Genetic Analysis of Human Cell Surface Structures

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Many studies indicate that cell surface components are important in the control of cellular growth and function and that alterations of these structures occur in the malignant state. The extreme complexity of the human cell surface makes difficult the exact determination of the role of specific cell surface structures in the malignant process. The strategy we have employed to simplify the genetic analysis of human cell surface structures takes advantage of two experimental facts.

1. When human cells are fused with the Chinese hamster ovary cell the resulting hybrids selectively lose human chromosomes (Kao and Puck, 1970) and it is possible to prepare human-Chinese hamster somatic cell hybrids which contain only a single human chromosome (Jones et al., 1972; Kao et al., 1976; Moore et al., 1977). Therefore human cell surface structures due to individual human chromosomes can be studied. Fig. 1 illustrates the presence of the single human chromosome number eleven in one of these hybrids.
2. Antisera elicited in experimental animals after the injection of human cells are toxic to human cells in the presence of complement but do not kill the Chinese hamster ovary cell (Oda and Puck, 1961). As illustrated in Fig. 2

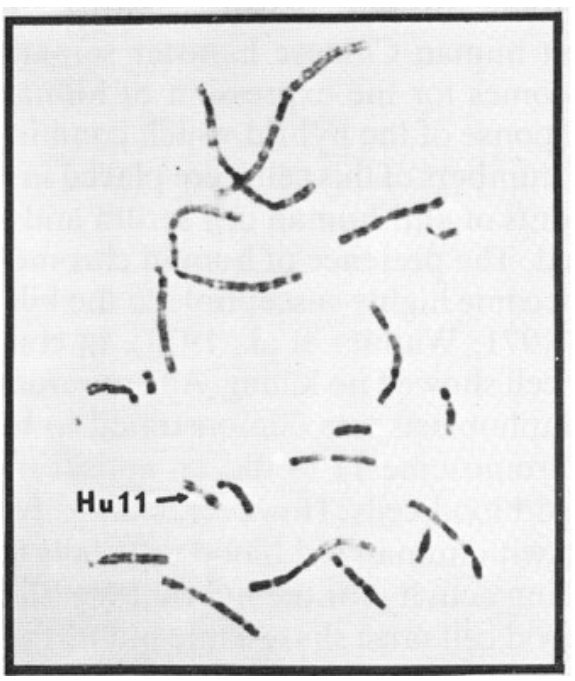

Fig. 1. Chromosomes of a human-Chinese hamster somatic cell hybrid containing all of the Chinese hamster ovary cell chromosomes and human chromosome 11

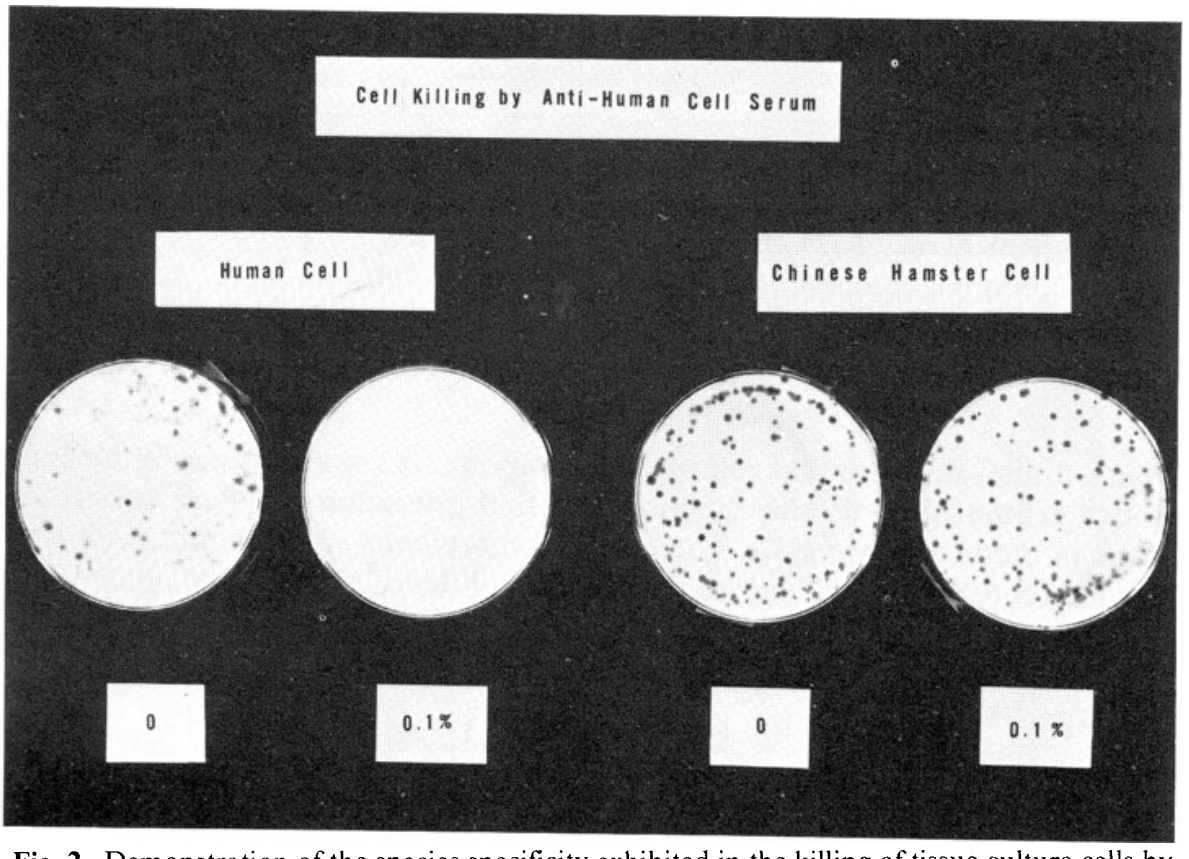

Fig. 2. Demonstration of the species specificity exhibited in the killing of tissue culture cells by antiserum. An antiserum to cultured human lymphoblasts was prepared by injecting these cells into a sheep. The antiserum kills human cells but the same concentration of antiserum has no effect on the plating efficiency of Chinese hamster cells. Normal rabbit serum was the source of complement which was present in all plates

when human cells are placed in a petri dish containing growth medium alone and are incubated the cells form large distinct colonies. In the presence of an antiserum produced against human cells all of the plated cells are destroyed. The Chinese hamster cell formed equal numbers of colonies in the presence and absence of the anti-human cell serum.

Consequently it becomes possible to test human-Chinese hamster somatic cell hybrids with single human chromosomes for the expression of human cell surface antigens. Fig. 3 shows the response of the hybrid which contains only human chromosome 11 when equal numbers of this cell were placed in a series of dishes to which increasing amounts of anti-human cell serum and a constant amount complement were added. The presence of human chromosome 11 in such hybrids causes them to become highly susceptible to the killing action of the antiserum (Puck et al., 1971; Wuthier et al., 1973). In contrast the parental Chinese hamster ovary cell showed no killing. An antiserum prepared in the horse against human lymphoblasts was demonstrated to be highly lethal to the hybrid containing chromosome 11 as did an antiserum prepared in the rabbit against human red blood cells. However, exhaustive adsorption of the antilymphoblast serum with human red blood cells fails to remove more than a small part of the killing activity for the hybrid (Wuthier et al., 1973). Therefore, the human red blood cell must share some but not all of the lethal antigens common to this hybrid and the human lymphoblast. It

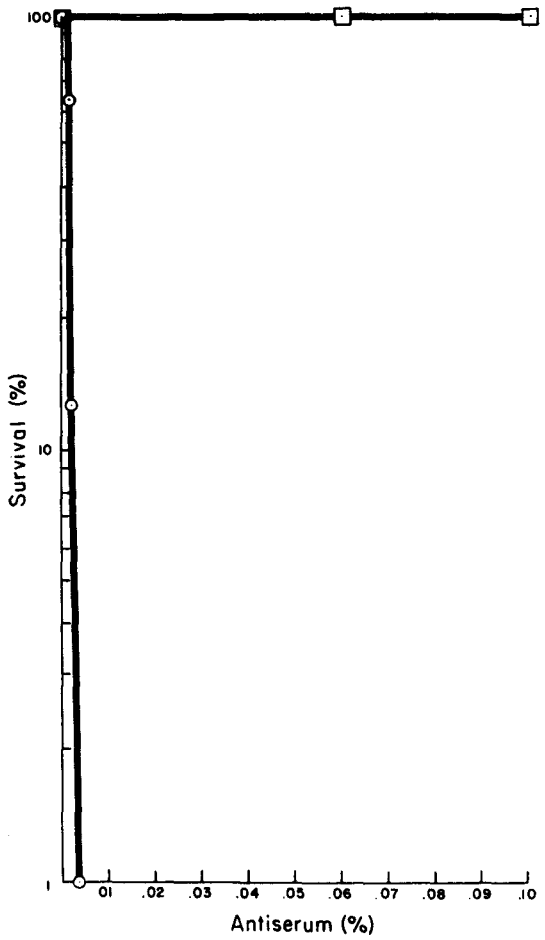

Fig. 3. Demonstration that sheep anti-human red blood cell serum kills the human-Chinese hamster somatic cell hybrid which contains the single human chromosome 11 (circles ⊙) but fails to kill the parental Chinese hamster ovary cell (squares ⊡)

has been possible using these different antisera to demonstrate that at least three human antigens (a_1, a_2 and a_3) are specified by human chromosome 11, and by mutagenesis and immunoselection to prepare clonal stocks of variants containing various combinations of the three antigen markers (Jones et al., 1975; Jones and Puck, 1977). For example a population of hybrid cells is treated with the mutagenic agent, ethyl methanesulfonate, and after removal of the mutagen the survivors which represent 10–20% of the original population are allowed to grow for several days. They are then placed in complement plus sheep anti-human red blood cell serum which has anti a_1 and a_3 activity. Clones which develop under these conditions are isolated and grown into large cultures and their resistance to anti a_1 and a_3 serum confirmed. They are also tested with anti a_2 serum and if they are killed their phenotype is by definition $a_1^- a_2^+ a_3^-$. Clones with other possible phenotypes have been prepared in a similar manner by selection in the appropriate antiserum.

Some of these variant clones were shown to contain deletions of specific segments of human 11 (Kao et al., 1977; Jones and Kao, 1978). These proved useful in the regional mapping of the antigen markers and of two human enzymes, lactate dehydrogenase A and acid phosphatase 2 which have also been assigned to this chromosome (Boone et al., 1972; Bruns and Gerald, 1974). Table 1 summarizes the phenotypic and cytogenetic characterization of some of these clones. Analysis of these data has led to the assignment of the a_1 and a_3 antigens to the short arm of chromosome 11, in the region 11pter

Table 1. Characteristics of clones isolated from the hybrid with human chromosome 11 and used for regional gene mapping

Clone number	Mutagenic agent used	Identified deletions in human chromosome 11	Presence of human markers				
			a_1	a_2	a_3	LDH-A	ACP_2
Parent (J1)	none	none	+	+	+	+	+
7	X-ray	pter → p11	−	+	−	−	−
9	UV	pter → p12	−	+	−	−	+
11	ICR-191E	qter → q13	+	−	+	+	+
23	X-ray	pter → p13	−	+	−	+	+

LDH-A – lactate dehydrogenase A
ACP_2 – acid phosphatase 2

→ 11p13 and the a_2 antigen to the long arm in the region 11qter → 11q13. The regional assignment for the human gene for lactate dehydrogenase A is 11p12 → 11p13 and for acid phosphatase 2, 11p11 → 11p12. Other variants which lack specific antigens without detectable chromosomal changes are being subjected to further genetic analysis. It has been demonstrated that the a_1 phenotype is made up of different complementation groups, mutation in any one of which results in a_1^- behavior. The distribution of these antigens on various normal and malignant cell types is being investigated. The human red blood cell has a_1 and a_3 antigenic activity on its cell surface but not a_2. Normal peripheral blood lymphocytes, cultured lymphoblasts and human amniotic fluid fibroblasts have a_1, a_2 and a_3. It has been found that a_1 is immunologically related to the glycophorin fraction of human red blood cells (Moore et al., 1976).

The ability to identify human cell surface components and to establish their corresponding genetic elements should provide a link in understanding the role of the cell surface in malignancy.

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