FACULTY OF ENGINEERING & TECHNOLOGY

LT.8. Biology & characterization of cultured cells, Maintenance of cellline

Outline

1. Biology of cultured cells
    a. Environmental effect
    b. Cell adhesion
    c. Intercellular Junction
    d. Extracellular Matrix
    e. Cytoskeleton
    f. Cell motility
    g. Cell proliferation
    h. Control of cell proliferation
    i. Differentiation & Dedifferentiation
    j. Relationship between cell proliferation & Differentiation
    k. Cell signaling
2. Characterization of cells and cell line
3. Maintenance of cell line

Environmental effect

Immediate environment and environment of surrounding medium governs the various properties of cell.

•It is very difficult to provide exact condition for growth and differentiation of cultured Cells invitro.

•Migration, proliferation and differentiation are dependent upon culture media composition, culture environment and absence or presence of stimuli in media.

•Cell's microenvironment needed that favor the spreading, migration, and proliferation of unspecialized progenitor cells

•The invitro condition markedly affects the cellular property of cultured cells. For e.g. Reduction in Cell–cell and cell-material interaction.

Influences of environment on the culture is expressed via 5 routes:

•The nature of substrate

•Degree of contact with other cells

•Physicochemical and physiological constitution of medium

•The constitution of gas phase

•The incubation temperature

Cell adhesion

•Untransformed cells prefer to grow as adherent monolayers.

•These cells need to attach and spread out on the substrate before they will start to proliferate.

•Cell adhesion is mediated by specific cell surface receptors for molecules in the extracellular matrix.

Three major classes of trans-membrane proteins have been shown to be involved in cell–cell and cell–substrate adhesion:

i. Cell–cell adhesion molecules, CAMs (Ca2+ independent), and *cadherins* (Ca2+ dependent)

ii. Transmembrane proteins: also interacting with matrix constituents such as other proteoglycans or collagen,

iii. Cell–substrate interactions are mediated primarily by *integrins*, receptor for matrix molecules such as fibronectin, entactin, laminin, and collagen, which bind to them via a specific motif usually containing the arginine–glycine–aspartic acid (RGD)

Intercellular Junctions

- These structures provide adhesion and communication between cells
- These are specialized regions of contact between the plasma membranes of adjacent cells. They ar essential to any multicellular organism, providing the structural means by which groups of cells can adhere and interact.

There are three types of junctions with varying roles:

(i) mechanical junctions such as the desmosomes and adherens junctions, which hold epithelial cells together,

(ii) tight junctions, which seal the space between cells such as between secretory cells in an acinus or duct or between endothelial cells in a blood vessel,

(iii) gap junctions, which allow ions, nutrients, and small signaling molecules such as cyclic adenosine monophosphate (cAMP) to pass between cells in contact.

Extracellular Matrix

•The extracellular matrix (ECM) is a three-dimensional network of extracellular macromolecules, such as collagen, enzymes, and glycoproteins, that provide structural and biochemical support of surrounding cells.

•Cells attached to the ECM control its composition, and in turn, the composition of the ECM regulates the cell phenotype.

•The use of ECM constituents can be highly beneficial in enhancing cell survival, proliferation, or differentiation, but unless recombinant molecules.

•Cultured cell line are allowed to generate their own ECM, but primary culture and propagation of some specialized cells, and the induction of their differentiation, may require exogenous provision of ECM

Constituent of Extracellular matrix

ECM is comprised variously of collagen, laminin, fibronectin, hyaluronan, and proteoglycans such as betaglycan, decorin, perlecan, and syndecan-1, some of which bind growth factors or cytokines.

Cytoskeleton

•The cytoskeleton is a complex, dynamic network of interlinking protein filaments present in the cytoplasm of all cells.

•It extends from the cell nucleus to the cell membrane and is composed of similar proteins in the various organisms..

Depending upon their size, shape and diameter, cytoskeleton is categorized in three types (i) Microtubules, (ii) Intermediate filaments, (iii) Actin

The cytoskeleton is a structure that helps cells maintain the shape and internal organization.

Functions:

•The attachment of integrins to actin microfilaments via linker proteins is associated with reciprocal signaling between the cell surface and the nucleus.

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•The microtubules appear to be related mainly to cell motility and intracellular trafficking of micro-organelles, such as the mitochondria and the chromatids at cell division.

Cell motility

•Cultured cells are capable of movement on substrate. *Cell motility* is defined as the capacity of cells to translocate onto a solid substratum.

•Cell migration is a central process in the development and maintenance of multicellular organisms

•Tissue formation during embryonic development, wound healing and immune responses all require the orchestrated movement of cells in particular directions to specific locations.

•The movement of cells requires energy.

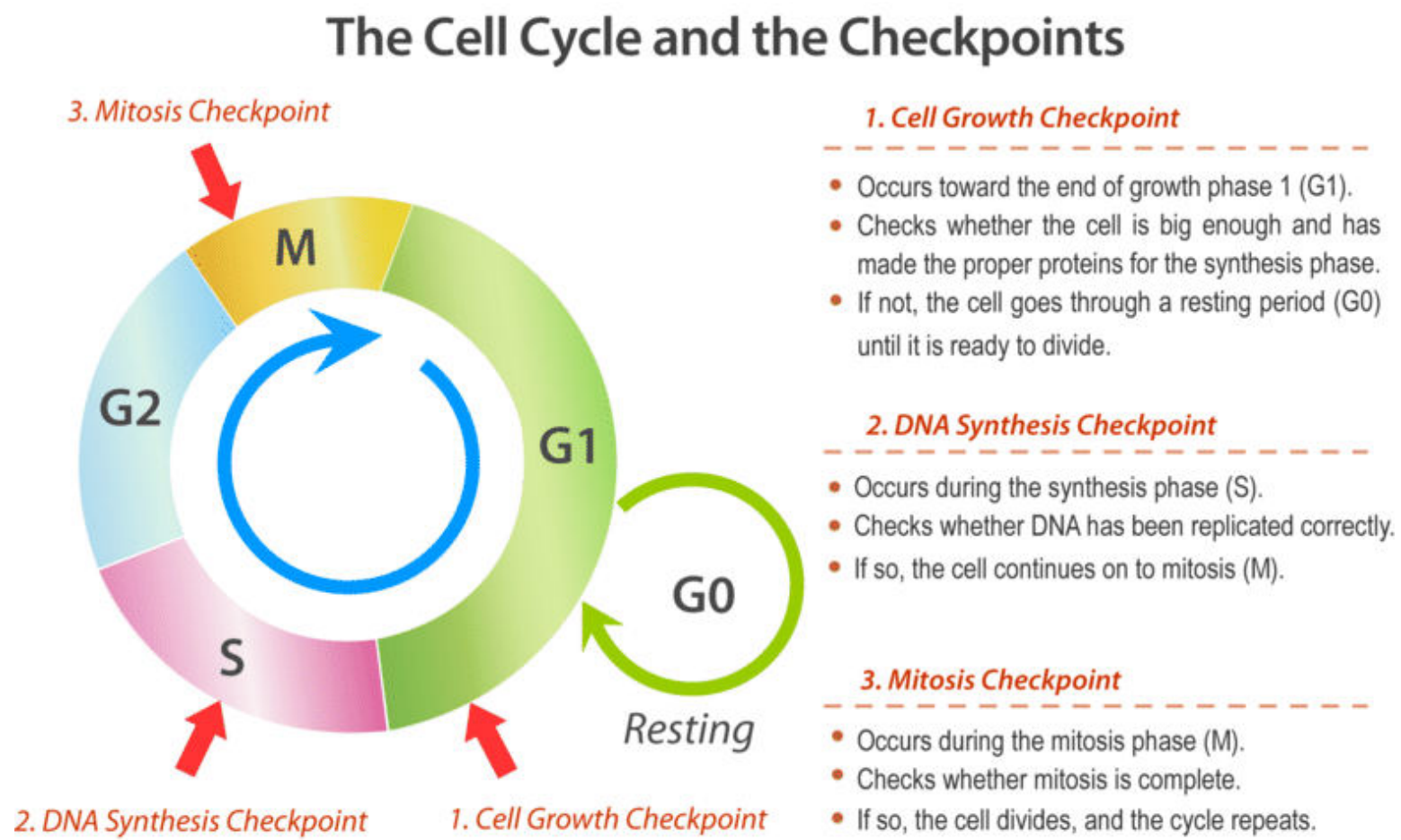
•The most motile are fibroblasts at a low cell density (when cells are not in contact), and the least motile are dense epithelial monolayers.

Contact inhibition: The cessation of movement at confluence, which is accompanied by a reduction in plasma membrane ruffling, is known as *contact inhibition* and leads eventually to withdrawal of the cell from the division cycle.

Cell Proliferation

•Cell proliferation is the process that results in an increase of the number of cells, and is defined by the balance between cell divisions and cell loss through cell death or differentiation. (Nature)

•Cell proliferation is regulated by a coordinated entry into the cell cycle



https://bio.libretexts.org/Bookshelves/Human_Biology/Book%3A_Human_Biology_(Wakim_and_Grewal)/07%3A_Cell_Reproduction/7.2%3A_Cell_Cycle_and_Cell_Division

Control of Cell Proliferation

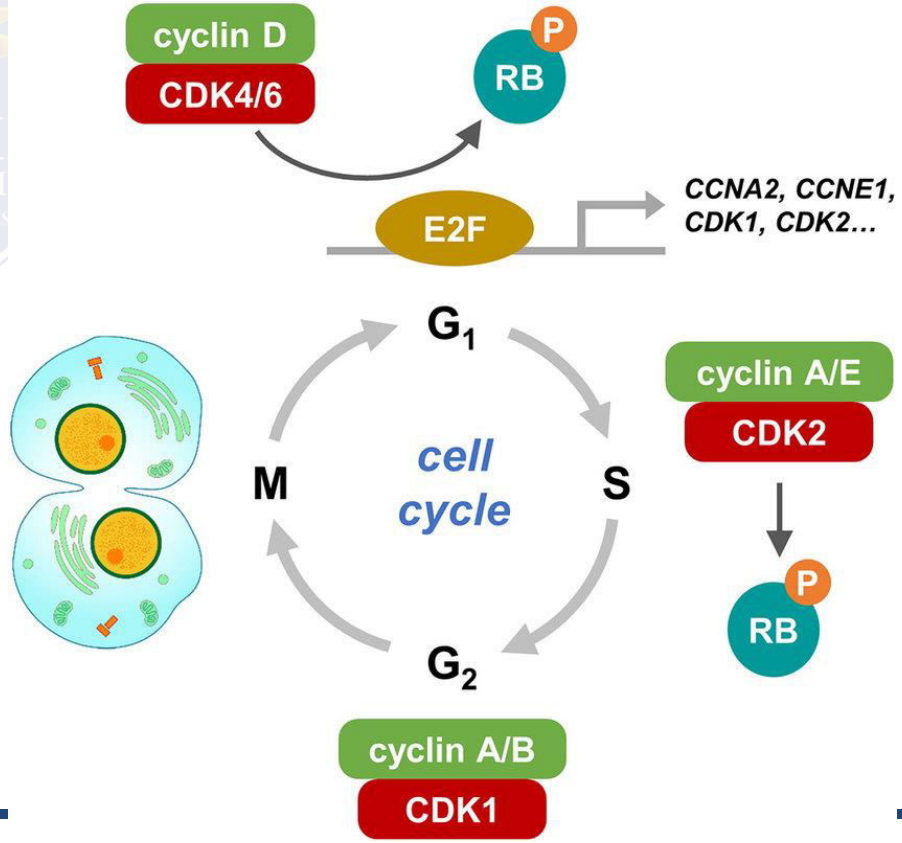
•It is during G1 that the cell is particularly susceptible to control of cell cycle progression at a number of restriction points, which determine whether the cell will re-enter the cycle, withdraw from it, or withdraw and differentiate.

•Checkpoints at the beginning of DNA synthesis and in G2 determine the integrity of the DNA and will halt the cell cycle to allow DNA repair or entry into apoptosis if repair is impossible

➢Control of proliferation is regulated by extracellular factors as well as intracellular factors.

➢Extracellular factors is related to environmental condition provided to cells.

➢Extracellular control are regulated by mitogenic growth factors, such as epidermal growth factor (EGF), fibroblast growth factors (FGFs), or platelet-derived growth factor (PDGF).



Example of extracellular signal

•Low cell density permit entry of cells into cell cycle, or negative acting such as high cell density which signals inhibition of proliferation of normal cells

Intracellular regulation of cell proliferation

•Intracellular control is mediated by positive-acting factors such as the cyclins.

•cell cycle progression are the cyclin-dependent kinases (CDKs). These are serine/threonine protein kinases that phosphorylate key substrates to promote DNA synthesis and mitotic progression.

•Cyclin-binding allows inactive CDKs to adopt an active configuration akin to monomeric and active kinases.

•Negative-acting factors such as p53, p16 and p21 or the Rb gene product block cell cycle progression at restriction points or checkpoints.

Role of regulatory gene elements in cell cycle regulations

p53: *p53*, also known as TP53 or tumor protein is a gene that codes for a protein that regulates the cell cycle and hence functions as tumor suppression. If damaged DNA is detected at any checkpoint, activation of the checkpoint results in increased **protein p53** production. In the cell, p53 protein binds DNA, which in turn stimulates another gene to produce a protein called p21 that interacts with a cell division-stimulating protein (cdk2). When p21 is complexed with cdk2 the cell cannot pass through to the next stage of cell division.

p21: *p21* mediates its various biological activities primarily by binding to and inhibiting the kinase activity of the cyclin-dependent kinases (CDKs) CDK2 and CDK1 (also known as CDC2) leading to growth arrest at G1/S phases in the cell cycle

pRb: The Rb protein is a tumor suppressor, which plays a pivotal role in the negative control of the cell cycle and in tumor progression. It has been shown that Rb protein (pRb) is responsible for a major G1 checkpoint, blocking S-phase entry and cell growth. t regulates cell growth and keeps cells from dividing too fast or in an uncontrolled way.

Differentiation & Dedifferentiation

•*Differentiation* is the process where a *cell* changes from one *cell* type to another. Usually, the *cell* changes to a more specialized type for e.g. hematopoietic differentiation of myeloid lineage.

•Differentiation in cell culture is governed by cellular characteristics, physiology, culture conditions, medium composition and presence or absence of differentiation factors.

•The conditions required for the induction of differentiation—a high cell density, enhanced cell–cell and cell–matrix interaction, and the presence of various differentiation factors —may often be antagonistic to cell proliferation, and vice versa.

•Soluble inducers, such as hydrocortisone, retinoids, cytokines, or planar polar compounds are some examples of differentiation inducer in medium.

Dedifferentiation

It refers to loss of specialized function of differentiated cells.

Dedifferentiation may result from any of the following:

•The wrong lineage of cells is selected in vitro,

• Undifferentiated cells of the same lineage overgrow terminally differentiated cells of reduced proliferative capacity.

• The absence of the appropriate inducers (hormones, cell or matrix interaction) causes an adaptive, and potentially reversible, loss of expression of differentiated properties or

•The differentiated cell reverts to a more primitive phenotype or even a stem cell.

Relationship between differentiation and proliferation

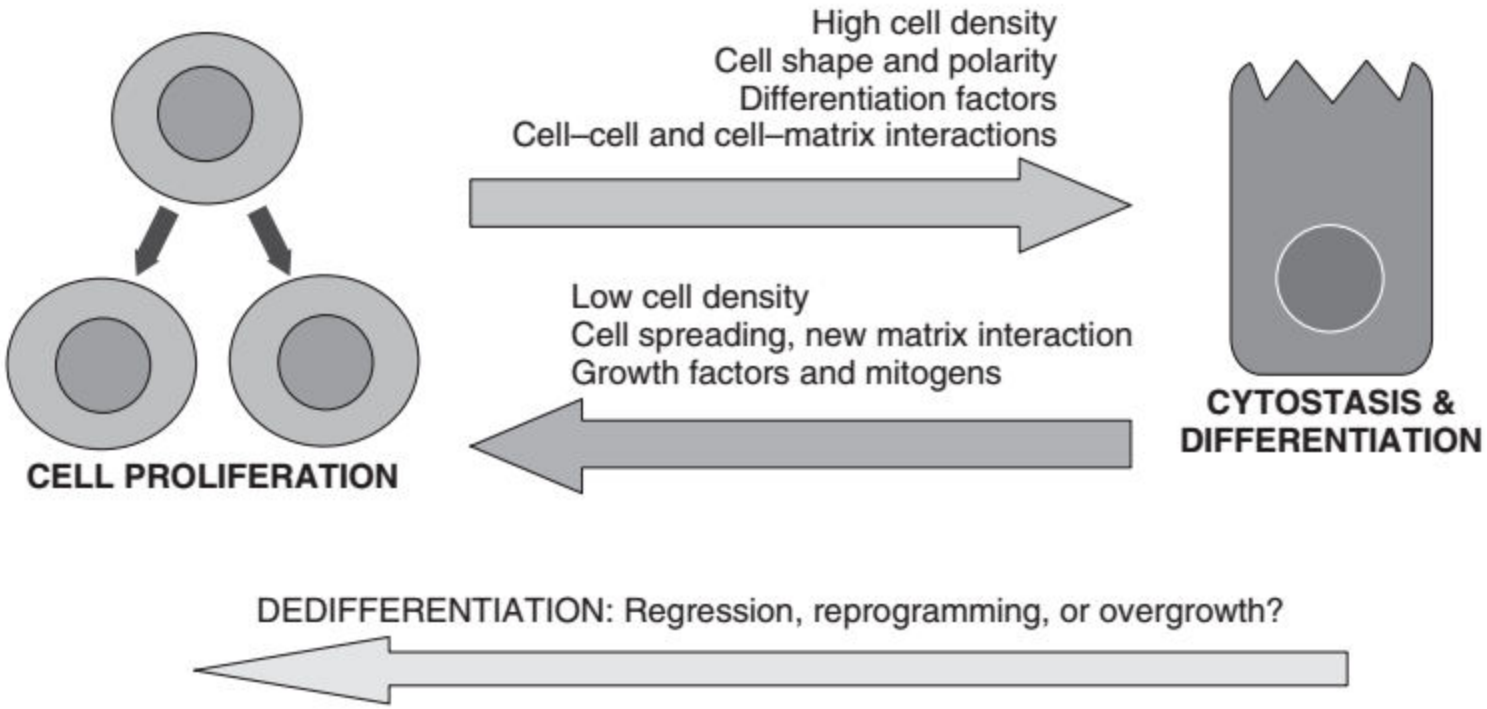
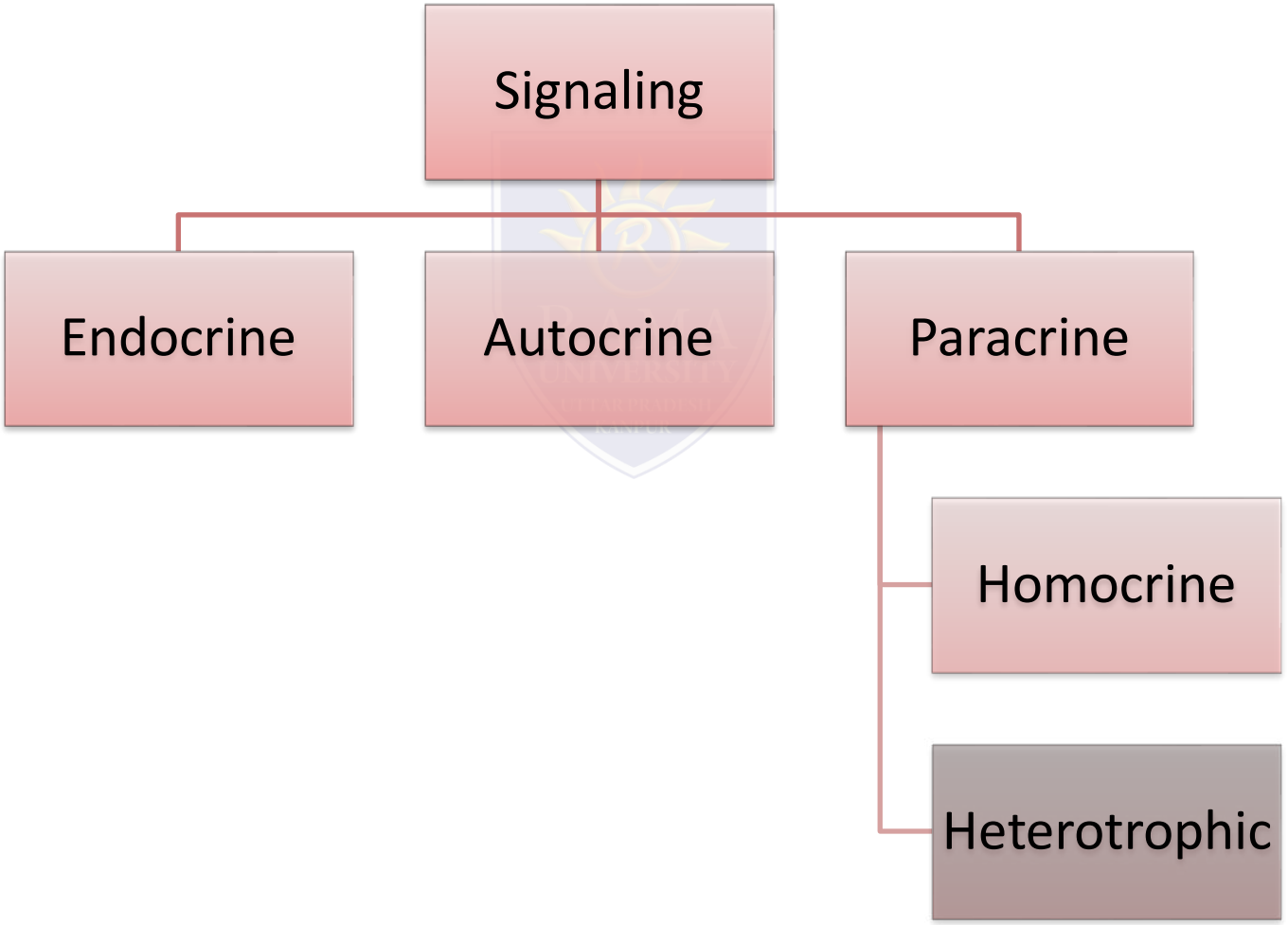


Figure adopted from Animal cell culture, Freshney

- Normal culture conditions (low cell density, mitogens in the medium) will favor cell proliferation.
- while high cell density and addition of differentiation factors will induce differentiation.
- The position of the equilibrium will depend on culture conditions.

Cell signalling

Cell proliferation, migration, differentiation, and apoptosis in vivo are regulated by cell–cell interaction, cell–matrix interaction, and nutritional and hormonal signals..



Endocrine: Signals that reach the cell from another tissue via the systemic vasculature

Paracrine : Signals that diffuse from adjacent cells without entering the bloodstream.

Autocrine : A cell can also generate its own signaling factors that bind to its own receptors or activate signal transduction pathways directly.

➢Paracrine and endocrine factors regulates the maintenance, proliferation, and the induction of differentiation in specialized cells, it should be identified and supplied in the medium.

Characterization of cells & cell line

➢Characterization refers to outlining/defining many traits of cells, some of which may be unique

•Characterization of cell line is essential to identify the cultured cells, to determine species of origin, and history of cell line functionality , sanctity or purity of growing cells, to determine

•Tumorigenecity and oncogenecity of cells.

There are six main requirements for cell line characterization:

•Authentication, i.e., confirmation that the cell line is not cross-contaminated or misidentified

•Confirmation of the species of origin.

•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).

•Determination of whether or not the cell line is transformed:

(a) Is the cell line finite or continuous (b) Does it express properties associated with malignancy

•Indication of whether the cell line is prone to genetic instability and phenotypic variation

• 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

Commonly used techniques for cell line characterization

The most commonly used techniques for cell line characterization and identification are :

- Cellular morphology
- DNA profiling or analysis of gene expression
- multiple isoenzyme analysis by agarose gel
- karyotype analysis i.e. chromosome analysis coupled with FISH
- chromosome painting
- MHC analysis (HLA typing).
- Cell surface antigen/ Cell surface marker

Characterization: Chromosome content

Chromosome content or *karyotype* is one of the most characteristic 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 chromosome number is more stable in normal cells.

(a). Chromosome banding

This group of techniques was devised to enable individual chromosome pairs to be identified when there is little morphological difference between them. Dyes are used to differentially stain the chromosomes. Thus each chromosome is identified by its banding pattern. For Giemsa banding, the chromosomal proteins are partially digested by crude trypsin, producing a banded appearance on subsequent staining. Trypsinization is not required for quinacrine banding. The banding pattern is characteristic for each chromosome pair.

(b).Chromosome painting

Chromosome '*painting*' refers to the hybridization of fluorescently labeled *chromosome*-specific, composite probe pools to cytological preparations. It is used to describe the direct visualisation using in situ hybridisation of specific *chromosomes* in metaphase spreads and in interphase nuclei.

Characterization: DNA analysis

DNA content can be measured by propidium iodide fluorescence with a CCD camera or flow cytometry. Hoechst 33258 and other DNA fluorochromes such as DAPI, propidium iodide, or Pico Green (Molecular Probes). Analysis of DNA content is particularly useful in the characterization of transformed cells that are often aneuploid and heteroploid.

(a).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. Thus strain-specific gene amplifications, such as amplification of the dihydrofolate reductase (DHFR) gene, may be detected in cell lines selected for resistance to methotrexate; amplification of the MDR gene in vinblastine-resistant cells.

(b).DNA fingerprinting

•DNA fingerprinting is based on sequence of polymorphisms that occur in the genome of every organism.

•When DNA are treated with restriction endonucleases, DNA fragment of variable length are generated, electrophoresis of these restricted fragment length polymorphic (RFLP) reveals variations in nfragment length in satellite DNA that are specific to the individual from which the DNA was derived.

•When analyzed by polyacrylamide electrophoresis, each individual's DNA gives a unique hybridization pattern as revealed by autoradiography with radioactive or fluorescent probe.

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Enzyme activity

•For cell line identification Isoenzymes are frequently used

•These enzymes usually display different kinetic parameters. These enzymes, may be separated chromatographically or electrophoretically, and the distribution patterns (zymograms) may be found to be characteristic of species or tissue.

•During electrophoresis, isoenzymes from different cell lines migrate at different rates and can be detected later by staining with chromogenic substrates. Stained gels can be read directly by eye and photographed, or scanned with a densitometer.

Characterization: Antigenic Marker

- Immunostaining and ELISA assays are among the most useful techniques available for cell line characterization.
- In Immunostaining, antibody localization is determined by fluorescence, whereinthe antibody is conjugated to a fluorochrome, such as fluorescein or rhodamine.
- Immunostaining uses primary and secondary antibodies

Maintenance of cell culture

➢Maintenance refers to act of keeping cell lines in their normal and healthy physiological conditions.

➢Periodic change of the medium is required for the *maintenance of cell lines* in *culture*, whether the *cells* are proliferating or non-proliferating.

Methods involved involved in maintenance

a. Examination of cell morphology
b. Determination of cell density
c. Determination of pH of medium
d. Exhaustion of medium

Examination of cell morphology

- Change in cell morphology may result from any signs of deterioration, such as granularity around the nucleus, cytoplasmic vacuolation, and rounding up of the cells with detachment from the substrate.
- Cell morphology can be determined by confirming against archival photographs at same cell density

Determination of cell density

•Actively growing cells increases their number and hence cell density increases. This increase in cell density causes rapid exhaustion of medium and growth inhibition.

• when they reach confluence (contact inhibition), and it takes them longer to recover when reseeded. Therefore, too high density is undesirable and dense cell culture should be sub-cultured immediately.

Determination of pH of medium and medium replacement

Drop in pH: The rate of fall and absolute level should be considered. Most cells stop growing as the pH falls from pH 7.0 to pH 6.5 and start to lose viability between pH 6.5 and pH 6.0, so if the medium goes from red through orange to yellow, the medium should be changed. A drop in pH may be due to accumulation of lactic acid (product of cellular metabolism) or a sign of contamination.

Cell concentration. Cultures at a high cell concentration exhaust the medium faster than those at a low concentration. This factor is usually evident in the rate of change of pH, but not always.

Cell type: Every cell behaves differently in laboratory condition, some cells stop dividing at high cell density while others keep dividing and growing. For Eg. Normal cells (e.g., diploid fibroblasts) usually stop dividing at a high cell density, Transformed cells, continuous cell lines, and some embryonic cells, however, deteriorate rapidly at high cell densities unless the medium is changed daily or they are subcultured.

Morphological deterioration: If deterioration is allowed to progress too far, it will be irreversible, as the cells will tend to enter apoptosis.

Exhaustion of medium

•The rapidly growing culture eats up all the available nutrients thereby necessitating medium replacement.

•The rate of change of pH is generally dependent on the cell concentration in that cultures at a high cell concentration exhaust medium faster than cells lower concentrations.

•You should subculture your cells if you observe a rapid drop in pH (>0.1 – 0.2 pH units) with an increase in cell concentration.

Volume, depth, and surface area

Gaseous exchange and requirement of O_2 governs the medium depth. Cells with a high O2 requirement do better in shallow medium (e.g., 2 mm), and those with a low requirement may do better in deep medium (e.g., 5 mm). If the depth of the medium is greater than 5 mm, then gaseous diffusion may become limiting.

Test your understanding

1.Influences of environment on the culture is expressed via Influences of environment on the culture is expressed via

a. The nature of substrate
b. Degree of contact with other cells
c. Physicochemical and physiological constitution of medium
d. All of the above

2.Characterization of cell line gives which of the following information

a. Origin of source cell used to create cell line
b. Sanctity and Purity of growing cells
c. Tumorigenecity & Oncogenecity of growing cells
d. All of the above

3. Periodic change of media is an act of

a. Cell maintenance
b. Characterization of cells
c. Cell to cell communication
d. None of the above

4. DNA fingerprinting is based on……

5. Drop in pH of cell culture media during cell culture indicates……

References & Further reading

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Further reading

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