ROLE OF HUMAN CHROMOSOME 11 IN DETERMINING SURFACE ANTIGENIC PHENOTYPE OF NORMAL AND MALIGNANT CELLS

Somatic Cell Genetic Analysis of Eight Antigens, Including Putative Human Thy-1

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Serological characterization of human cell surface phenotypes through typing of cultured cell panels and tissues with monoclonal antibodies (mAb)[1] has revealed a high degree of antigenic diversity on somatic cells. Expression of a large number of antigenic systems has been correlated with differentiation pathways (providing markers for cell lineages or distinct stages within a differentiation pathway), with growth characteristics and cell proliferation, or with the transformed phenotype of tumor cells (1–4). Coordinate expression of a series of surface antigenic systems was found to be characteristic for certain differentiated cell types, and a cell type–specific sequence of changes in these antigenic patterns was found to accompany cellular differentiation and maturation steps. These principles were established through serological analysis of normal and malignant cells of hematopoietic origin, and have been extended more recently to other human cell types (2, 5). While phenotypic variability on the human cell surface has been studied in considerable detail, little is known about the genes coding for most surface antigens and genes involved in coordinating the expression of multiple surface components to generate specific phenotypic patterns.

Somatic cell genetic analysis of antigen expression in rodent-human hybrid cells has provided several types of information concerning genes controlling surface antigens and their interaction and organization in the human genome: (*a*) chromosomal assignment of genes coding for surface antigens (6); (*b*) identification of subunit components of multimeric antigens coded for by different chromosomes (7–9); and (*c*) definition of regulatory controls of antigen expression related to cell lineage and growth characteristics, including the permis-

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[1] *Abbreviations used in this paper:* mAb, monoclonal antibody; MHA, mixed hemadsorption assay.

sive/nonpermissive and the inducing/noninducing character of differentiated rodent cells for the expression of human differentiation antigens (8).

In this study, we describe the analysis of eight distinct antigenic systems controlled by human chromosome 11, including the putative human Thy-1 antigen, K117 (25 kilodalton [kD] glycoprotein), and T43 (85 kD glycoprotein), a marker of malignant tumors derived from $T43^-$ epithelia (4). Our results, and those described by others (10, 11) indicate that a large number of known gene loci coding for cell surface components are clustered in human chromosome 11, and that this chromosome carries several genes differentially expressed on cells undergoing hematopoietic and neuroectodermal differentiation.

Materials and Methods

Cell Lines. 19 mouse-human hybrid clones were derived in the laboratory of X. Breakefield (Yale University, New Haven, CT), from fusions between SK-N-BE(2) human neuroblastoma cells and N4TG-1 mouse neuroblastoma cells (NSK hybrid series), or between BE(2)-C human neuroblastoma cells and NS20TG11E mouse neuroblastoma cells (NBE hybrid series) as described (12). Additional hybrid cell lines derived from fusions between human melanoma (LC and CE hybrid series) or normal kidney epithelial cells (LNK, ANK, and RC hybrid series) with mouse RAG, A9, or L cells, or with YH21 Chinese hamster ovary cells, have been described (12–14). SK-N-MC human neuroblastoma cells were fused with mouse L cells to generate the LMC hybrid series.

Chromosome Analysis of Hybrid Cells. The presence of human genetic material in hybrid cell lines was determined by four methods: (*a*) Isozyme analysis (enzymes listed in 12). (*b*) Karyotype analysis; metaphase spreads were stained by trypsin-Giemsa techniques (15, 16), or by the G11 method (17), or sequentially with both techniques. ≥25 metaphases were evaluated for each NSK, CE, and LC hybrid, and 10–25 metaphases for NBE hybrids. (*c*) Cell surface markers; hybrid cells were tested in mixed hemadsorption assays (MHA) (see below) with mAb directed against 21 chromosomally mapped cell surface antigens (8, 12–14). (*d*) Southern blot analysis; presence of the distal end of the short arm of chromosome 11 was confirmed by DNA hybridization techniques with the pT22 (*HRAS1*) and pβpstd (β-globin) probes, obtained from M. Wigler (Columbia University College of Physicians and Surgeons, New York) and T. Maniatis (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), respectively, using standard procedures, as described (18).

mAb and Serological Procedures. mAb AbG344, AbK117, AbMC139, and AbNP13 were newly derived from mice immunized with cultured human astrocytoma (AbG344, AbK117), or neuroblastoma cells (AbMC139), or from a tissue extract (AbNP13), following standard fusion, hybrid selection, and cloning procedures (19). The derivation of the other antibodies has been described (4, 5, 8, 19). MHA rosetting for the detection of surface antigens on cultured cells have been described (5, 12, 19).

Immunohistochemical Procedures. Tissues were quick-frozen in isopentane precooled in liquid nitrogen. Frozen 5-μm sections were cut, mounted on gelatin-coated slides, air-dried, and fixed in cold acetone. Avidin-biotin immunoperoxidase tests were carried out as described (20), using biotinylated horse anti-mouse IgG (Vector Labs, Burlington, CA) as second antibody.

Immunochemical Procedures. Cells were metabolically labeled with [^{35}S]methionine or [^{3}H]glucosamine (New England Nuclear, Boston, MA), and used for immunoprecipitation experiments following published procedures (19).

Results

Serological and Biochemical Characteristics of Cell Surface Antigenic Systems. Eight antigenic systems defined by mouse mAb are analyzed in this study,

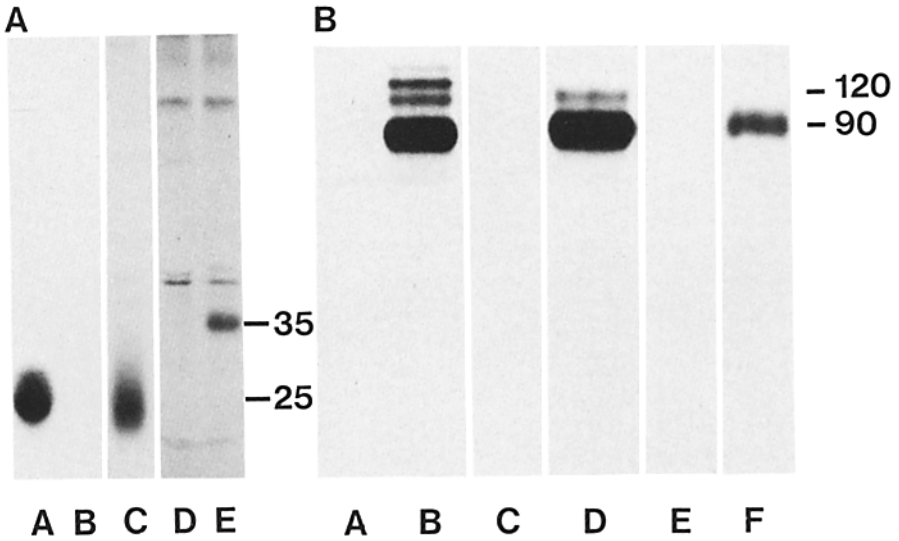

FIGURE 1. Fluorogram of immunoprecipitates obtained with mAb and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions. (*A*) Extracts of [^{3}H]glucosamine-labeled SK-GS-1 (lanes A and B), U251MG cells (lane C), or concanavalin A–bound fractions of [^{35}S]methionine-labeled LA-N-1 cells (lanes D and E) were tested with AbG344 (lane A), AbK117 (lane C), AbMC139 (lane E), or unrelated mouse mAb (lanes B and D). (*B*) Extracts of SK-RC-6 renal cancer cells (lanes A and B), SK-LC-8 lung cancer cells (lanes C and D), or normal lung fibroblasts (lanes E and F) were tested with AbJF23 (lanes B, D, and F) or unrelated mouse mAb (lanes A, C, and E). Molecular masses of immunoprecipitated components are indicated at the right (kD).

including four antigens (K117, MC139, G344, and NP13) detected by newly derived antibodies. The antigenic systems can be distinguished by three criteria: (*a*) differential expression on a cultured human cell panel, (*b*) biochemical properties, and (*c*) differential expression on a panel of reduced rodent-human somatic cell hybrids.

K117 Antigenic System. K117 is a 25 kD glycoprotein (Fig. 1*A*) expressed on a small range of cultured cell types. Fig. 2 summarizes the results of typing a panel of 120 human tumor cell lines and short-term cultures of normal cells for K117 expression. Neuroblastomas, astrocytomas, sarcomas, teratocarcinomas, and fibroblasts are K117$^+$, whereas most melanocytic, epithelial, and hematopoietic cell lines tested are K117$^-$. Frozen sections of normal human tissues were tested for K117 expression using the avidin-biotin immunoperoxidase method. Strong K117 reactivity was found with neurons, neuropil, and glial cells in both gray matter and white matter of adult cortex and cerebellum (Fig. 3). Blood vessels (endothelium, smooth muscle cells) and connective tissue cells were K117$^+$ in all tissues tested. In the liver, hepatocytes and bile ducts were antigen-negative. In fetal thymus, K117$^+$ cells were found in the septi (blood vessels, connective tissue cells); a proportion of thymocytes in the cortical areas was also K117$^+$, whereas fewer K117$^+$ cells were located in medullary areas (Fig. 3).

MC139 Antigenic System. MC139 is a 35 kD glycoprotein (Fig. 1*A*) expressed on a distinct range of cultured human cells. All normal and malignant cells of neuroectodermal origin tested, most sarcomas, choriocarcinomas, and teratocar-

Cell type	Monoclonal antibody K117	MC139
Human cells		
Neuroectoderm-derived cells		
Neuroblastoma	●●●●●●●●●●	●●●●●●●●●●
Astrocytoma	●●●●●●●●●●	●●●●●●●●●●
Melanoma	○●○○○○●○○●	●●●●●●●●●●
Melanocytes	○○	●●
Sarcomas	●●●●●●●●●●	●●●●●●●●○●
Epithelial cells		
Normal kidney epithelial cells	○○○	○○○
Renal cancer	○○○○○○○○○	○○○○○○○○○
Bladder cancer	○○○○○	●○○○○
Colon cancer	○○○○○	○○○○○
Breast cancer	●○○○○○○○	●○○○○○○○
Lung cancer	●○○○○	●●○○○
Other cancers	○○○○○○○○○○	○○○○○○○●○○
Choriocarcinoma	○○○	●●●
Teratocarcinoma	●●●	●●●
Hematopoietic cells		
B cell leukemia	○○○○○○	○○○○○○○
T cell leukemia	○○○○○○	●●●●●●●●
Null cell leukemia	●○○	○○○
Myeloma	○	○
Myeloid leukemia	○○	○○
EBV- B cells	○○○○○	○○○○○
Skin fibroblasts	●●●●●	●●●●●
Rodent cells	○○○○○	○○○○○

FIGURE 2. Reactivity of mAb AbK117 and AbMC139 with cell surface antigens of cultured human and rodent cells. Summary of results of MHA assays using serial dilutions of antibodies with 127 established human and rodent cell lines and short-term cultures of normal cells: Neuroblastomas [SK-N-SH, -BE(1), -BE(2), -MC, LA-N-1, -2, CHP212, -234, NAP, and MC-NB-1]; astrocytomas (SK-MG-1–9, and U251MG); melanomas (MeWo, SK-MEL-13, -28, -29, -113, -174, -131, -147, -178, and -186); sarcomas (RD-2, A673, SK-ES-1, 5838, 8387, A2394, TE-85, U20S, SAOS, and SK-CS-M); renal cancers (CAKI-1, SK-RC-1, -2, -4, -6, -7, -9, -10, -29, and -45); bladder cancers (253J, T24, SW780, RT4, and TCC-SUP); colon cancers (HT-29, SW403, -837, -1116, and SK-CO-10); breast cancers (AlAb, MCF-7, Cama, BT-20, SK-BR-3, -5, -7, and MDA-MB-361); lung cancers (SK-LC-1, -6, -8, -9, and -LL); other cancers (A431, SW626, 2774, SK-OV-3, -4, -6, A10, SK-UT-1, KNS62, and MKN45); choriocarcinomas (GCC-SV, OCC-MM, and JEG-3); teratocarcinomas (Tera-1, -2, and 833KE); B cell leukemias (SK-LY-16, -18, SK-DHL-2, -10, BALL-1, DAUDI, and RAJI); T cell leukemias (MOLT-4, T45, CCRF-CEM, -HSB2, P12, RPMI 8402, PEER, and HPB-ALL); null cell leukemias (NALM-1, -16, and NALL-1); myeloma (SK-MY-1); myeloid leukemias (HL-60 and K562); and rodent cell lines (mouse L, A9, N4TG-1, NS20TG11E, and RAG cells; and Chinese hamster ovary cells YH21). Results are indicated as follows: ● , antigen-positive (titration endpoints 5×10^{-5}–1×10^{-7}; ◐ , weak antibody reactivity (titration endpoint 2×10^{-3}–1×10^{-4}); ○, antigen-negative.

cinomas are strong MC139 expressors (Fig. 2). Fibroblasts show weak reactivity with AbMC139, and a large proportion of normal and malignant epithelial cells are MC139$^-$. Among hematopoietic cell lines, MC139 shows a highly characteristic distribution, being expressed on all eight T cell leukemias, but on none of 18 cell lines derived from B cell, null cell, or myeloid leukemias, a myeloma, or Epstein-Barr virus–transformed normal B cells.

T43, Q14, JF23, A124, G344, and NP13 Antigenic Systems. Antigens T43 (85 kD glycoprotein), Q14 (130 kD glycoprotein), JF23 (90 kD glycoprotein), and A124 have been described (4, 5, 18, 19). We have now examined the biochemical nature of the JF23 antigen, and Fig. 1 *B* shows that AbJF23 precipitates a variable set of glycoproteins from extracts of different cell types, with 90 kD glycopeptides as a major component in all cell lines tested. The newly derived mAb AbG344 and AbNP13 were tested on the cell line panel shown in Fig. 2, and were found

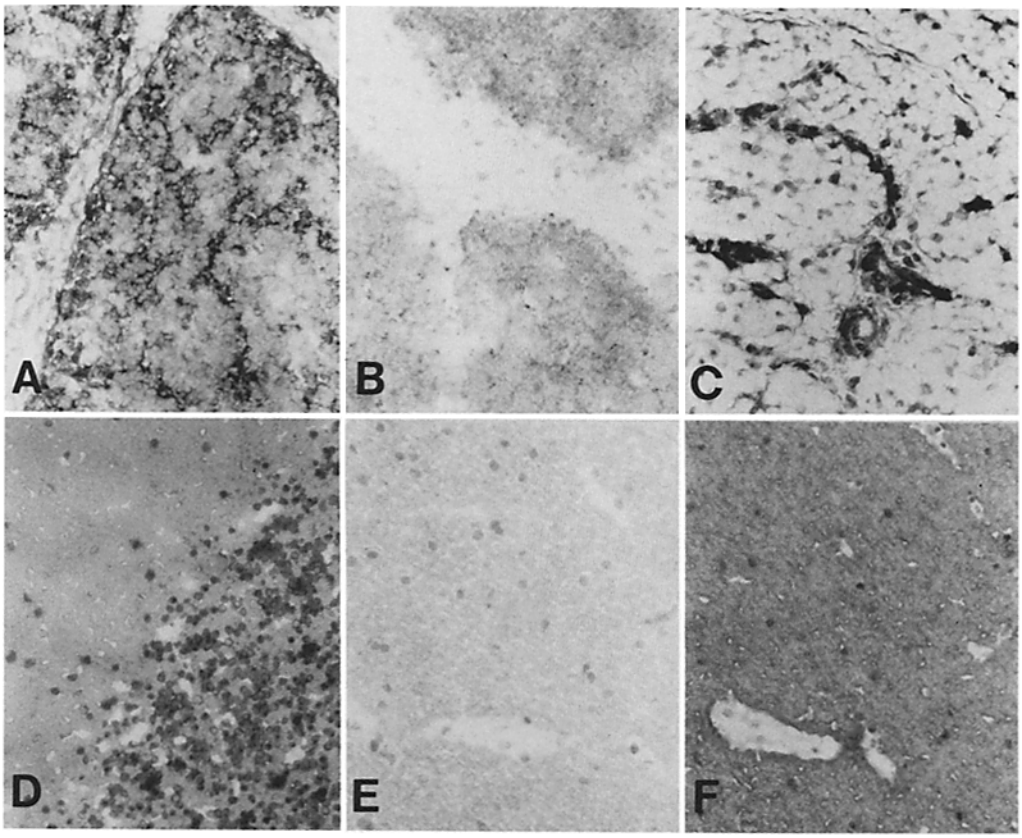

FIGURE 3. Immunoperoxidase staining of frozen sections of fetal human thymus (*A* and *B*), subcutaneous tissue (*C*), and adult cerebellum (*D–F*) with AbK117 (A, C, D, and F) or unrelated antibody (B and E). Note the strong K117 reactivity in the septi and in a subpopulation of thymocytes (*A*), blood vessels and connective tissue cells (*C*), neuronal cell bodies and neuropil of cerebellar cortex (*D*) and white matter (*F*). Hematoxylin counterstaining. Original magnification × 400.

to react with all cultured cell types (data not shown). AbG344 precipitated 25 kD glycoproteins from [^{3}H]glucosamine and [^{35}S]methionine-labeled cell extracts (Fig. 1*A*). Immunoprecipitation experiments with AbNP13 did not yield any specific components in tests with metabolically labeled cell extracts.

Chromosomal Assignments. mAb AbK117, AbMC139, AbT43, AbQ14, AbJF23, AbA124, AbG344, and AbNP13 do not react with the rodent cells used for the generation of somatic cell hybrids, but they were reactive with characteristic subsets of the panel of 32 rodent-human hybrid clones tested. Table I illustrates the results of MHA assays with a series of 13 mouse-human neuroblastoma hybrid clones, and Fig. 4 shows the human chromosome content of the hybrids. For the eight antigenic systems studied, two patterns of reactivity were observed in this hybrid panel: AbJF23, AbG344, AbA124, AbT43, and AbNP13 cotyped and reacted only with the hybrids containing a complete human chromosome 11. In contrast, AbMC139, AbK117, and AbQ14 reacted with hybrids containing one or more copies of either a normal human chromosome 11 or the

TABLE I

Serological Typing of Rodent-Human Hybrid Clones with mAb Against Human Cell Surface Antigens

Hybrid	Monoclonal antibody							
	JF23	G344	A124	T43	NP13	MC139	Q14	K117
NBE-C2	—	—	—	—	—	750	150	1,000
NBE-D4	—	—	—	—	—	150	150	1,000
NBE-E1	1,000	150	10	1,000	250	750	150	1,000
NBE-G1	—	—	—	—	—	150	150	1,000
NBE-H1	—	—	—	—	—	150	150	1,000
NBE-J2	—	—	—	—	—	750	750	1,500
NBE-K1	—	—	—	—	—	750	150	1,500
NBE-M1	—	—	—	—	—	750	150	100
NBE-N1	—	—	—	—	—	750	150	100
NSK-3	—	—	—	—	—	—	—	—
NSK-4	150	1,500	10	100	750	750	750	150
NSK-5	5,000	1,500	50	3,000	3,000	750	3,000	1,500
NSK-6	—	—	—	—	—	—	—	—

Serial dilutions of antibody were tested on target cells by MHA assays. Numbers indicate reciprocal of highest serum dilution ($\times 10^{-3}$) giving rosette formation with target cells in the MHA assay. Negative results (—) indicate no rosette formation at starting dilutions of antibody (1:500 *nu/nu* serum or ascites).

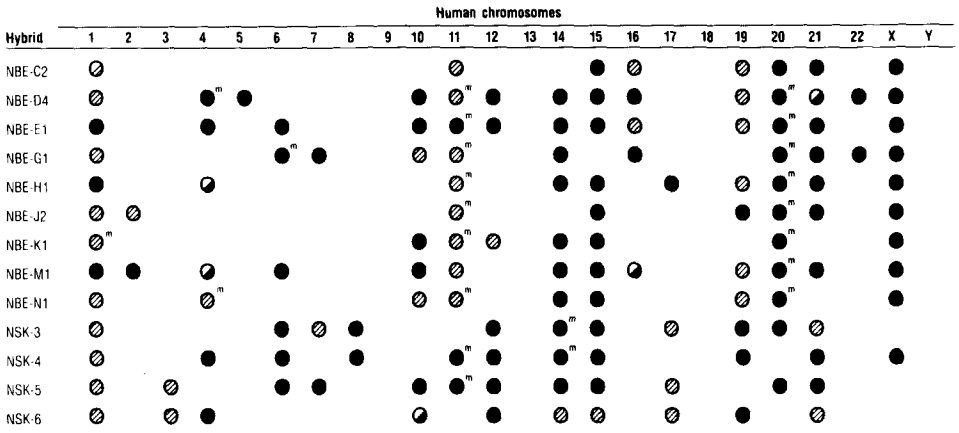

FIGURE 4. Human chromosome content of mouse-human neuroblastoma hybrid clones. Presence of human chromosomes determined by karyotyping, isozyme analysis, and testing of hybrid cells with mAb directed against 21 previously assigned cell surface markers (see Methods and Materials). ●, normal chromosome present; no entry, chromosome not present; ◕, chromosome present in <50% of cells of a culture; ◍, segment of chromosome present in marker chromosome; ⊘, segment present in <50% of cells. Superscript m indicates that 2–4 copies of the chromosome are present per hybrid cell.

t(11;20)(11qter-11q13::20p13-20qter) marker chromosome previously observed in the human parental BE(2)-C cells. The segregation of no other human chromosome followed either of these two distinct patterns of reactivity in the hybrid clones. Analysis was then extended to the entire hybrid panel (32 clones) available for study. Patterns of antibody reactivity and distribution of human chromosomes in the hybrid clones were compared separately for each antigenic

system. For this purpose, data were collected only from hybrids derived from fusions between antigen-positive human cells, and rodent cells known to be permissive for the expression of the respective antigen (see below). The numbers of hybrid clones showing a discrepancy between antibody reactivity and presence of individual human chromosomes were used as a measure of concordant/discordant segregation, and are presented in Table II. These results show unequivocally that all eight antigens studied cosegregated with human chromosome 11. The presence of no other human chromosome was necessary or sufficient for antigen expression. For Q14 and JF23, these results confirm our previous findings, which had mapped gene loci *MSK13* (controlling Q14) and *MSK14* (controlling JF23) to human chromosome 11 (8).

Regional Assignment of Gene Loci on Human Chromosome 11. Presence of identifiable segments of human chromosome 11 (in the absence of normal homologs of this chromosome) in at least 14 hybrid clones (shown in Figs. 4 and 5) has permitted a subregional assignment on chromosome 11 of gene loci controlling antigen expression. Fig. 5 presents results of the serological typing with a discriminating panel of seven hybrid clones, each clone containing a different, unique portion of human chromosome 11. These typing results can be compared with the specific segments of chromosome 11 genetic material present in the hybrids, as determined by serological typing, karyotype analysis, and testing of hybrid cell DNA for the presence of human *HBBC* and *HRAS1* genes (Fig. 6), which have been mapped to the distal portion of chromosome 11p (6).

Five distinct patterns of surface antigen expression could be distinguished on the hybrid panel for the eight antigenic systems. Three pairs of antigens, JF23/G344, A124/T43, and Q14/K117 segregate independently, and their patterns of distribution are different from both the NP13 and MC139 patterns. NP13 and MC139 show very similar patterns, with only one discrepancy, the $NP13^-/MC139^+$ hybrid clone NBE-K1. These results permit the regional assignment of genes controlling JF23, G344, A124, T43, and NP13 to human chromosome 11pter-q13, and genes controlling MC139, Q14, and K117 to human chromosome 11q13-qter (Fig. 7). Assuming that a minimum number of separate segments of chromosome 11 are present in the hybrid cells, these results suggest a tentative arrangement of the gene loci on chromosome 11 in the order 11pter–*MSK14/MSK21–MSK22/MSK23–MSK24*–11q13–*MSK25–MSK13/MSK26*–11qter.

K117 and MC139 Are Differentially Expressed on Hybrid Cells Containing Chromosome 11. Antigens K117, MC139, JF23, and Q14 are not expressed on all human cell types used to construct the hybrid cell panel. K117 is expressed on neuroblastomas, but is absent from melanoma lines SK-MEL-29, SK-MEL-28, and MeWo, and from normal kidney epithelial cells. MC139 is expressed on neuroblastomas and melanomas, but not normal kidney epithelial cells. JF23 is expressed on all cell lines used except for SK-N-MC neuroblastoma cells, and Q14 is expressed on all cell lines used except for MeWo melanoma cells. Nevertheless, we have examined all chromosome 11–positive hybrid clones for the expression of all eight chromosome 11–controlled antigens. We have found that JF23 is induced in the LMC hybrid clones, which are derived from $JF23^-$

TABLE II

Discordancy Analysis for Chromosomal Assignments

Surface antigen	Hybrid clones*	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
JF23	32	8	11	6	8	15	10	11	8	9	8	0	9	10	17	16	13	5	15	11	18	21	10	22	11
G344	23	4	6	4	6	7	4	6	5	3	8	0	5	5	10	10	9	5	6	7	13	9	7	14	6
A124	25	3	7	6	7	11	10	7	8	5	8	0	6	7	13	14	9	7	11	7	16	14	8	17	7
T43	32	8	10	8	8	11	12	10	9	10	8	0	10	11	16	17	13	7	14	10	18	20	10	23	9
NP13	26	5	9	6	8	12	10	7	10	7	8	0	8	9	12	11	9	7	12	8	16	15	10	16	10
MC139	22	3	14	13	10	13	10	11	15	13	8	0	11	15	4	3	5	13	16	8	5	4	13	4	16
Q14	32	9	19	13	9	19	16	15	16	21	8	0	16	18	13	11	9	11	23	14	7	16	14	14	18
K117	19	3	12	11	7	11	10	10	13	15	8	0	11	13	6	5	5	11	14	10	3	4	11	4	14

Numbers of hybrid clones showing discordance between presence of chromosomes indicated above and expression of antigen listed on the left. Rearranged chromosomes were not assessed. Hybrid clones included in the analysis were 19 independently derived NBE and NSK clones (for all antigens), and in addition, clones CE12, CE25, and CC4 (for MC139 and G344); clones RC4 and RC6 (for G344); clone LC1/3/45 (for G344, A124, JF23, T43, and Q14); clone LC2/4 (for NP13, A124, JF23, T43, and Q14); clone RAG4Y (for A124, JF23, T43, and Q14); clones RC2, RC2/13, and RC2/17 (for NP13, A124, JF23, T43, and Q14); clones LNK1, RC1/7, and RC1/9 (for NP13, JF23, T43, and Q14); and clones LNK1/6, LNK1/8, LNK1/15, and RC1/2 (for JF23, T43, and Q14).

* Total numbers of hybrid clones evaluated for individual antigenic systems.

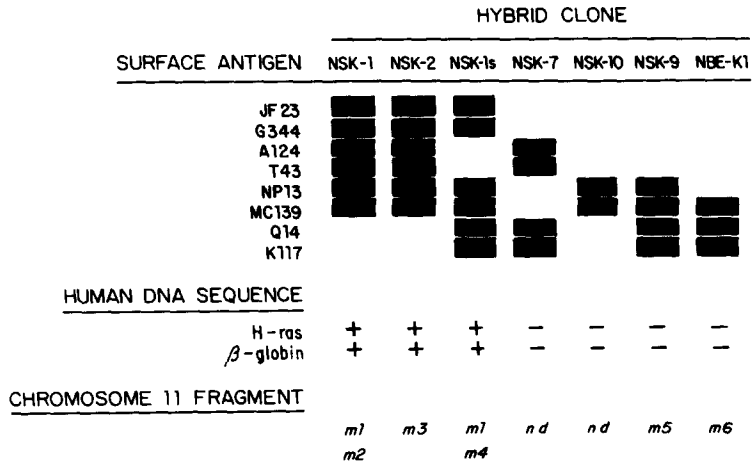

FIGURE 5. Differential expression of chromosome 11–controlled human cell surface antigens on a discriminating rodent-human hybrid panel. Surface antigen expression on hybrid cells is indicated by filled rectangles, no entry indicates lack of antigen expression. Presence of karyotypically defined segments of chromosome 11 is indicated as m1–6. Segment m1, del(11)(pter-q13), comprises chromosome 11p and the proximal portion of 11q; m2 is probably a rearranged m4 marker chromosome, including bands 11q13-11q22; m3 is a small marker chromosome possibly derived from chromosome 11; m4 is the marker chromosome, t(11;20), present in the parental SK-N-BE(2) cells; m5 and m6 comprise the segment 11q13-11qter. Hybrid clones NSK-7 and NSK-10 contain no markers karyotypically identified as comprising 11 material, although both contain several rearranged chromosomes with small unidentified chromosomal segments. The human LDH-A isoenzyme is not consistently detectable in human × mouse neuroblastoma hybrids containing a normal human chromosome 11 (N. Dracopoli, unpublished observations), and therefore results are not included here.

SK-N-MC cells (Fig. 8). No induction was observed for K117, MC139, and Q14 in the hybrids. However, differences were found in the levels of K117 and MC139 expression in chromosome 11–containing hybrids derived from $K117^+/MC139^+$ human neuroblastomas (NBE, NSK, and LMC hybrid series) and $MC139^+$ human melanomas (LM and AM series), depending on the cell type of the rodent fusion partner. NBE and NSK hybrids, constructed with mouse neuroblastoma cells, were strong K117 and MC139 expressors. In contrast, LMC hybrids, constructed with mouse L cells, showed little or no reactivity with AbK117 and AbMC139, and AM and LM hybrids, also constructed with L cells or A9 cells, showed no reactivity with AbMC139 (Fig. 8). The finding that seven independently derived LMC hybrid clones showed weak AbK117 reactivity, and five LMC clones showed weak AbMC139 reactivity (rather than a complete absence of the antigens) is significant in the interpretation of these results. It demonstrates that the K117 and MC139 coding sequences are present in the hybrid cells, and that regulatory mechanisms characteristic for the rodent fusion partner control antigen expression in the hybrids.

Discussion

The Thy-1 antigen of mouse thymocytes (21) was one of the first examples of a group of serologically defined cell surface components designated as differentiation antigens (22). Studies by several investigators (23, 24) have subsequently

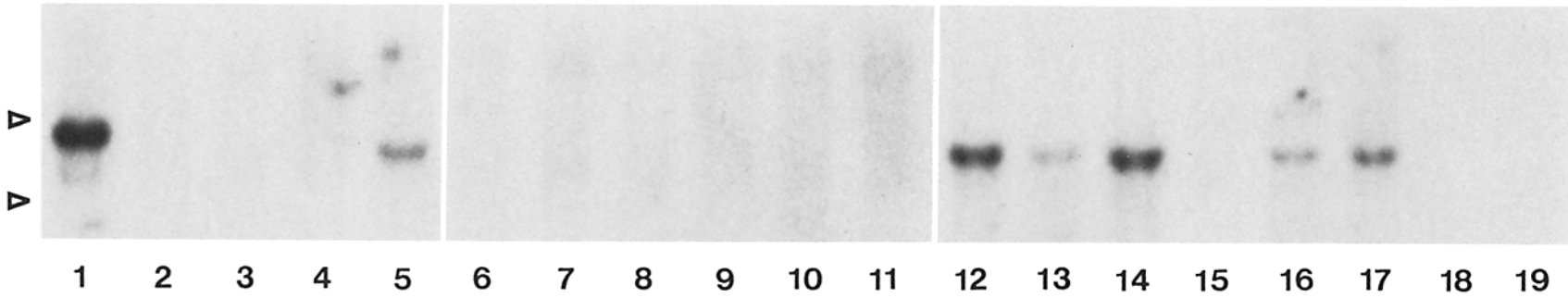

FIGURE 6. Southern blot analysis of Bam HI–digested total cellular DNA from human, mouse, and hybrid cell lines for detection of human *HRAS1* genes using the pT22 probe. Results are shown for human neuroblastoma line SK-N-BE(2) (lane 1), mouse neuroblastoma line N4TG-1 (lane 2), and mouse-human hybrid clones NBE-C2, -D4, -E1, -H1, -J2, -K1, -M1, -N1, and -G1, (lanes 3–11), and clones NSK-1, -1s, and -2–7 (lanes 12–19). The 6.0 kb human Bam HI fragment is present in 6 of the 17 hybrid clones tested. Arrowheads indicate positions of 6.4 and 4.0 kb markers. The same six hybrid clones were also positive for human β-globin genes when tested with the *pβpstd* probe (*not shown*).

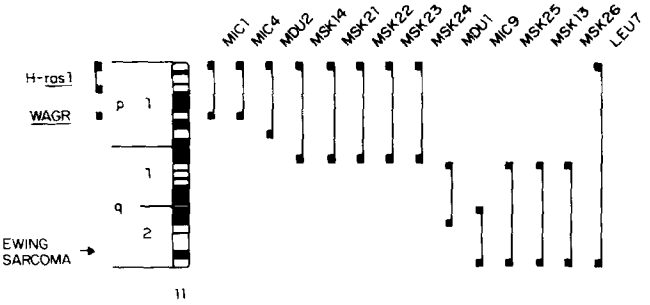

FIGURE 7. Regional assignment of gene loci on human chromosome 11 controlling cell surface antigens. Bars indicate shortest regions of overlap. The location of the *HRAS1* gene, the *WAGR* locus of Wilms' tumor and the breakpoint on chromosome 11 in the t(11;22) marker chromosome of Ewing sarcomas (arrowhead) are indicated.

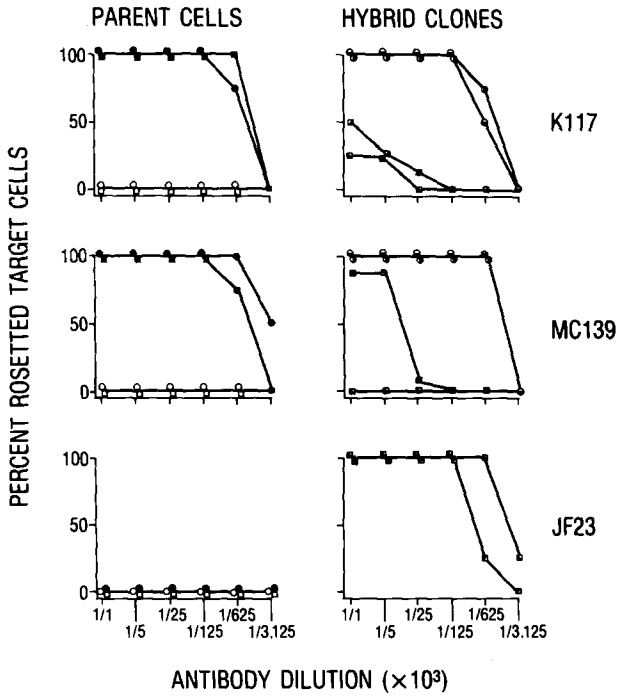

FIGURE 8. Differential expression of K117, MC139, and JF23 cell surface glycoproteins on rodent-human hybrid cells containing human chromosome 11. Diagrams show results of representative titration experiments with AbK117, AbMC139, and AbJF23 on human and rodent parental cell lines, and on hybrid cell lines using the MHA assay. Cell lines are indicated as follows: ■, ●, SK-N-BE(2) and SK-N-MC human neuroblastoma lines; O, N4TG-1 and NS20TG11E mouse neuroblastomas; □, mouse L cells. Hybrid clones were: ◒, NBE-J2; ◑, NSK-1s; ◪, LMC5; ◫, LMC4, ◨, LMC18.7; and ◩, LMC17.

identified putative rat, human, and canine Thy-1 molecules. mAb Ab390, which detects a 24 kD glycoprotein in human brain extracts, has been reported (25) as recognizing human Thy-1. The reactivity of Ab390 with a panel of 29 human cell lines and a range of normal tissues has been described (25), and closely resembles the pattern defined for K117 in the more extensive analysis presented

here. The molecular masses of the K117 and 390 molecules are also comparable. Using the immunoperoxidase method, we found that AbK117 shows the same staining pattern in fetal thymus and several other tissues that has been described for F15-42-1, another mAb reported to detect human Thy-1 (26). Therefore, AbK117 most likely also recognizes human Thy-1. Our finding that the *MSK26* gene locus (controlling K117 expression) maps to human chromosome 11q13-qter is consistent with this conclusion, since it places human *Thy-1* into the same linkage group (including loci *APOA1, UPS*, and *ESA4* in man and *Ap1-1, Ups*, and *Es-17* in the mouse) that resides on the *Thy-1*-carrying chromosome 9 in the mouse (27, 28).

The chromosomal assignment of antigens K117, MC139, Q14, and JF23, which show a restricted or intermediate distribution on the cultured human cell panel and which follow differentiation patterns, permits the analysis of antigen expression in human chromosome 11–containing hybrids constructed with antigen-positive or antigen-negative human cells and rodent cells of distinct differentiation lineages. The results demonstrate different regulatory principles governing surface expression of these antigens in hybrid cells. For K117 and MC139, antigens strongly expressed on human neuroblastomas, we have found that human-mouse neuroblastoma hybrids (NBE and NSK clones) are strong K117 and MC139 expressors (see Fig. 8). In contrast, hybrids derived from human neuroblastoma cells and mouse L cells showed little or no K117 and MC139 expression, and hybrids derived from human melanoma cells ($MC139^+/K117^-$ phenotype) and mouse A9 or L cells were consistently $MC139^-$, suggesting tissue-specific regulation of K117 and MC139 in hybrid cells, determined by the cell type of the rodent fusion partner. Mouse neuroblastoma cells are permissive for K117 and MC139, but mouse A9 and L cell fibroblasts are not. Similar results have been reported for the expression of mouse Thy-1 in rodent cells transfected with a cloned mouse *Thy-1* gene, with mouse lymphoma and rat neural tumor cells being permissive, and L cells being nonpermissive for Thy-1 (29). For JF23, we have found that fusion of cells of the $JF23^-$ human neuroblastoma line SK-N-MC with mouse L cells results in cells expressing JF23 (Fig. 8). This inducing phenotype of L cells for JF23 expression resembles the F8 induction described (8) for L cell hybrids containing human chromosome 19, and contrasts with the permissive/noninducing phenotype of L cells for Q14 (8), confirmed in this study. Among the neuroblastoma hybrids, induction of F8 and JF23 is seen in clones characterized by fibroblastic morphology and increased substrate adhesiveness compared to the human parental cells (LMC and certain NSK hybrid clones) (8). Typically, cultured human neuroblastoma cells show a neuroblastic morphology, with round cell bodies and neuritic processes, and adhere poorly to the culture flask substratum. However, variant cells with an epithelial or fibroblast-like morphology have been derived from several of the neuroblastoma cell lines (30). These variants are strongly substrate-adherent and exhibit an $F8^+/JF23^+$ surface phenotype. As F8 and JF23 expression seem to be related to the same cellular growth characteristics in both neuroblastoma variants and hybrid cells, these antigens may have a direct role in cell adhesion. Thus, different regulatory principles determining surface antigen expression in hybrid cells have been identified in our analysis. Some human differentiation antigens are tightly

TABLE III

Human Cell Surface Antigenic Systems Controlled by Genes on Human Chromosome 11

Surface antigenic system		Chromosome assignment	Gene designation*	Reference
Name	Description			
JF23	90 kD glycoprotein	11pter-q13	*MSK14*	8
G344	25 kD glycoprotein	11pter-q13	*MSK21*	
A124		11pter-q13	*MSK22*	5
T43	85 kD glycoprotein	11pter-q13	*MSK23*	4
NP13		11pter-q13	*MSK24*	
MC139	35 kD glycoprotein	11q13-qter	*MSK25*	
Q14	130 kD glycoprotein	11q13-qter	*MSK13*	8, 19
K117	25 kD glycoprotein	11q13-qter	*MSK26*	
W6/34	glycolipid	11pter-p13	*MIC1*	7
F10.44.2	80 kD polypeptide	11pter-p13	*MIC4*	38, 39
A3D8	80 kD glycoprotein	11pter-p11	*MDU2*	11
4F2	80/40 kD glycoprotein	11q13-q22	*MDU1*	11, 39, 40
4D12	100 kD polypeptide	11q22-qter	*MIC9*	10, 39
LEU7	200/140 kD glycoprotein	11	*LEU7*	31, 41

* Chosen according to conventional nomenclature rules (6).

controlled by the phenotype of the rodent fusion partner (inducing and permissive/nonpermissive modes of expression; see K117 and MC139, or A0122 and F23 [8]). Other antigens are controlled by the growth characteristics of the hybrid cells (inducing and conditionally permissive modes of expression; see JF23 or F8 [8]). Some antigens show no such regulatory influence (generally permissive and noninducing modes of expression; see Q14). Fundamentally different genetic mechanisms may be involved in the control of these groups of antigens, not only in hybrid cells, but also in human parental cells.

The distinctive nature of each of the eight antigens described in this study has been demonstrated by a combination of serological, biochmical, and genetic methods. Using this approach, the antigens can be compared with cell surface antigens previously assigned to human chromosome 11. Antigens K117, MC139, T43, Q14, G344, and NP13 are readily distinguished from previously assigned antigens based on their characteristic distribution, their biochemical characteristics, and their regional assignments on chromosome 11 (see Table III). The relationship between JF23, A3D8/A1G3, and F10.44.2 remains to be established, particularly since AbJF23 recognizes a variable set of glycopeptides in different cell types (Fig. 1). A124 has biochemical characteristics of a glycolipid, and resembles in this regard and in its wide distribution the W6/34 and E7 antigens (A. Houghton, Memorial Sloan-Kettering Cancer Center, personal communication). However, human erythrocytes are $A124^-$, $E7^+$, and $W6/34^+$, and therefore AbA124 seems to define a new antigenic system. Our results and those described by others (10, 31) show that human chromosome 11 controls the expression of at least 12 cell surface antigens (Table III). This contrasts with the small number of surface antigens known to be controlled by other human chromosomes (6). At least two general mechanisms can account for this clustering of assignments for surface antigens defined by mouse mAb: Human chromosome 11 may have an important role in constructing the human cell surface, and may code for a

large number of surface antigens. Alternatively, chromosome 11–controlled cell surface components may be particularly immunogenic in mice and therefore be more readily detected by mouse mAb technology than surface components encoded by other human chromosomes.

Considerable attention has been directed recently towards the occurrence of nonrandom chromosome rearrangements in a number of human malignancies, and the possible involvement of gene loci spatially related to chromosome deletions or break points in generating the malignant phenotype (32). Cellular oncogenes and genes involved in the control of cell proliferation and cellular differentiation have been of particular interest in this regard. Various mechanisms have been proposed (32, 33) that could result in increased expression of these genes, or in the expression of altered gene products, both leading to a disturbance of cellular differentiation or growth control. Nonrandom rearrangements of chromosome 11 have been associated with hematopoietic malignancies, Wilms' tumors, and other cancers. So far, no close association has been found between the regions on chromosome 11 involved in specific rearrangements and any known gene locus, including the *HRAS1* protooncogene (34). Therefore, it seems important to define new gene loci on chromosome 11, especially gene loci that are actively transcribed in specific tumor cells, and genes that specify proliferation or transformation-related cellular traits. In addition, gene loci that are closely related to breakpoints may provide markers for further genetic studies, even if they are not involved in disturbances of cellular proliferation or differentiation. Certain antigenic systems described here deserve special attention with regard to the transformed phenotype. A specific translocation, t(11;22)(q24;q12), has been described for Ewing sarcomas (35, 36); moreover, Geurts van Kessel et al. (37) have shown that the c-*sis* oncogene is translocated from chromosome 22 to the rearranged chromosome 11 in a Ewing sarcoma cell line. Conceivably, such a translocation could bring c-*sis* into the proximity, and possibly under the influence of genes located on chromosome 11q. Both *MSK25* (controlling MC139) and *MSK26* (controlling K117) map to this region (11q13-qter), and MC139 and K117 are expressed at high levels in Ewing sarcoma cells. Therefore, it is important to determine more precisely the localization of these genes relative to the breakpoint region, and to investigate their possible effects on c-*sis* expression. The translocation of c-*myc* to the Ig gene cluster provides a precedent for such an interaction (33), and it may be important that Ig and Thy-1 belong to a common family of related cell surface and secreted glycoproteins (38). In the case of the T43 antigenic system, it may be significant that we have now mapped *MSK23* (controlling T43) to chromosome 11pter-q13. Fradet et al. (4) have shown, in an immunohistochemical study of normal and malignant human tissues, that T43 is a marker of malignant tumors derived from $T43^-$ epithelia. 15 malignant tumors of bladder, colon, lung, and breast all had a $T43^+$ phenotype, while the corresponding normal epithelia, and nine cases of benign tumors derived from these tissues were $T43^-$ (4). It is tempting to speculate that T43 is not only a marker of malignant tumors, but is directly involved in generating the malignant phenotype, and the rearrangements of chromosome 11 may contribute to the malignant behavior of tumor cells by changing the pattern of T43 expression, or through expression of an altered T43 molecule.

Fine mapping of *MSK23* on chromosome 11, through serological analysis of somatic cell hybrids, including hybrids constructed with cells from Wilms' tumor patients (34), will be an important next step in this analysis.

Summary

The expression of eight serologically and biochemically distinct human cell surface antigens defined by monoclonal antibodies was examined on a panel of rodent-human somatic cell hybrids. Cosegregation was observed for human chromosome 11, and surface expression of all eight antigens was studied. Serological analysis of hybrids containing defined segments of human chromosome 11 permitted the regional assignment of genes controlling antigens JF23 (90 kD glycoprotein), G344 (25 kD), T43 (85 kD), A124, and NP13 to chromosome 11pter-q13, and of genes controlling Q14 (130 kD), MC139 (35 kD), and K117 (25 kD) to chromosome 11q13-qter. K117, the putative human Thy-1 antigen, was expressed at high levels in chromosome 11–containing hybrids constructed with mouse neuroblastoma cells, but showed little or no expression in hybrids constructed with mouse L cells. A similar pattern of expression in hybrids was found for MC139, an antigen shared by neuroectoderm-derived cells and normal and malignant T lymphocytes. T43 is a marker of malignant tumors (but not benign tumors) derived from a number of $T43^-$ epithelia, and the regional assignment of the T43 locus on chromosome 11 raises questions about its possible involvement in the specific rearrangements of this chromosome seen in human malignancies.

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