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Brief Communication

Genes Controlling gp25/30 Cell-Surface Molecules Map to Chromosomes X and Y and Escape X-Inactivation

N. C. DRACOPOLI,[1] W. J. RETTIG,[1] A. P. ALBINO,[1] D. ESPOSITO,[1]
N. ARCHIDIACONO,[2] M. ROCCHI,[2] M. SINISCALCO,[1] AND L. J. OLD[1]

SUMMARY

The monoclonal antibody AbO13 defines a cell-surface antigen that is expressed on most cultured human cells, but not on rodent cells. AbO13 precipitates glycoproteins of 25,000 and 30,000 mol. wt. from lysates of [^{3}H]glucosamine-labeled human cells. Results of the serological typing of a panel of 25 rodent-human somatic cell hybrid clones show that reactivity with AbO13 segregates with the human X and Y chromosomes. The presence of either of these chromosomes is sufficient for O13 expression on the hybrid cell surface. Analysis of hybrid clones containing human X chromosomes with karyotypically defined deletions permitted the regional assignment of the X-linked gene locus controlling the expression of O13 to Xp22-pter. In addition, AbO13 is reactive with Chinese hamster-human hybrids derived from fibroblasts of a 49,XXXXX individual that contained only inactivated copies of the human X chromosome. These results suggest that the X-linked locus determining the expression of O13 is not subject to X-inactivation.

INTRODUCTION

The chromosomal assignment of loci determining the expression of human cell-surface antigens has been greatly facilitated through the analysis of interspecies somatic cell hybrids with monoclonal antibodies (reviewed in [1]). We have

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[1] Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.
[2] Department of Medical Genetics, University of Trieste, Italy.

recently mapped genes for a series of newly defined cell-surface antigens to human chromosomes 1, 2, 3, 5, 10, 11, 12, 15, 17, and 19 [2–4]. In the present study, we have characterized the specificity of a new monoclonal antibody on a panel of human cells and have mapped two gene loci controlling antigen expression to Xp22-pter and to the Y chromosome. In addition, we report that the locus on the X chromosome escapes X-inactivation.

MATERIALS AND METHODS

Cell Lines

Short-term cultures of human fibroblasts, kidney epithelial cells, and established tumor cell lines were taken from our human cell bank. Cell culture conditions have been described [5]. The derivation of the rodent-human somatic cell hybrids listed in table 2 and the five mouse-human hybrid clones with partially deleted human X chromosomes have also been described [2, 3, 6]. The RAG/4Y clone was derived from a cell line originated by Marcus et al. [7]. The X8 hybrid clones were derived from the fusion of hypoxanthine phosphoribosyltransferase (HPRT)-deficient Chinese hamster YH21 cells with skin fibroblasts obtained from an individual with a 49,XXXXX karyotype [8]. These hybrids were initially selected in medium supplemented with HAT (hypoxanthine 2×10^{-4} M, aminopterin 4×10^{-7} M, thymidine 3.2×10^{-5} M). Subsequently, hybrid cells containing only inactivated copies of the human X chromosome were isolated by reverse-selection in medium containing 6-thioguanine (3×10^{-5} M). All cells were tested for mycoplasma and found to be free of contamination.

Serological Techniques

The mixed hemadsorption assay (MHA) for detection of cell-surface antigens, using rabbit antimouse Ig conjugated to human O erythrocytes, was performed according to published procedures [9]. The assessment of the heat stability of antigenic determinants and antigen expression on erythrocytes was determined by absorption analysis [10].

Karyotype Analysis

Metaphase spreads were stained using the trypsin-Giemsa and a modification of the alkaline Giemsa (G11) techniques [2, 3]. Differential staining of metaphases from cells grown in medium supplemented with 5-bromodeoxyuridine (BrdU) was used to distinguish between the euchromatic and heterochromatic human X chromosomes [11].

Isozyme Analysis

Isozyme analyses were performed for at least one locus on each human chromosome, except for the Y chromosome [2, 3]. Glucose-6-phosphate dehydrogenase (G6PD) and hypoxanthine phosphoribosylphosphate (HPRT) isozymes were used as markers for the human X chromosome. Retention of human phosphoglycerate kinase (PGK) isozymes in the X8 hybrids was tested by standard electrophoretic methods [12].

Derivation of Monoclonal Antibodies

(BALB/c × C57BL/6)F_1 mice were immunized with MeWo human melanoma cells. Spleen cells were fused with mouse myeloma SP2 cells, and antibody producing hybrids were selected and cloned as described [5]. Hybridomas were grown in nu/nu mice (Swiss background), and ascites fluid was used for serological and biochemical analysis.

Immunoprecipitation Procedures

Extracts of [^{3}H]glucosamine- and [^{35}S]methionine-labeled cells were used for immunoprecipitation tests. Isolation of immunoprecipitates, analysis by SDS-polyacrylamide gel electrophoresis, and fluorography were carried out as described [5].

RESULTS

Serological and Biochemical Analysis

A panel of 44 human cell lines, short-term cultures of human skin fibroblasts and kidney epithelial cells, human erythrocytes, and five rodent cell lines (listed in table 1) was tested for O13 expression. All human cells in the panel were found to react with AbO13, and most cell lines showed positive reactivity in the MHA assays at antibody dilutions as low as 10^{-5}–10^{-7}. In contrast, the five rodent cell lines used for hybridization (table 1) did not express O13.

The O13 determinant is heat-labile, and immunoprecipitation tests with [^{3}H]glucosamine-labeled lysates of SK-LC-6 lung cancer cells showed that AbO13 reacts with glycoproteins of 25,000 and 30,000 mol. wt. (fig. 1). When lysates of [^{3}H]glucosamine-labeled SK-MEL-28 and [^{35}S]methionine-labeled SK-MEL-147 melanoma cells were tested, only the 25,000 mol. wt. component was detected.

Chromosomal Assignment

Serological typing of the 25 somatic cell hybrid clones listed in table 2 demonstrates that reactivity with AbO13 segregates with the human X chromosome.

TABLE 1

HUMAN AND RODENT CELL LINES TESTED FOR REACTIVITY WITH AbO13

Cell lines reactive with AbO13	
Human tumor cell lines:	
Melanoma	SK-MEL-13, -19, -28, -29, -31, -64, -75, -93, -119, -129, -131, MeWo
Astrocytoma	SK-MG-3, -9, -11, -12, U251MG
Renal carcinoma	SK-RC-7, -11, -28
Bladder carcinoma	5637, 253J, 647V, J82, T24, SCABER
Lung carcinoma	SK-LC-6, Calu-1
Breast carcinoma	BT-20, AlAb, MCF-7
Colon carcinoma	HT-29
Cervical carcinoma	ME-180
Choriocarcinoma	GCC-SV(c), JEG-3
Teratocarcinoma	Tera-1, Tera-2
Neuroblastoma	SK-N-BE(2), SK-N-MC, SK-N-SH, IMR-32, SMS-SAN, LA-N-1
T-cell leukemia	MOLT-4
Normal human cells:	
Skin fibroblasts	(n = 5)
Normal kidney	(n = 2)
Erythrocytes	(n = 2)
Cell lines not reactive with AbO13	
Rodent cell lines	N4TG-1, L cells, A9, RAG, YH21

NOTE: All human cells were reactive in the MHA assays. Most human cell lines were reactive with AbO13 with reciprocal antibody dilutions between 10^{-3}–10^{-7}. The five rodent cell lines used in the construction of somatic cell hybrids were not reactive with AbO13 at the starting dilution of 1:250 in direct MHA tests and by absorption tests.

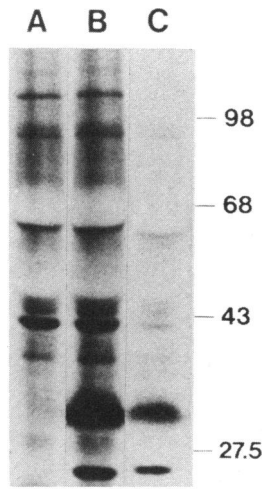

FIG. 1.—Fluorogram of immunoprecipitates obtained from lysates of [^{3}H]glucosamine-labeled SK-LC-6 lung carcinoma cells separated on an SDS-PAGE gel under reducing conditions. Precipitation was carried out with: AbK8 (*lane A*), an unrelated monoclonal antibody of IgG1 subclass serving as a negative control; AbO13 (*lane B*); AbO13 precleared with AbK8 (*lane C*). Molecular weights are indicated *at the right of the figure*.

In addition, the RAG/4Y hybrid clone, which contains Y chromosomes as its only human genetic material [7], also reacts with AbO13. Consequently, the presence of either the X or Y chromosome is sufficient for the expression of O13 on the hybrid cell surface. This demonstrates that both the X and Y chromosomes carry genes, which have been designated *MSK5X* and *MSK5Y,* that control the expression of O13. The involvement of any human autosomes in O13 expression is excluded by at least eight discordancies for each chromosome (table 2).

Five hybrid clones containing only fragments of the human X chromosome were tested with AbO13 to determine the regional localization of *MSK5X*. Two clones were O13 positive, and three O13 negative, permitting the assignment of *MSK5X* to the shortest region of overlap (SRO), Xp22-pter (fig. 2).

MSK5X *Escapes X-inactivation*

AbO13 was tested on four hybrid clones (X8/6T2, X8/6T6, X8/6T7, X8/6T12) that had been derived from the fusion of HPRT-deficient Chinese hamster YH21 cells and fibroblasts from a female with X-chromosome pentasomy, and were subsequently back-selected in 6-thioguanine. The hybrid clones contained different numbers of inactivated human X chromosomes, but no active copies, as shown by the differential staining of heterochromatic X chromosomes (fig. 3) and by their failure to express human G6PD, HPRT, and PGK. All X8 hybrid clones were reactive with AbO13. In titration experiments, these hybrids showed the same level of reactivity as the human parental cells and hybrids containing active X chromosomes, indicating that *MSK5X* completely escapes inactivation and that there is no quantitative change in O13 expression.

TABLE 2

EXPRESSION OF O13 CELL-SURFACE ANTIGEN AND HUMAN CHROMOSOMAL CONTENT OF 24 RODENT-HUMAN SOMATIC CELL HYBRIDS

HYBRID CLONE	TITER × 10^{-3} AGO13	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
NSK-1	1500	●*				●	●*	●		●	●	●	●		●	●				●				●
NSK-1s	312	●		●*	●*	●	●	●		●	●	●	●	●.3	●	●		●			●	●.1	●.1	●
NSK-2	—	●*								●		●			●	●*						●		
NSK-3	—	●*					●	●*	●				●		●	●		●*		●	●	●		
NSK-4	312	●*			●*		●		●			●	●		●	●				●		●		●
NSK-5	—	●*		●			●	●			●	●	●		●	●		●*			●	●		
NSK-7	—	●*			●	●		●			●	●			●						●	●		
NSK-9	3000		●.3		●		●					●*			●	●	●				●	●		●
NSK-9/1	—		●		●		●					●*			●	●	●				●	●		
NSK-10	3000	●*									●				●	●				●		●		●
CE12	150	●*		●	●	●	●		●				●	●.2		●	●	●			●	●	●	●
CE25/13/17	500												●											●
A9/1492	62.5	●		●	●		●								●	●	●	●	●	●	●	●	●	●
A9/GM10/1	—	●									●*													
LC2/4	—					●	●	●										●	●			●		
LC1/3/45	—		●															●	●			●		
ANK 3	1500																							●
ANK 4/14	1500		●													●								●
LNK 1	—			●	●		●	●	●		●	●					●	●		●				
RC1.3	62.5	●		●						●							●							●
RC1.7	62.5	●		●						●			●				●			●	●	●		●
RP1/9	500													●			●							●
RP2	12.5																●					●		●
RAG/194/1	312				●						●						●				●		●	●
Concordant with O13	8	8	11	10	10	15	6	9	12	9	7	13	10	10	13	15	7	8	13	10	10	11	24
Discordant with O13	8	15	12	12	14	8	17	15	12	14	15	11	12	14	10	9	15	16	11	14	13	12	0

NOTE: The strength of serological reactivity of AbO13 is indicated by the reciprocal of the highest antibody dilution giving rosette formation in the MHA assays. Key: ●, presence of at least one of the relevant human chromosomes in the hybrid cells; ●*, presence of a chromosome carrying a karyotypically defined abnormality (which does not involve the human X chromosome) in the absence of the normal chromosome homolog; ●.2, proportion of cells containing relevant human chromosomes, if less than 70%. Rearranged chromosomes indicated by ●*, and mixed hybrid cell populations containing a particular chromosome in less than 50% of the cells are excluded from the comparison of antigen expression and chromosome content. Consequently, the sum of discordant and concordant hybrids does not always equal 24.

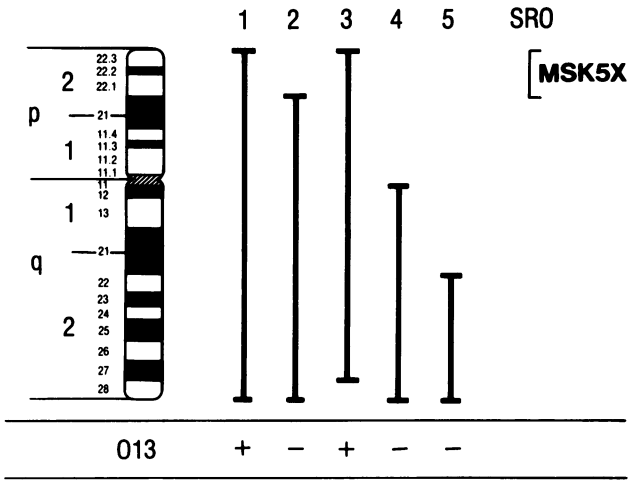

FIG. 2.—Regional assignment on the human X chromosome of gene locus, *MSK5X*, which controls the expression of the O13 cell-surface antigen. *Vertical bars* indicate chromosome fragments present in mouse-human hybrids A9/GM10 (*1*), A9/GM1695 (*2*), RAG/194.7 (*3*), A9/GM73 (*4*), A9/GM89 (*5*). The SRO for *MSK5X* is Xp22-pter.

DISCUSSION

Although more than 200 gene loci have been assigned to the human X chromosome [1], few loci controlling the expression of cell-surface antigens have been defined on this chromosome. The Xg^a blood group determinant, which is defined by heterologous antisera, has been mapped to Xp22.3-pter [1]. More recently, antigens defined by monoclonal antibodies 12E7 [13, 14] and R1 [15] were shown to be determined by genes on the X chromosome. Antibody 12E7 defines a 28,000 mol. wt. protein that is expressed on almost all human cells [14, 16]. The *MIC2X* gene, which controls 12E7 expression, has been assigned to the same portion of the X chromosome (Xp22.3-pter) as Xg^a, but the relationship between these two antigens is not yet fully understood [14]. The R1 antigen is controlled by a gene, *MIC5*, which is located on Xcen-qter [15].

The O13 and R1 antigenic systems can be easily distinguished by the regional assignment of *MSK5X* and *MIC5*. However, O13 and 12E7 share a number of properties. Both antigens show a widespread distribution on human cells, and the gene loci *MSK5* and *MIC2* have been assigned to the same region on the X chromosome and to the Y chromosome. The results of immunoprecipitation tests obtained with AbO13 differ from those reported for 12E7 [16]. Antibody 12E7 has been shown to precipitate polypeptides of 28,000 mol. wt. from ^{125}I-labeled leukemia cells [16]. However, using [^3H]glucosamine-labeled lung cancer cells, both AbO13 and 12E7 precipitate two components of 25,000 and 30,000 mol. wt. instead of a single band. The serological and biochemical properties and the regional assignments of *MSK5X* and *MIC2X* suggest that O13 and 12E7 are related. However, since the antibodies detect at least two distinct polypeptides, it will be difficult to establish the exact nature of this relationship. Although preliminary results from sequential immunoprecipitation experiments have shown that AbO13

and 12E7 partially preclear each other, indicating an association of O13 and 12E7 epitopes on the cell surface, it is not evident whether these epitopes reside on the same or different polypeptides. It will require further analysis to determine the exact relationship between the O13 and 12E7 epitopes, and also the relationship between them and the Xg^a blood group determinant.

In addition to the chromosomal assignment of *MSK5X*, we have examined the influence of X-chromosome inactivation on O13 expression in hybrid cells. Previous studies of rodent-human somatic cell hybrids have shown that inactivated human X chromosomes remain heterochromatic and continue to replicate asynchronously

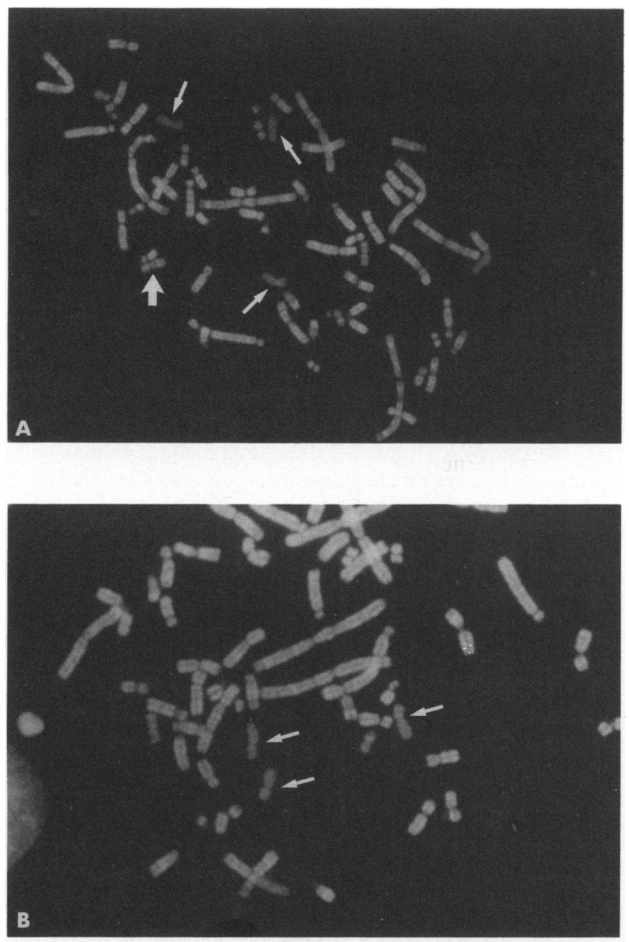

FIG. 3.—Visualization of late-replicating human X chromosomes in Chinese hamster-human somatic cell hybrids [11]. *A*, Metaphase of hybrid clone X8/6T6 grown in medium supplemented with HAT. This metaphase contains three late-replicating inactive human X chromosomes (*small arrows*) and one active human X chromosome (*large arrow*). *B*, Metaphase of the same hybrid clone after reverse-selection in medium supplemented with 6-thioguanine. The active X chromosome has been lost, and the three inactive X chromosomes (*small arrows*) are still present.

and that localized derepression of the HPRT locus on the inactivated X chromosome occurs very rarely [17, 18]. The X8 hybrid clones tested in this study were shown to contain the typical human late-replicating heterochromatic X chromosomes (fig. 3), and in contrast to their failure to express HPRT, G6PD, or PGK, they strongly expressed the O13 antigen. These findings demonstrate that *MSK5X* escapes X-inactivation in these hybrid cells and suggest that this is also true for human cells.

It is noteworthy that *MSK5X* has been assigned to the same chromosomal region as two other loci, including the Xg^a blood group locus, which have also been reported to escape X-inactivation [19]. These observations indicate that *MSK5X* is part of a small region at the tip of the short arm of the heterochromatic X chromosome that escapes inactivation. In addition, the assignment of genes for the O13 cell-surface molecules to both the X and Y chromosomes is in agreement with previous evidence for the presence of homologous regions on the two human sex chromosomes.

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