The Turkish Journal of Pediatrics
1973 / Vol. 15 / No. 2 / pp. 108 - 117

Somatic Cell Hybrids and Human Genetics*

Charlotte Boone Halıcıoğlu, Ph.D.**

The study of genetics in man is made difficult by his long generation and gestation time, the fact that he is a diploid organism (which hinders the appearence of new mutations) and his understandable objection to controlled matings. Genetic mapping studies in the classical sense are limited to families possessing detectable variant gene products as markers. So far, human genes have been chromosomally assigned in large number only to the X chromosome, since X-linked genes are present in hemizygous condition in males. The possibility of "repairing" genetic defects by something like a virus infection has come nearer to being a reality in recent years. If this repair is to be brought about by a virus-like infection, with the "virus" containing genetic material for the "correct" gene, then it may contain as well other, neighboring genes. A neighboring gene in excess may produce deleterious effects. Therefore, it becomes more important to know the exact order of genes on chromosomes.

The two basic requirements for genetic linkage studies are: 1. Various dependable genetic markers and 2. Some way of demonstrating recombination or segregation of these markers.

Genetic markers may be of various types in whole organisms, ranging from eye color to enzymes of the red blood cells. Recombination and segregation in the whole organism are usually brought about by events at meiosis: independent assortment and exchange between chromosomes as a result of cross-over events.

The discovery that cells grown in tissue culture could be made to fuse, and that cells of very different types (even different species) could be fused has provided an approach to human genetics that circumvents

* From Hacettepe University Faculty of Medicine, Department of Pediatrics, Hacettepe Children's Hospital, Ankara, Turkey
** Visiting Research Associate in Pediatrics and Clinical Genetics

some of the problems mentioned above. Cell fusion may result in two different products: 1. Heterokaryon or, 2. "Hybrid" type cell. In the heterokaryon, nuclei from two different types of cell are combined in one cytoplasm. In a truly hybrid type cell, the nuclear contents of two different cells have combined in one nucleus in one cytoplasm.

The first demonstration of fusion of cells in tissue culture was made by Barski and colleagues in 1960.[1] They were looking for the transfer of characteristics in mixed cultures of two lines of mouse tumor-producing cells which differed in culture morphology, chromosome markers and tumor-producing ability. Eventually they detected chromosomally "hybrid" cells; that is, a single cell containing a chromosome number approximately equal to the sum of the two input cells and containing as well marker chromosomes from each each of the two input lines.

Ephrussi and co-workers expanded the field by showing that such fusion was not limited to the same strain of mouse[2] and eventually showing that is was even possible to form hybrids between cultured cells derived from different species: mouse x rat.[3]

Two important advances were made in the next few years. First was the development of culture techniques which selected for hybrid type cells and against the input cell lines. The second was the discovery of ways to increase the frequency of hybrid cell formation. Left alone, cells appeared to fuse at a rate similar to spontaneous mutation rates.

Selective Techniques

Littlefield's "Aminopterin" selective technique[4] and its modifications[5] have been the most widely used in hybrid selection. The Littlefield technique involves using two single-enzyme-deficient cell lines as parents of the hybrid. Aminopterin blocks the *de novo* synthesis of DNA precursors, and if a cell lacks the enzymes thymidine kinase (TK) or hypoxanthine-guanine phosphoribosyl transferase (HGPRT), it can not survive in the presence of aminopterin. It has been possible to obtain cell lines which lack TK and others which lack HGPRT. If two such cell cultures are mixed in the presence of aminopterin, hypoxanthine and thymidine (HAT) the only types of cells which can grow are genetic revertants (back mutations,e.g.: $TK^- \rightarrow TK^+$) or fusion products. These two events can be distinguished by the use of separate markers (chromosome number and morphology, enzyme differences, etc.).

Since it is difficult to obtain reliable, non-revertant cell lines deficient for enzymes such as TK and HGPRT, the introduction of the

"half system" has been helpful.[5] This technique uses only one enzyme-deficient line in combination with a strain of normal fibroblasts which usually can be distinguished by morphology in culture. Established cell lines (from which biochemically deficient lines are usually derived) are often rapidly growing, highly refractile cells which can pile up on top of each other in culture; whereas, normal fibroblastic cells are less refractile, slowly growing and generally stop increasing when they have formed a single cell layer on the culture vessel. When such cell lines are mixed in HAT medium, the deficient line is unable to grow. The normal cell line can grow, but grows more slowly with a distinctive appearance. Hybrids that may form can be selected as more refractile colonies in which cells are piled up on top of one another. (In general the hybrids that are selected resemble the deficient line in morphology, and growth pattern, but this may be a prejudice imposed by the nature of the selective system.)

One attempt to overcome the difficulty in obtaining deficient cell lines has been to use fibroblasts grown from individuals known to lack certain enzymes. For example, cells grown from individuals with the Lesch-Nyhan Syndrome are deficient for HGPRT.

A further selective technique involves peripheral leukocytes in combination with a TK^- or $HGPRT^-$ tissue culture line in aminopterin-containing medium. Peripheral leukocytes do not attach to the culture vessel, although they can grow in HAT medium; the deficient cell line can attach, but can not grow in the presence of aminopterin; the hybrid presumably would grow and attach to the culture surface.[6]

Methods to Increase Hybrid Formation Frequency

The best results have been obtained by the use of beta-propriolactone inactivated Sendai virus.[7] This virus is known to cause cell fusion but when live virus is used, the fused cells usually die. With a properly inactivated virus and a carefully controlled virus titer, viable hybrid cells composed of only two input cells can be obtained in larger number. It may be that non-virally induced hybrids are caused by the spontaneous activation of a latent virus in one of the parent cells.

Marker Systems

Cultured cells usually retain enzymes and surface antigens, and to some extent, chromosome complements characteristic of the species of origin. These can all be used as markers, if the two cells to be hybridized come from different species. So far, differences in electrophoretic mobility

of various enzymes has been one of the most frequently used genetic marker systems in hybridization studies. Sometimes tumor cells adapted to tissue culture continue to produce specific end products.[8] If such cells are hybridized with non-specific types of cultured cells, the specific products can then be used as markers.

Segregation

Ephrussi and co-workers[2] were among the earliest to look for segregation of chromosomes (i.e., selective loss of certain chromosomes) in hybrid cells. They were hindered in their early work, however, by the fact that both input cells were from the mouse.

The introduction of interspecific hybrids[3] simplified the search for segregation of chromosomes. The discovery by Weiss and Green[9] that in mouse x human hybrids, human chromosomes were preferentially lost (or excluded) has provided a much-needed convenience for assignment of genes to specific human chromosomes. In this system a mouse established cell line deficient for TK or HGPRT is combined with a line of human cells. The gene for TK or HGPRT must come from the human and therefore, the human chromosome containing that gene should be retained even if no others are. In this way, the gene for TK has been localized to human chromosome E_{17}[10], and the chromosome localization of HGPRT in humans has been verified.[11]

The recent development of banding techniques for specific identification of chromosomes[12 13] has aided in the discrimination between mouse and human chromosomes.

USES OF HYBRIDS IN GENETICS

Genetics Linkage and Chromosome Mapping

If two human genes are linked, i.e., on the same chromosome, then whenever one is found in a hybrid, the other should be found as well. Conversely, whenever one is absent, or lost, the other should be lost as well. This constant correlation of two human genes has been used to establish linkage relationships, especially for enzymes which can be visualized electrophoretically. It can be used to establish both negative or positive linkage relationships. If, for example, we examined five different mouse x human hybrids for several different human enzymes, as in Table I, we could make the following conclusions: the human genes for IDH, MOD and Peptidase A were not linked to each other and were not linked either to LDH-A or to Aryl-Esterase.

However, we would be led to suspect linkage between LDH-A and Aryl-Esterase. Furthermore, we could also conclude that none of the genes for these enzymes was linked to the genes for TK, since linkage with TK would mean that enzyme should be present in all of the hybrids.

TABLE I

HUMAN ENZYME DISTRIBUTION AMONG VARIOUS MOUSE X HUMAN HYBRIDS (HYPOTHETICAL)

Hybrid	TK	IDH	MOD	LDH-A	Aryl Esterase	Peptidase A
1	+	+	−	−	−	−
2	+	−	+	−	−	−
3	+	−	−	+	+	+
4	+	+	−	+	+	−
5	+	+	+	−	−	+

TK = Thymidine kinase; IDH = isocitrate dehydrogenase; MOD = malate oxidoreductase decarboxylase; LDH-A = Lactate dehydrogenase, A subunit.

If we now examine the chromosomes of the different hybrids of Table I, we can attempt to correlate the presence or absence of a parti cular human chromosome with the presence or absence of a particular human enzyme; that is, if the number of human chromosomes present is sufficiently small. Frequently, the human chromosome number in mouse x human hybrids is no greater than five.[14] The original hybrid populations isolated are not likely to be homogeneous (pure) populations with regard to chromosome complements. During the early divisions of the hybrid cell, a certain amount of chromosome loss is to be expected, and since this loss may be different in different cells, a certain degree of heterogeneity is to be expected. Thus, we may find variation around a modal chromosome number, depending on the greater or lesser loss of human chromosomes.

From the hypothetical results given in Table II, we would expect the human genes for LDH-A and Aryl Esterase to be located on C_{11}, the gene for IDH to be on F_{20} and the gene for Peptidase A on B_5.

These expectations can be reinforced by cloning experiments, which should reveal karyotype heterogeneities in the original hybrid. The results of such a hypothetical cloning experiment for hybrid number 3 of Table II are given in Table III. Using such methods, Boone, *et al.*[14] were able to show that the human gene for LDH-A was associated

TABLE II

HYPOTHETICAL CHROMOSOMAL LOCALIZATION OF HUMAN ENZYMES IN MOUSE X HUMAN HYBRIDS

Hybrid	Human Chromosomes						Human Enzymes				
	A_1	B_5	C_9	C_{11}	E_{17}	F_{20}	TK	LDH-A	Pep-A	Aryl-Es	IDH
1	+	−	+	−	+	−	+	−	−	−	−
2	−	+	−	−	+	−	+	−	+	−	−
3	−	−	−	+	+	+	+	+	−	+	+
4	+	−	−	−	+	+	+	−	−	−	+
5	+	+	−	−	+	+	+	−	+	−	+
6	−	−	−	+	+	−	+	+	−	+	−

TABLE III

HYPOTHETICAL CLONAL ANALYSIS OF HUMAN CHROMOSOMES AND ENZYME DISTRIBUTION FROM MOUSE X HUMAN] HYBRID NUMBER THREE

Clone	Human Chromosomes			Human Enzymes			
	C_{11}	E_{17}	F_{19}	TK	LDH-A	Aryl Es	IDH
a	+	+	+	+	+	+	+
b	+	+	−	+	+	+	−
c	−	+	+	+	−	−	+
d	−	+	+	+	−	−	+
e	−	+	−	+	−	−	−
f	−	+	+	+	−	−	+
g	+	+	−	+	+	+	−

with C_{11} and IDH with F_{20}. Separately, Shows[15] reported constant correlation between the presence of Aryl Esterase A-4 and LDH-A in mouse x human hybrids.

There is, of course, a risk involved in such linkage and chromosome mapping studies. It is possible that chromosomal rearrangements may take place and thereby disrupt normal linkage relationships. This difficulty may be overcome by examining large numbers of different hybrids, and by using the new banding techniques for fine analysis of chromosome rearrangements[16]. In fact, such rearrangements may even be used to advantage in localizing a gene to a specific region of a human chromosome, as pointed out for the TK gene.[14]

OTHER USES

Complementation

Somatic cell hybrids (especially heterokaryons) have provided valuable information about genetic heterogeneity among certain clinically similar enzyme-deficient individuals. If cells grown from two clinically similar individuals are combined in a heterokaryon and examined for the enzyme in question, we can determine whether the two cases result from similar or different mutations. If the enzyme remains deficient in the heterokaryon, we may conclude that the mutations involved are similar. If, however, enzyme activity is restored in the heterokaryon, we would conclude that the two similar phenotypes result from dissimilar mutations.

Such studies have been performed in cases of galactosemia.[17] Cultures were derived from seven different patients with galactosemia. Various two by two combinations of cultures were made to produce different types of heterokaryons. One of the fibroblast cultures was able to complement all the other six in hybrid combination (that is, enzyme activity was restored in the heterokaryon), indicating that at least two different mutations may be involved in galactosemia.

Virology

When a fibroblast cell culture is exposed to virus, the virus may infect, proliferate in the cells and eventually destroy the cells, or the virus may transform the cell cultures into heteroploid type cell lines. Transformation is thought to result from incorporation of the virus genome within the genome of the host cell. This incorporation has been difficult to demonstrate experimentally. The virus is thought to be responsible for production of new antigens in the transformed cell, but recovery of virus from such cells was not demonstrated.

Koprowski[10] and co-workers formed a heterokaryon between a cell line transformed by SV-40 virus and a normal cell. Although no live virus had ever been recovered from the SV-40 transformed parent, SV-40 virus infections were found to be produced by the hybrid cell. The change in internal cellular environment caused by the hybrid condition apparently was sufficient to activate the latent virus.

Weiss[19] has attempted to locate the incorporated SV-40 genome to a particular human chromosome (or chromosomes) by using an SV-40 transformed human cell hybridized with a mouse established cell line. Again the human chromosomes are preferentially lost. In this case, the

marker is the antigen associated with transformation. Hybrids are analyzed for presence of this specific antigen as well as for human chromosome complement, in a manner analogous to the one used for enzyme mapping. As yet she has been unable to localize the region responsible for the SV-40 antigen to any one particular chromosome.

Gene Regulation

The study of gene regulation (i.e., what causes one gene to function at a certain time and place in development and another to function at another time and place) is a very important and difficult problem. Let us take hemoglobin production as an example. Normally in the human fetus gamma and alpha chains are produced to form fetal hemoglobin. After birth beta chains predominate over the gamma, and combine with alpha chains to produce adult hemoglobin. Why does the synthesis of one molecular species (the gamma chain) subside, and another (the beta chain) begin ? If we knew some way to control this genetic activity, we might be able to overcome such diseases as thalassemia (in which abnormal beta chains are produced) by causing the synthesis of gamma chains to continue in place of beta chains.

Somatic cell hybrids have been used in attempts to understand genetic regulation (See review by Davidson[20]). Fusion of "differentiated" type cells with undifferentiated cells usually results in a more or less undifferentiated type hybrid cell, and has led to the idea of a "repressor" type substance acting in such hybrids. Furthermore, Klebe et al.[21] found evidence for a regulator element in hybrids between a mouse renal adenocarcinoma and human fibroblasts. The production of a kidney specific enzyme of the *mouse* cell was apparently correlated with the absence of a specific *human* chromosome. When this human chromosome was present, the mouse enzyme was not produced. This is even more interesting because the regulator element apparently acts across a species boundary.

Tumor Studies

Cell hybrids are also being used in the study of tumors. When the highly malignant Ehrlich ascites mouse tumor is injected into healthy mice it is usually lethal, primarily because of the tumor's weak antigenic properties. If, however, the Ehrlich tumor cells are fused with hamster cells in a hybrid and the hybrid cells are injected into mice, the mice apparently acquire immunity to the Ehrlich tumor cells.[22] The implications of such studies for their possible use in the treatment of human tumors are obviously exciting.

REFERENCES

1. Barski, G., Sorieul, S., and Cornefert, F.: Production dans des cultures in vitro de deux souches cellulaire en association, de cellules de caractere hybride, Compte. rend. Acad. d. Sc. 251: 1825, 1960.
2. Ephrussi, B., Stenchever, M. A., and Scaletta, L. J.: Hybridization as a tool for Cell Genetics. pp 85-93 in Second International Conference on Congenital Malformations, Morris Fishbein, Ed., International Medical Congress, Ltd., N. Y., 1964.
3. Ephrussi, B., and Weiss, M. C.: Interspecific hybridization of somatic cells, Proc. Natl. Acad. Sci. U.S.A. 53: 1040, 1965.
4. Littlefield, J. W.: Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants, Science 145: 709, 1964.
5. Davidson, R. L., and Ephrussi, B.: A selective system for the isolation of hybrids between L cells and normal cells, Nature (Lond.) 205: 1170, 1965.
6. Miggiano, V., Nabholz, M., and Bodmer, W.: Hybrids between human leukocytes and a mouse cell line: production and characterization, Wistar Institute Symposium, 1968.
7. Klebe, R. J., Chen, T., and Ruddle, F. H.: Controlled production of proliferating somatic cell hybrids, J. Cell Biol. 45: 74, 1970.
8. Sato, G., Augusti-Tocco, G., and Posner, M.: Hormone-secreting and hormone-responsive cell cultûres, Recent Prog. Horm. Res. 26: 539, 1970.
9. Weiss, M. C., and Green, H.: Human-mouse hybrid cell lines containing partial complements of human chromosomes and functioning human genes, Proc. Natl. Acad. Sci. U.S.A. 58: 1104, 1967.
10. Migeon, B. R., and Miller, C. S.: Human-mouse somatic cell hybrids with single human chromosome (group E): link with thymidine kinase activity, Science 162: 1005, 1968.
11. Nabholz, M., Miggiano, V., and Bodmer, W.: Genetic analysis with human-mouse somatic cell hybrids, Nature (Lond.) 223: 358, 1969.
12. Caspersson, T., Zech, L., and Johansson, C.: Analysis of human metaphase chromosome set by aid of DNA-binding fluorescent agents, Exp. Cell Res. 62: 490, 1970
13. Sumner, A. T., Evans, H. J., and Bucklane, R. A.: A new technique for distinguishing between human chromosomes, Nature (New Biol.) 232: 31, 1971.
14. Boone, C., Chen, T., and Ruddle, F.: Assignment of three human genes to chromosomes (LDH-A to 11, TK to 17, and IDH to 20) and evidence for translocation between human and mouse chromosomes in somatic cell hybrids (thymidine kinase-lactate dehydrogenase A-isocitrate dehydrogenase-C-11, E-17, and F-20 chromosomes), Proc. Natl. Acad. Sci. U.S.A. 69: 510, 1972.
15. Shows, T. B.: Genetics of human-mouse somatic cell hybrids: linkage of human genes for lactate dehydrogenase-A and esterase-A 4, Proc. Natl. Acad. Sci. U.S.A. 69: 348, 1972.
16. Seabright, M.: The use of proteolytic enzymes for the mapping of structural rearrangements in the chromosomes of man, Chromosoma 36: 204, 1972.
17. Nadler, H. L., Chacko, C. M., and Rachmeler, M.: Interallelic complementation in hybrid cells derived from human diploid strains deficient in galactose-1-phosphate uridyl transferase activity, Proc. Natl. Acad. Sci. U.S.A. 67: 976, 1970.

18. Koprowski, H., Jensen, F. C., and Steplewski, Z.: Activation of production of infectious tumor virus SV 40 in heterokaryon cultures, Proc. Natl. Acad. Sci. U.S.A. 58: 127, 1967.

19. Weiss, M. C.: Further studies on loss of T-antigen from somatic hybrids between mouse cells and SV 40-transformed human cells, Proc. Natl. Acad. Sci. U.S.A. 66: 79, 1970.

20. Davidson, R. L.: Regulation of gene expression in somatic cell hybrids: a review, In Vitro 6: 411, 1971.

21. Klebe, R. J., Chen, T., and Ruddle, F. H.: Mapping of a human genetic regulator element by somatic cell genetic analysis, Proc. Natl. Acad. Sci. U.S.A. 66: 1220, 1970.

22. Watkins, J. F., and Chen, L.: Immunization of mice against Ehrlich ascites tumor using a hamster-Ehrlich ascites tumor hybrid cell line, Nature (Lond.) 223: 1018, 1969.