A42-associated molecule or direct steps in the processing or intracellular targeting of the A42 molecule (neither surface-A42$^-$ human nor hybrid cells show detectable intracellular antigen when tested by immunoprecipitation assays or immunofluorescence tests of permeabilized cells; unpublished observations). Cell surface expression of human HLA class I antigens shows some analogies to the A42 antigenic system in that it also depends on two gene loci, the HLA heavy chain locus on chromosome 6 and the β_2-microglobulin locus on chromosome 15 (39) and cell surface expression of HLA molecules in rodent–human hybrids requires the synthesis of β_2-microglobulin in hybrid cells containing HLA genes (40, 41). However, rodent β_2-microglobulin is capable of substituting for its human homologue (42) and masks the two-gene requirement. The observation of an increase of 9,000 in the M_r of the A42 glycopeptide under nonreducing conditions raises the possibility that the 57,000 M_r molecule may be linked by disulfide bonds to an as yet unidentified second subunit. Alternatively, a noncovalently linked A42-associated molecule may have remained undetected due to dissociation during cell extraction procedures.

The second type of information derived from somatic cell genetic analysis is the identification of regulatory controls determining surface antigen expression. Previous studies have shown that the differentiation program of the rodent fusion partner determines the expression of a number of introduced human genes, presumably through intrinsic, *trans*-acting signals. For instance, cell surface expression of human EGF-R (43), nerve growth factor receptor (16), HLA-DR (44), mel-CSPG (10), and the F23 and JF23 glycoproteins (13) has been described in rodent–human hybrids derived from fusions between antigen-negative human cells and appropriate rodent cell types (inducing rodent phenotype). Conversely, lack of human Thy-1 and MC139 expression has been described (14) in a subset of hybrids derived from antigen-expressing human cells that have retained the Thy-1 and MC139 encoding human chromosome 11 (nonpermissive rodent phenotype). In the present study we show that A42 and J143 are two new examples of inducible human cell surface antigens. The molecular nature of the intrinsic, inducing, or restricting signals is not known; conceivably, they are provided by the rodent recipient cell, or, alternatively,

result from the loss of human signals through chromosome segregation or inhibition in the rodent recipient cell.

In addition to these intrinsic regulatory signals, a number of extrinsic signals have been shown to modulate surface antigenic phenotypes (29–34). In the case of the A42 and J143 antigens, surface expression was found to be modulated by ECM-derived extrinsic signals, and together with mel-CSPG (10), these glycoproteins represent the first examples of human cell surface molecules regulated in this way. Previous studies with soluble differentiation factors and mitogens have provided precedents for extrinsic control of surface antigenic phenotype. For instance, IFN-γ is known to induce HLA class I and class II antigen expression in many cell types (31, 32, 45); tumor necrosis factor induces HLA class I expression (30); and mitogens and lymphokines modulate surface antigen expression in distinct lymphocyte subsets (29). We propose that ECM-derived signals play a similar role in determining antigenic phenotypes, as has been defined for soluble factors. Specificity of the ECM-derived signals may stem from the variable composition of the matrix surrounding cells in vivo or from selective expression of individual ECM receptors in cells of different cell lineages or cells at distinct stages of differentiation within a cell lineage. Because A42 and J143 expression in the ECM-responsive neuroblastoma hybrids and IARC-EW1 cells is induced by biochemically complex native BCE-ECM and also by purified plasma FN, it will be possible now to examine which of the known domains of the FN molecule carry regulatory signals and whether A42 and J143 are modulated by the same signals. Based on the results of this and previous studies (10), it seems likely that divergent molecular mechanisms control cell adhesion, cell spreading, cell morphology, and expression of specific cell surface antigens in ECM-responsive cells, and that the diverse phenotypic effects of ECM are target cell–specific. For instance, BCM-ECM is equally effective in changing cell morphology and growth characteristics in neuroblastoma hybrids and in IARC-EW1 cells but induction of mel-CSPG expression is seen only in neuroblastoma hybrids (10) and not in IARC-EW1 cells. Furthermore, BCE-ECM and FN-induced changes in cell–substrate adhesion and cell spreading are observed within 1–2 h after transfer from plastic surfaces, whereas induction of A42 and J143 cell surface expression is first detected after 12–24 h (Figs. 5 and 6; unpublished results). Finally, EHS-BM promotes substrate adhesion of Ewing's sarcoma cell aggregates but not cell spreading or A42/J143 surface expression in these cells (Figs. 5 and 7).

The diversity of ECM-controlled traits and the fact that the genes encoding the three known ECM-modulated cell surface antigens, A42, J143, and mel-CSPG, are located on different human chromosomes suggest that the ECM-derived signals activate target cell–specific programs of alternative differentiation rather than the expression of single gene products. Characterization of the distinct ECM signals, the ECM receptors, and the intracellular signalling pathways controlling A42, J143, and mel-CSPG will be important next steps in our analysis of extrinsic signals that govern tissue-specific patterns of cell surface antigen expression.

Summary

We have used serologic, biochemical, and genetic methods to characterize two stage-specific human differentiation antigens of neural and melanocytic cells: A42 (57,000 M_r glycoprotein) and J143 (140,000/30,000 M_r glycoprotein). The genes determining A42 and J143 cell surface expression in rodent–human hybrids were chromosomally mapped, and the respective human chromosomes were introduced into rodent cells derived from distinct differentiation lineages. Serologic analysis of the resulting hybrid clones has permitted the identification of two types of regulatory signals determining A42 and J143 expression. First, both antigens are expressed in hybrids constructed with antigen-positive human cells and also in certain hybrids constructed with antigen-negative human cells, indicating that intrinsic signals provided by the differentiation program of the rodent fusion partner induce antigen expression. Second, a series of human–mouse neuroblastoma hybrids, which are $A42^-$ or $J143^-$ when cultured on plastic surfaces, can be induced to express the antigens when cultured on substrates coated with extracellular matrix (ECM) produced by bovine corneal endothelial cells or fibronectin. This induction of antigen expression by extrinsic, ECM-derived signals is accompanied in the neuroblastoma hybrids by increased substrate adhesiveness and cell spreading and by characteristic changes in cell morphology. A similar program of phenotypic changes is also seen in spontaneous variants of human neuroblastoma and Ewing's sarcoma cells and in ECM-induced Ewing's sarcoma cells. These findings suggest that ECM-derived signals have a role analogous to mitogens and soluble differentiation factors in modulating differentiation phenotypes and tissue-specific patterns of cell surface antigen expression.

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