HUMAN CELLS IN CULTURE: REVISITED*

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SUMMARY

Human cells in vitro *offer considerable advantages in the study and detection of defects in human chromosomes and gene products. Of all the euploid cells of the mammalian organism the fibroblasts are capable of reflecting many different metabolic disorders. These methods, combined with recently developed techniques for the early detection of genetic disorders allow the assessment of a 'high risk' pregnancy.*

Cell hybridization has become of paramount importance to genetic analysis and has proved very useful in assigning gene information to certain chromosomes.

Over the past 10 years, human somatic cells have for various purposes, especially with regard to genetic analysis, been grown *in vitro*. All the cells of the body, except sperm or egg cells, are somatic cells, of which many can be grown in culture as micro-organisms. Human cells in culture, as opposed to 'man' *in toto*, offer considerable advantages, e.g. the generation time is reduced to about 1/10 000 that of the whole organism; the number of progeny derived from a single cell by far exceeds the progeny derived from a single individual, and no moral or ethical commitments are involved. In addition, cells in culture can be exposed to an unlimited number of environmental conditions, and with the number of culture techniques and methods available a remarkable degree of repeatability can be maintained.

In order to provide a working hypothesis it is assumed that the biology of *in vitro* somatic cells in culture more or less reflects the cellular biology of the donor tissue.[1,2] In the case of homoploid or homonuclear cells especially, this assumption can be considered valid and only seldom, e.g. when individuals are genetic mosaics, can the assumption be debated.

Some of the major reasons for utilizing man's somatic cells are: the identification and characterization of the genome; the detection of defects in the genome and the identification of factors causing changes in the genome.

It suffices to point out that cultivated mammalian cells impose a limitation on the number of gene loci that can be studied in these cells. Unfortunately, serially propagated mammalian cells do not normally carry out all the specific functions of the tissue from which they were isolated. This limitation removes many important gene-markers. Although only in experimental animals, there is some indication that the loss of tissue-specificity can be overcome.[3] Nevertheless, it is reasonable to assume that if an enzyme is present in white or red blood cells and in parenchymatous tissue other than the spleen, it is likely to be ubiquitous and therefore present in cultured cells.[4]

A few examples, pertaining especially to medical problems, will serve to demonstrate some of the more important applications of somatic cell genetics. Only results obtained from man's homonuclear somatic cells rather than heteronuclear cell lines, which are less relevant to the problems in medical genetics will be considered.

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The standard methods involved in growing somatic cells *in vitro* are relatively easy, and several reviews summarize the basic principles.[5,6] Fibroblasts, obtained by skin biopsy, are very often used for cell cultures. A small portion of skin is removed and put into a suitable culture medium under sterile conditions. The medium contains all the essential amino acids, vitamins, sugar, minerals and protein in the form of foetal serum. The addition of an antibiotic prevents bacterial infection, and for optimal growth a pH of 7·4 and a temperature of 37°C is usually maintained. Subsequently a large number of subcultures can be made from the initial explant.

Testing Mutagenic Agents

As mentioned, human cells *in vitro* can be subjected to virtually any artificial or environmental factor, e.g. chemicals, viruses, radiation and other physical factors. With the aid of cytogenetic analysis the qualitative and quantitative effects of the agents can be assayed at the chromosome level, e.g. the number of chromosome aberrations caused per cell, which may vary significantly when the dose increase of X-rays is compared with the increase of neutrons.[7]

Diagnosis of Genetic Defects

An individual's chromosome set can be examined with the aid of appropriate staining techniques after adequate growth in fibroblast culture has been obtained. Many structural changes in the chromosome set are often caused by phenotypic imbalances. In cases where no adequate supply of blood can be gained (e.g. in stillbirths), analysis can readily be carried out on fibroblasts (Table I).

TABLE I. INDICATIONS FOR CHROMOSOME ANALYSIS

I. Detected in: Fibroblasts, lymphocytes and bone marrow.
   1. Combination of general growth retardation, mental retardation, deformities and dermatoglyphic abnormalities.
   2. Confirming clinical diagnosis of congenital chromosomal syndromes.
   3. Familial occurrence of mongolism.
   4. Parents under the age of 30 with mongoloid children.
   5. Parents with recurrent irregularities in reproduction (familial occurrence of normal children with deformed or aborted foetuses at an early gestation).
   6. Radiation accidents.
   7. Disparity between clinical finding and sex chromatin finding.
   8. Myeloproliferating syndromes, Bloom syndrome, Fanconi anaemia and hereditary ataxia telangiasia.

II. Detected in: Amniotic cells.
   1. Translocation heterozygote in one parent.
   2. Pregnancy in elderly mothers.

Gene Mutations—Point Mutations

Point mutations can be assessed by measuring the enzyme activity, biochemical analysis of the phenotype in selective culture media, analysis of incorporation studies using radio-active metabolites, or direct histological staining techniques. Already more than 30 defects, resulting from point mutations which involve defective enzymes in the normal metabolism, can be determined from fibroblasts in culture.

In most cases the genes involved in point mutations are located on the autosomal chromosomes and seldom on the X-chromosome (Table II). The significance of these detection methods is especially realized when not only homozygotes but also heterozygote carriers can be ascertained.

TABLE II. EXAMPLES OF GENE MUTATIONS DEMONSTRABLE IN CULTURED FIBROBLASTS[32,33]

Disease	*Enzyme defect*	*Genetics*
Acatalasia Type I	Catalase	Autosomal recessive
Type II		
'Maple-syrup' disease	Oxydative decarboxylation of α-ketoisocaproic acid α-keto-β-methyl valeric acid and α-ketoisovaleric acid	Autosomal recessive
Citrullinaemia	Argininosuccinate synthetase	Autosomal recessive
Chediak-Higashi syndrome	*	Autosomal recessive
Argininosuccinic-aciduria	Argininosuccinase	Autosomal recessive
Cystinosis	*	Autosomal recessive
Cystathioninuria	Cystathionase	Autosomal recessive
Galactosaemia	UDP-galactose transferase	Autosomal recessive
Hurler's disease	*	Co-dominant
Glycogen storage disease: Type II	Lysosomal α -1-4 glucosidase	Autosomal recessive
Lesch-Nyhan syndrome	Hypoxanthine guanine phosphoribosyl transferase	X-linked
Gaucher's disease	Glucocerebrosidase	Autosomal recessive
Niemann-Pick disease	*	Autosomal recessive
Oroticaciduria	Orotidine-5′ - monophosphopyrophosphorylase and orotidine- 5′ - monophosphate decarboxylase	Autosomal recessive
Refsum disease	Pythanic acid α-hydroxylase	

*Unspecific test

In order to distinguish between different genotypes the utilization of selective media is also heavily relied on. Under these conditions individual genotypes may be identified according to a characteristic growth rate and the difference between a normal person, a homozygote defective, or heterozygote, for in these the mutated gene can be recognized. Cases in which these tests have been successfully applied are citrullinaemia, galactosaemia, argininosuccinicaciduria and oroticaciduria.[8-11]

With recently developed techniques, it is to be expected that further examples will be added to the ever-increasing list of 'inborn errors of metabolism', where the mechanism of the defective gene, even in the heterozygote state, will be characterized. An already classic example is the syndrome: choreoathetosis, spastic paraplegia, oligophrenia and self-mutilation. Besides the typical symptoms, this syndrome is further identified by the elevated level of uric acid in the blood of affected children.[12] This disease, the first in which a direct relationship is found between a behavioural disturbance and a defective gene, is prevalent only in male individuals who are carriers of the recessive X-linked gene mutation.[13] As in the case of haemophilia, the females are the carriers of the defective gene. The biochemical defect in this case is the inability of cells to convert the purine bases hypoxanthine and guanine to metabolites necessary for nucleic acid synthesis. This defect can be demonstrated in cells *in vitro* by supplying the cells in culture with radio-active purine bases. Normal cells will incorporate and convert these substances into radio-active nucleic acids whereas cells from a Lesch-Nyhan patient will remain unmarked.[14] Cells grown from the mothers of affected males, however, show that only 50% of the cell population is defective, which furnishes an excellent example in support of the Lyon X-inactivation theory.[15]

Storage Diseases Identified by Metachromasia

In some cases of storage diseases the phenotype can be identified by the phenomenon of metachromasia, i.e. when cultured fibroblasts are stained with certain basic dyes. This technique has proved very useful in supporting the suspected diagnoses in cases such as cystic fibrosis, Hurler's disease, syndrome, Chediak-Higashi syndrome and Morbus Gaucher's disease.[16-19]

Kinetics of Cells in Culture

The kinetics and characteristics of cells in culture are studied more readily with the method of cloning (i.e. a single cell is isolated and allowed to multiply into a colony) and the growth rate, for instance, of particular cell types can be studied. There appears to be a close correlation between the *in vivo* age of the donor individual and the *in vitro* age of the cell culture,[20] as there is an indication that fibroblasts obtained from diabetic or pre-diabetic individuals exhibit a reduced growth capacity as compared with normal individuals.[38] Utilizing the growth rate of clones, i.e. the plating efficiency of fibroblasts, could have far-reaching consequences in cases where prognoses depend on early diagnosis.

THE EARLY DETECTION OF GENETIC DEFECTS

With the increase in therapeutic procedures it has become increasingly necessary to rely on the early detection of genetic diseases for prognoses. Treatment at an early stage is especially indicated in cases such as phenylketonuria, galactosaemia, 'maple-syrup' disease and the adrenogenital syndrome, where irreversible damage can be prevented. The resulting degree of mental retardation to a large extent depends on the stage at which therapy is administered to newborn infants.

The method of amniocentesis at and beyond the 32nd week of pregnancy, has for many years been applied as a routine procedure in the diagnosis of Rh-incompatibility. It has further been shown that amniocentesis can be performed very successfully prior to the 20th week of gestation.[1,33,35-37] Amniotic fluid obtained in this manner contains desquamated cells derived from the foetus and the amnion[21] and consequently contains the intact genome of the developing individual. The ability to culture amniotic fluid cells has been reviewed extensively by several investigators.[22,23] Amniotic cells in culture represent epithelial cells at first and later take on the characteristics of fibroblasts. The cells are then subjected to cytological and biochemical analysis.[28] A very promising development currently in progress will allow a skin biopsy to be taken from a foetus at an early age.[24] The cell growth from a skin biopsy is much more efficient and tests can be carried out within one week of the punctuation, and still leave ample time for a repeat if necessary. Defects ranging from chromosome to point mutations can subsequently be assessed at an early stage, thus enabling positive eugenics to be exercised in countries which comply with the demands of modern legislation. The method of analysis is summarized in Fig. 1.

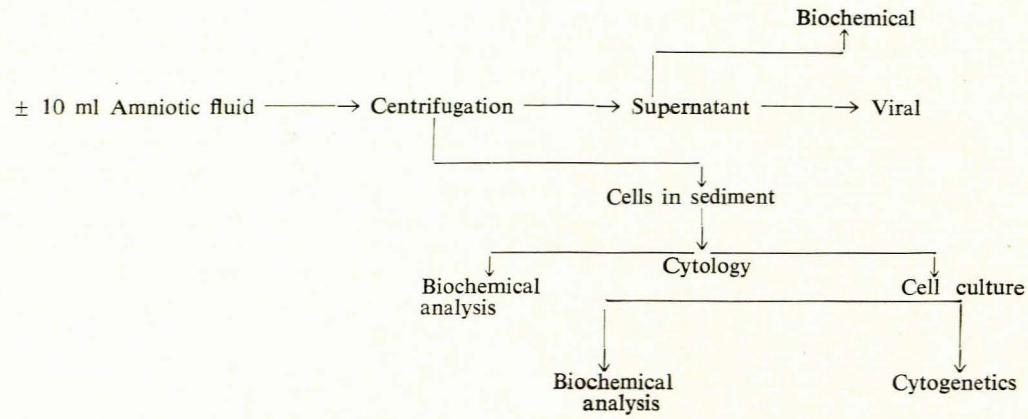

Fig. 1. Possible analyses after amniocentesis. (Adapted from Nadler.[25])

The activity of several enzymes can also be assayed in amniotic-fluid cells directly after amniocentesis prior to culturing[26] (Table III). There appears to be a direct correlation between the level of enzyme activity and foetal age,[25,27] and potentially this could serve as an aid to assess the maturity of the foetus in cases of uncertainty.

TABLE III. ENZYMES POSSIBLE TO ASSAY IN NON-CULTIVATED AMNIOTIC FLUID CELLS[34]

Acid phosphatase
Alkaline phosphatase
α - glucosidase
Galactose - 1 - phosphate uridyl transferase
Glucose - 6 - phosphate dehydrogenase
Hypoxanthine guanine phosphoribosyl transferase
Lactate dehydrogenase
Ornithine transcarbamylase
6 - phosphogluconic dehydrogenase
Valine transaminase

CELL HYBRIDIZATION

Although the method of cell hybridization has until recently proved to be of limited value in clinical practice, it has become of paramount importance to genetic analysis. Cells of different mammalian species are capable of fusing under suitable conditions *in vitro* (e.g. low temperature, and UV-inactivated viruses).[2,27] The consequent hybrid cells exhibit properties very useful to the geneticist:

(a) The chromosome complement of both parent strains remains functionally intact and therefore exhibits properties of both parents. Significant differences in chromosome morphology of the two parents can subsequently be recognized in the hybrid cells.[29]

(b) With subsequent divisions of the hybrid, a gradual decrease in chromosome numbers is experienced. This phenomenon is considered with corresponding changes in assigning genetic information to certain chromosomes, i.e. gene mapping.[30]

(c) Although still in its infancy, this technique is presently being applied in cancer research. With hybrid, cancer/normal fibroblasts the loss of certain chromosomes could be correlated with the sudden loss of malignant cell growth.[31]

Of all the euploid cells of the mammalian organism, the fibroblasts are capable of reflecting many different metabolic disorders (Table II). It would, however, be of further benefit if more specialized cells such as liver parenchyma, kidney epithelium or highly specialized cells of acini endocrine glands could be grown *in vitro*, a better assessment of the *in vivo* situation could then be attained.

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