Cell growth characteristics and kinetics

THE NEED FOR CHARACTERIZATION :

There are six main requirements for cell line characterization:

(1) Demonstration of the absence of cross-contamination .

(2) Confirmation of the species of origin

(3) Correlation with the tissue of origin, which comprises the following characteristics:

a) Identification of the lineage to which the cell belongs

b) Position of the cells within that lineage (i.e., the stem, precursor, or differentiated status)

(4) Determination of whether the cell line is transformed or not:

a) Is the cell line finite or continuous

b) Does it express properties associated with malignancy

(5) Indication of whether the cell line is prone to genetic instability and phenotypic variation

(6) Identification of specific cell lines within a group from the same origin, selected cell strains, or hybrid cell lines, all of which require demonstration of features unique to that cell line or cell strain.

Characterization of a cell line is vital, not only in determining its functionality but also in proving its authenticity; special attention must be paid to the possibility that the cell line has become cross-contaminated with an existing continuous cell line or misidentified because of mislabeling or confusion in handling.

TABLE 16.1. Characterization of Cell Lines and Cell Strains

Criterion	Method
Karyotype	Chromosome spread with banding
Isoenzyme analysis	Agar gel electrophoresis
Cell surface antigens	Immunohistochemistry
Cytoskeleton	Immunocytochemistry with antibodies to specific cytokeratins
DNA fingerprint	Restriction enzyme digest; PAGE; satellite DNA probes
DNA Profile	PCR of microsatellite repeats

SPECIES IDENTIFICATION :

Chromosome analysis is otherwise known as karyotyping is one of the methods for distinguishing between species. Chromosome banding patterns can be used to distinguish human and mouse chromosomes and chromosome painting, i.e., using combinations of specific molecular probes to hybridize to individual chromosomes , adds further resolution and specificity to this technique. These probes identify individual chromosome pairs and are species specific. The availability of probes is limited to a few species at present, and most are either mouse or human, but chromosome painting is a good method for distinguishing between human and mouse chromosomes in potential cross-contaminations and interspecific hybrids. Isoenzyme electrophoresis , is also a good diagnostic test and is quicker than chromosomal analysis. A simple kit is available that makes this technique readily accessible . A combination of the two methods is often used and gives unambiguous results .

LINEAGE OR TISSUE MARKERS :

Individual organs are comprised of tissues, e.g., skin is made up of an outer epidermis and underlying dermis, and tissues, in turn, are made up of individual lineages, e.g., the dermis contains connective tissue fibrocytes, vascular endothelial cells and smooth muscle cells, and the mesenchymal cells of the dermal papillae, among others. Each cell type can be traced back, via a series of proliferating cell stages, to an originating stem cell , forming a treelike structure. Each ''branch'' of that ''tree'' can be regarded as a *lineage*, as in a basal cell of the epidermis following a differentiation path to a mature cornified keratinocyte. Some lineages, e.g., the myeloid lineage of hematopoietic differentiation, may branch into sublineages (neutrophilic,

eosinophilic, and basophilic), so lineage marker expression is also influenced by *differentiation*, i.e., the position of the cell in the lineage differentiation pathway .Lineage markers are helpful in establishing the relationship of a particular cell line to its tissue of origin.

Cell surface antigens. These markers are particularly useful in sorting hematopoietic cells and have also been effective in discriminating epithelium from stroma with antibodies such as anti-EMA and anti-HMFG 1 and 2 and neuroectodermally derived cells (e.g., anti-A2B5) from cells derived from other germ layers.

Intermediate filament proteins. These are among the most widely used lineage or tissue markers. Glial fibrillary acidic protein (GFAP) for astrocytes and desmin for muscle are the most specific, whereas cytokeratin marks epithelial cells and mesothelium .Neurofilament protein marks neurons and some neuroendocrine cells ,although usually restricted to mesodermally derived cells *in vivo*, can appear in other cell types *in vitro*.

Differentiated products and functions. Hemoglobin for erythroid cells, myosin or tropomyosin for muscle, melanin for melanocytes, and serum albumin for hepatocytes are among the best examples of specific cell type markers, but, like all differentiation markers, they depend on the complete expression of the differentiated phenotype.

Enzymes. Three parameters are available in enzymic characterization: (1) the constitutive level (i.e., in the absence of inducers or repressors); (2) the response to inducers and repressors; and (3) isoenzyme polymorphisms.

TABLE 16.2. Enzymic Markers

Enzyme	Cell type	Inducer	Repressor
Glutamyl synthetase	Astroglia (brain)	Hydrocortisone	Glutamine
Tyrosine aminotransferase	Hepatocytes	Hydrocortisone	
Sucrase	Enterocytes	NaBt	
Alkaline phosphatase	Type II pneumocyte (in lung alveolus)	Dexamethasone, oncostatin, IL-6	TGF-β
Alkaline phosphatase	Enterocytes	Dexamethasone, NaBt	
Nonspecific esterase	Macrophages	PMA, Vitamin D_3	
Angiotensin-converting enzyme	Endothelium	Collagen, Matrigel	
Neuron-specific enolase	Neurons, neuroendocrine cells		
Tyrosinase	Melanocytes	cAMP	
DOPA-decarboxylase	Neurons, SCLC		
Creatine kinase MM	Muscle cells	IGF-II	FGF-1,2,7
Creatine kinase BB	Neurons, neuroendocrine cells, SCLC		

Unique Markers

Unique markers include specific chromosomal aberrations (e.g., deletions, translocations, polysomy); major histocom-patibility (MHC) group antigens (e.g., HLA in humans), which are highly polymorphic; and DNA fingerprinting or profiling.

CELL MORPHOLOGY :Observation of morphology is the simplest and most direct technique used to identify cells.

Microscopy : The inverted microscope is one of the most important tools in the tissue culture laboratory.

Staining :A polychromatic blood stain, such as Giemsa, provides a convenient method of preparing a stained culture. Giemsa stain is usually combined with May–Grunwald¨ stain when staining blood, but not when staining cultured cells. Alone, it stains the nucleus pink or magenta,

the nucleoli dark blue, and the cytoplasm pale gray-blue. It stains cells fixed in alcohol or formaldehyde, but will not work correctly unless the preparation is completely anhydrous.

CHROMOSOME CONTENT : Chromosome content or *karyotype* is one of the most charac-teristic and best-defined criteria for identifying cell lines and relating them to the species and sex from which they were derived. Chromosome analysis can also distinguish between normal and transformed cells ,because the chro-mosome number is more stable in normal cells.

CHROMOSOME BANDING : This group of techniques was devised to enable individual chromosome pairs to be identified when there is little morphological difference between them.

CHROMOSOME ANALYSIS : The following are methods by which the chromosome complement may be analyzed:

Chromosome Count. Count the chromosome number per spread for between 50 and 100 spreads. (The chromosomes need not be banded.) Closed-circuit television or a camera lucida attachment may help. You should attempt to count all of the mitoses that you see and classify them (a) by chromosome number or (b), if counting is impossible, as ‘‘near diploid uncountable’’ or ‘‘polyploid uncountable.’’ Plot the results as a histogram .

Karyotype. Digitally photograph about 10 or 20 good spreads of banded chromosomes.

CHROMOSOME PAINTING : With the advent of fluorescently labeled probes that bind to specific regions, and even specific genes, on chromosomes, it has become possible to locate genes, identify translocations, and determine the species of origin of chromosomes.

DNA CONTENT :DNA can be measured by propidium iodide fluorescence with a CCD camera or flow cytometry. Analysis of DNA content is particularly useful in the characterization of transformed cells that are often aneuploid and heteroploid.

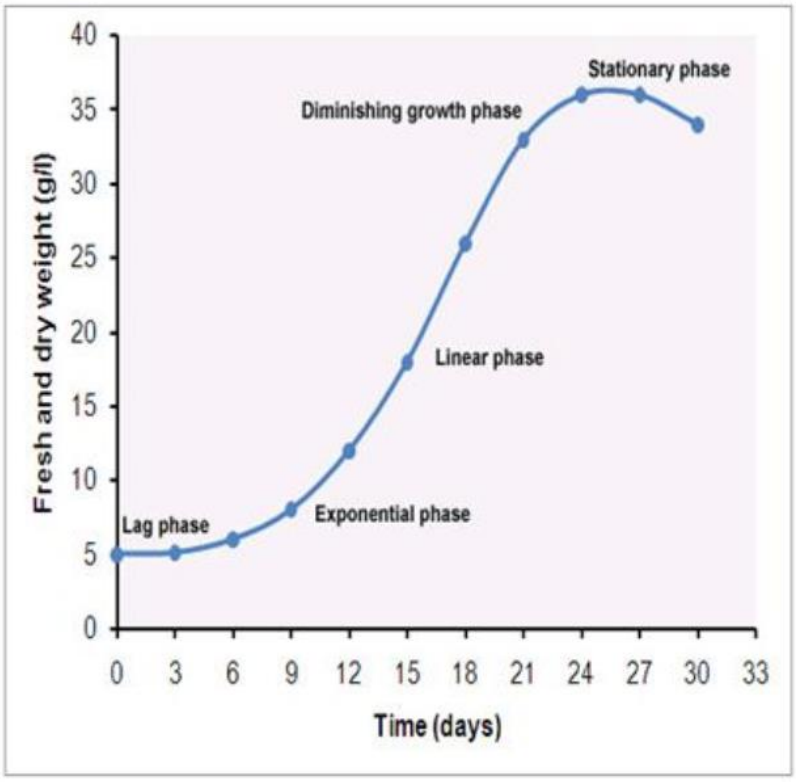
DNA HYBRIDIZATION : Hybridization of specific molecular probes to unique DNA sequences (Southern blotting) can provide information about species-specific regions, amplified regions of the DNA, or altered base sequences that are characteristic to that cell line.

DNA FINGERPRINTING :DNA contains regions known as satellite DNA that are apparently not transcribed. These regions are highly repetitive, and their lengths vary, with minisatellite

DNA having 1- to 30-kb repeats and microsatellite DNA having only 2–4 bases in repeating sequences. The functions of these regions are not fully understood; they may be purely structural or may provide a reservoir of potentially codable regions for genetic recombination in further evolution. Regardless of their function, however, these regions are not highly conserved, because they are not transcribed, and they give rise to regions of hypervariability. When the DNA is cut with specific endonucleases, specific sequences may be probed with cDNAs that hybridize to these hypervariable regions or they may be amplified by PCR with specific templates. The probes were originated by Jeffreys et al. . Electrophoresis reveals variations in fragment length in satellite DNA (restriction fragment length polymorphisms, RFLPs) that are specific to the individual from which the DNA was derived. When analyzed by polyacrylamide electrophoresis, each individual's DNA gives a specific hybridization pattern as revealed by autoradiography with radioactive or fluorescent probes. These patterns have come to be known as DNA fingerprints and are cell line specific, except if more than one cell line has been derived from one individual or if highly inbred donor animals have been used.

RNA AND PROTEIN EXPRESSION : Cells of a particular characteristic phenotype can be recognized by analysis of gene expression by Northern blotting [using radioactive, fluorescent, or luminescent probes.

KINETICS OF CELL GROWTH:



$$N = N_0 2^X$$

$$\log_{10} N = \log_{10} N_0 + X.\log_{10} 2$$

$$t_D = \frac{T}{X}$$

$$\mu = \frac{dN}{dt} \cdot \frac{1}{N}$$

$$\ln N = \ln N_o + \mu.t$$

$$\mu = \frac{\ln 2}{t_D} = \frac{0.6931}{t_D}$$

SCALE UP OF ANIMAL CELL CULTURES:

Modifying a laboratory procedure, so that it can be used on an industrial scale is called scaling up. Laboratory procedures are normally scaled up via intermediate models of increasing size. The larger the plant, the greater the running costs, as skilled people are required to monitor and maintain the machinery.The first pre-requisite for any large scale cell culture system and its scaling up is the establishment of a cell bank. Master cell banks (MCB) are first established and they are used to develop Master Working Cell Banks (MWCB). The MWCB should be sufficient to feed the production system at a particular scale for the predicted life of the product. The cell stability is an important criteria so MWCB needs to be repeatedly subcultured and each generation should be checked for changes. A close attention should be paid to the volume of cultured cells as the volume should be large enough to produce a product in amounts which is economically viable. The volume is maintained by a) increasing the culture volume, (b) by increasing the concentration of cells in a reactor by continuous perfusion of fresh medium, so that the cells keep on increasing in number without the dilution of the medium.

A fully automated bioreactor maintains the physicochemical and biological factors to optimum level and maintains the cells in suspension medium. The most suitable bioreactor used is a compact-loop bioreactor consisting of marine impellers. The animal cells unlike bacterial cells, grow very slowly. The main carbon and energy sources are glucose and glutamine. Lactate and ammonia are their metabolic products that affect growth and productivity of cells. So, the on-line monitoring of glucose, glutamate, and ammonia is carried out by on line flow injection analysis (FIA) using gas chromatography (GC), high performance liquid chromatography (HPLC) etc

.Cell cultures are used for obtaining useful products like bio-chemicals (interferon, interleukins, hormones, enzymes, antibodies, etc.) and virus vaccines (polio, mumps, measles, rabies, foot and mouth, rinderpest etc.).For these objectives, large scale cell cultures are essential; fermenters of 5,000 to 20,000 L are used for this purpose.

The scaling up of cell cultures may be done as follows:

(1) as monolayer cultures,

(2) as suspension cultures, or

(3) as immobilized cell systems.

For obvious reasons, scaling up of monolayer systems is more difficult than that of others.

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