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CELL LINE: A REVIEW

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ABSTRACT

A cell culture that is derived from one cell or the set of cells derived from the same type and in which under certain conditions the cells will proliferate indefinitely in the laboratory. Cells are separated in two classes : Eukaryotic and
rokaryoticCells.The clone or clones of cells derived from a small piece of tissue develope in culture. Cell is the basic structural unit of life that are bounded by plasma membrane i.e divided on the basis of the presence of cell or plasma membrane. Cell lines were the clones of animal or plant cells that grow on a suitable nutrient media in the laboratory which has various applications in the field of biochemistry and cell cell biology and biotechnology.

Keywords: Cells, clone, nutrient media, plasma membrane, tissues.

I. INTRODUCTION

Origin of cell line-1950-55. Hela cell, is a cell type in an immortal cell line that was used in scientific research. It is the oldest cell and most commonly used human cell line[1].Early, the primary cell line said to be named after a "Helen Lane" to conclude the fact that cells were taken without her knowledge or consent by Gey. Despite this attempt, her name was used by the press within a few years of her death. These cells were treated as cancerous, as they are obtained from a biopsy taken from a lesion on the cervix as part of diagnosis of cancer. A conflict still continues on the classification of these cells.Culture that were derived from main tissue is known as primary culture. A primary culture has becomes a cell line when it is transferred into the other culture vessel. Adherent cultures, the cells were separated using a protease, such as trypsin, and/or a chelating agent, such as EDTA, and subdivided — that process was known as passaging. For cells that will grow in suspension, the culture was split into new culture vessels. Under these circumstances the specialized culture conditions are used, within a few passages a relatively uniform population of proliferative cells was selected. This population was probably representative agent of the cells that divide when the tissue of origin is suffered, and will carry on growing until the end of the natural proliferative lifespan were reached and senescence occurs. As far as the cells proliferate, they show little or no law of tissue-specific differentiation. However, given the suitable signals, they can regenerate a functional tissue. Culture derived from primary subculture is known as cell lines and from continous culture or derived from passage of cell lines is known as subclones.

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1.1 CELL

1. the fundamental structural unit of living organisms.

2. a little more or less enclosed space.

All living cells result from other cells, either by division of one cell to make two, as in mitosis and meiosis, or binary fusion of two cells to make one, as in the union of the sperm and ova to make the zygote results by sexual reproduction.All cells are bounded by a structure called the plasma membrane, which is a lipidbilayer composedof two layers of phospholipids. Every layer was one molecule thick with the charged, hydrophilic end of the lipidmolecules on the surface of the membrane and the uncharged hydrophobic fatty acid tails in the interi or of the membrane.

Cells are seperated into two classes, eukaryotic cells and prokaryotic cells:Eukaryotic cells have a good nucleus, which contains the genetic material, defined with chromosome. Where every chromosome having a long linear deoxyribonucleicacid (DNA) molecule associated with protein. The nucleus was not bounded by nuclear membrane, which is composed of two lipid bilayer membranes.

Prokaryotic cells, the bacteria, unnucleus, and their genetic material, consisting of a single circular naked DNA molecule, which was not separated from the rest of the cell by a nuclear membrane.

Eukaryotic cells are larger and more complicated than prokaryotic cells. They also have membrane-bounded structures,such as mitochondria, chloroplasts, Golgi apparatus, endoplasmic reticulum and lysosomes, t hat prokaryotic cells don’t have.

The contents of a cell are collectivelyreffered as the protoplasm. In eukaryotic cells the contents of the nucleus are known to as nucleoplasm and the rest of the protoplasm are as the cytoplasm.The lipidbilayer of eukaryotic cells is nonperrmeable to many substances, such as ions, sugars and amino acids,whereas, membrane proteins selectively move specific substances through the cell membrane p ermeabilityy active or passivetransport. Water, gases such as oxygen and carbon dioxide, and nonpolar compoun ds pass through the cellmembrane by diffusion. Materials can also be engulfed and taken into the cell enclosed i n a portion of the cellmembrane. This is called phagocytosis when solids are ingested and pinocytosis when liqu ids are ingested. Thereverse process is called exocytosis. All of these processes permit the cell to maintain an int ernal environmentdifferent from its exterior.

The cells of the body discriminate the cells during development into many specialized types with specific tasks to perform.Cells are organized into tiss ues and tissues into organs. Embedded in the cell membrane are a wide range ofmolecules that vary with the cell type and are typically composed of proteins or glycoproteins that have acytoplasmic transmembrane and extern al domains. These molecules serve as cell receptors and are involved insignal transduction for a wide range of li gands, including hormones, cytokines and incidentally serve as receptors forviruses and drugs.

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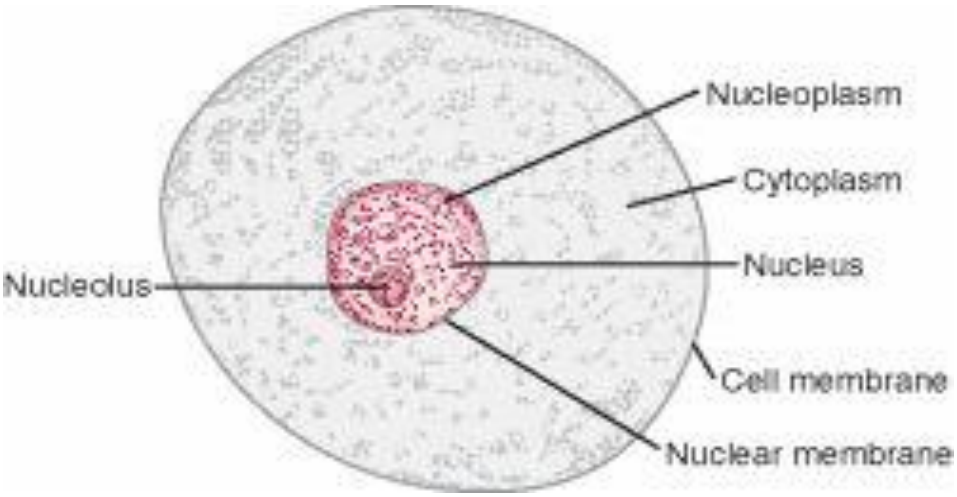

Fig1:Structure of the cell [2]

1.2 LINES

1. a narrow, streak, mark, or narrow ridge; often an imaginary line connecting different landmarks.

2. conversion of a broad part of x-rays to a pencil beam.

3. a single consignment of livestock from one farm. Said of a group of cattle or sheep notable for their homogen eity.

An **immortalised cell line** was a population of cells arise from a multicellular organism which would normally not proliferate indefinitely but, due to mutation, have evaded normal cellular senescence and instead can keep undergoing division[3]. The cells can therefore be grown for prolonged periods. The mutations required for immortality can occur naturally or be intentionally induced for experimental purposes[4]. Immortal cell lines are main tool for research into the biochemistry and cell biology of multicellular organisms. Immortalised cell lines have also found uses in biotechnology.

An immortalised cell line not be confused with stem cells, which can also divide indefinitely, but form a normal part of the development of a multicellular organism[5].

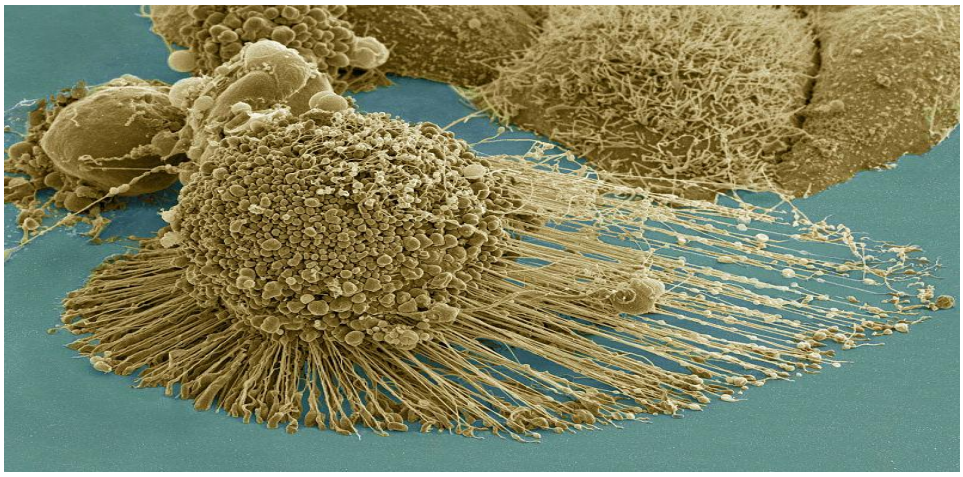

Fig2: HELA Cells

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II .BACKGROUND

The term cell line refers to the propagation of culture after the first subculture.

In other words, once the primary culture is sub-cultured, it becomes a cell line. A given cell line contains several cell lineages of either similar or distinct phenotypes.

It is possible to select a particular cell lineage by cloning or physical cell separation or some other selection method. Such a cell line derived by selection or cloning is referred to as cell strain. Cell strains do not have infinite life, as they die after some divisions.

2.1Types of Cell Lines:

Finite Cell Lines :

The cells in culture divide only a limited number of times, before their growth rate declines and they eventually die. The cell lines with limited culture life spans are referred to as finite cell lines. The cells normally divide 20 to 100 times (i.e. is 20-100 population doublings) before extinction[6][7]. The actual number of doublings depends on the species, cell lineage differences, culture conditions etc. The human cells generally divide 50-100 times, while murine cells divide 30-50 times before dying.

Continuous Cell Lines :

A few cells in culture may achieve a different morphology and get altered. Such cells are capable of growing faster resulting in an independent culture. The progeny originated from these altered cells has unlimited life (unless the cell strains from which they originated). They are designated as continuous cell lines.

The continuous cell lines are transformed, immortal and tumorigenic. The transgenic cells for continuous cell lines may be obtained from normal primary cell cultures (or cells strains) by treating them with chemical carcinogens or by infecting with cancer causing viruses. In the Table., the discriminating features of finite cell lines and continuous cell lines are compared.

Property	*Finite cell line*	*Continuous cell line*
Growth rate	Slow	Fast
Mode of growth	Monolayer	Suspension or monolayer
Yield	Low	High
Transformation	Normal	Immortal, tumorigenic
Ploidy	Euploid (multiple of haploid chromosomes)	Aneuploid (not an exact multiple of haploid chromosomes)
Anchorage dependence	Yes	No
Contact inhibition	Yes	No
Cloning efficiency	Low	High
Serum requirement	High	Low
Markers	Tissue specific	Chromosomal, antigenic or enzymatic

2.2Nomenclature of Cell Lines:

It is a world wide practice to give codes or designations to cell lines for their identification. For instance, the code NHB 2-1that represents the cell line obtained from normal human brain, followed by cell strain (or cell line number) 2 and clone number 1[8]. The general practice in a culture laboratory was to provide a log book or computer database file for each of the cell lines.When naming of cell lines was to be, it is absolutely necessary

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to ensure that each cell line designation is unique so that there were no confusion occurs when reports are given in literature. Further, at the time of publication, the-cell line should be prefixed with a code that was designating the laboratory from which it was obtained e.g. NCI for National Cancer Institute, Wl for Wistar Institute.

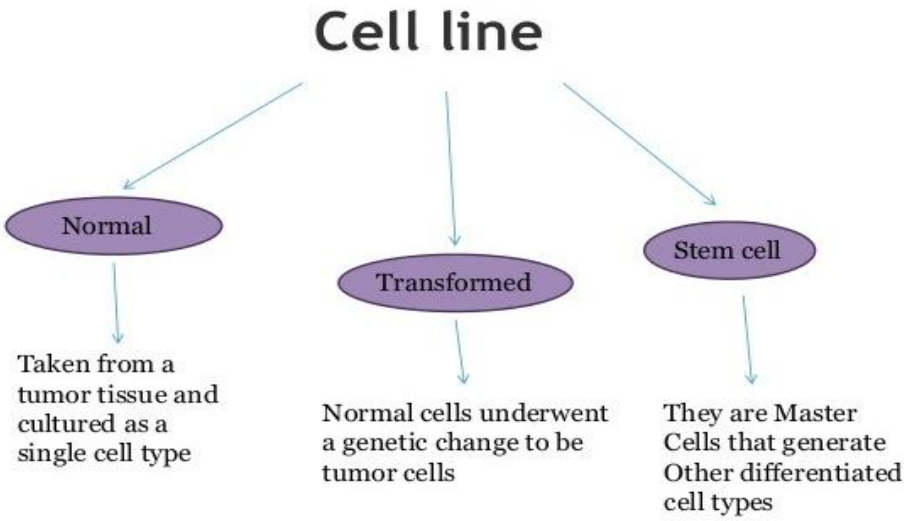

Fig3: Selection of Cell lines

Some of them are briefly described:

1. Species:

In general, non-human cell lines with low risk of biohazards, was preferred. However, different need to be taken into account while extrapolating the data to humans.

2. Measurable or continuous cell lines:

Cultures with continuous cell lines were domninating as they grow faster, easy to clone and maintain, and produce better and good yield. But it is unlike or uncertain whether the continuous cell lines express the right and appropriate functions of the cells. Therefore, some workers suggest the use of measured cell lines, although it is difficult.

3. Transgenic cells:

The transformed cells are democrated as they are immortalized and grow rapidly.

4. Availability:

The readily availability of cell lines is also important. Sometimes, it may be necessary to develop a particular cell line in a laboratory.

5. Characteristics appropriate for growth of cells:

The following growth parameters need to be considered:

i. Doubling time of population

ii. growth in liquid medium

iii. Saturation density (yield per flask)

iv. Cloning efficiency.

6. Stability & reliability:

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The stability & eliability of cell line with particular reference to cloning, generation of adequate stock and storage are important.

7. Phenotypic expression:

It is important that the cell lines having cells with the right phenotypic expression.

2.3 Maintenance of Cell Cultures & subcultures:

For the routine and good conditions of cell lines in culture (primary culture or subculture) the examination of cell morphology and the periodic change of medium was very important.

2.4 Cells structure:

The cells were in the culture must be focused regularly to check the health status of the cells, the absence of contamination, and any other serious complications (like toxins in medium, inadequate nutrients etc.).

2.5 Replacement of Medium for cell lines:

Periodicly the change of medium was required for the maintaining the cell lines in culture, whether the cells are proliferating or non-proliferating. Meanwhile the proliferating cells, the medium required to be changed more frequently as compared to non-proliferating cells. The time interval between medium changes depends onmany vitals like (the rate of cell growth and metabolism).

For a case, rapidly growing transformed cells (e.g. HeLa), the medium wants to be changed twice a week, while for a case of slowly growing non-transformed cells (e.g. IMR-90) the medium might be changed once a week. Further, for rapidly &instantly proliferating cells, the sub-culturing has to be carried out more frequently than for the slowly growing cells.

The following factors need to be considered for the replacement of the medium:

1. Cell concentration & nutrients:

The cultures with high cell concentration assume , the nutrients in the medium faster than those with low concentration; hence the medium is required to be changed more rapidly for the former.

2. A decrease in pH:

A decrease in the pH of the medium is an indication for change of medium. Most of the cells can grow better at optimally pH 7.0, and they almost stop growing when the pH fallens to be 6.5. A further drop in pH (between 6.5 and 6.0), the cells may lose their viability & reliability.

The rate of fall in pH is generally estimated for each cell line with a chosen medium. If the fall is less than 0.1 pH units per day, there is no harm even if the medium is not immediately changed. But when the fall is 0.4 pH units per day, medium should be changed immediately.

3. Cell type:

Embryonic cell, transgenic cells and continuous cell lines grow rapidly and require more frequent sub-culturing and change of medium. This is in contrast to normal cells, which grow slowly.

4. Structural changes:

Frequent examination of cell morphology is very important in culture techniques. Any deterioration in cell morphology may lead to an irreversible damage to cells. Change of the medium has to be done to completely avoid the risk of cell damage.

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2.6 Developing a new cell line:

Deriving a new cell line, especially when human, from fresh tissue is an expensive and time-consuming exercise. The subsequent value of the new cell line will depend on the ability to authenticate its origin and on the associated information that is available.

Tissue

In addition to tissue taken for culture, if donor or patient consent and/or ethical review permit it is recommended that additional material is stored for:

Confirmation of origin (authentication) A small portion of the sample used for primary culture (or a blood sample or DNA derived from the donor) should be frozen or processed immediately. The tissue or DNA can then be used to demonstrate unequivocally that the cell line is derived from the putative donor. Short tandem repeat (STR) profiling is most useful method for the purpose of authentication and identification, although additional information on genotype seuencing(karyotype, copy number variation (CNV) mapping, or even whole-genome sequence) will sometimes help ensure identity.

Histopathological confirmation. A small part of the sample being used to develope the culture should be fixed in formalin and used for histopathological assessment, ideally by the same histopathologist reports the surgical specimen if this is from a patient. This step is particularly important if a patient sample is supplied to the laboratory directly by a clinician, because it may not be representative of the surgical specimen sent to the histopathologist[12]. For a case or accordingly to, it may be taken at some distance from a tumour and consequently devoid cancer cells, or it may be from a region that is not affected by a specific pathology caused by a genetic or epigenetic defect.

Normal tissue for comparative study. A small quantity of blood (e.g., 10 ml) or normal tissue should be frozen. This tissue can after be used to look for genetic differences and could also be used for authentication and identification. In the case pleuripotent cell lines of iPSC lines, or when direct reprogramming is used to derive one somatic cell type from another, it is also good practice to cryopreserve stocks of the original cells used. These could be more useful to derive additional cell lines using new reprogramming technology and methods, but also to provide original donor material for validation of later discoveries made using the cell line. If somatic cell nuclear transfer (SCNT) or 'cloning' technology were mainly to derive cell lines, such as ES cells, then cells or tissue from both the somatic cell donor and oocyte donor should be kept in order to match nuclear and mitochondrial DNA, respectively.

2.7 Storage and banking of cell lines:

Once a primary cell line has been developed or acquired and assured (i.e., shown to be authentic and uncontaminated) the first step to ensuring a reliable and reproducible supply of cells is the cryopreservation of about 20 1-ml ampoules, each containing $1–5 \times 10^6$ cells. This will rise the vast majority of laboratories a ready supply for many years. Depending on the size and duration of the operation and law it is often useful to have a tiered system: (a) an MCB or seed stock, containing 10–20 ampoules, which should be protected and not distributed, a distribution stock generated from the seed stock, which is used to give the end users with cultures [9] from which they will generate their own frozen stock. This stock contain sufficient ampoules was to provide at least one ampoule for every 3 months of the proposed experimental period plus sufficient ampoules for

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contingencies;(b) these cells should not be distributed other than to those within the group for whom they were frozen. inadequate or serial banking (as occurs for cultures passed from one laboratory to another in a chain) results in increase in the population doubling number and additional risk of contamination or loss of key characteristics and to selection for abnormal growth characteristics accompanied by genetic and/or epigenetic changes.

2.9 Cryopreservation of cell lines:

Cell lines were preserved by freezing samples slowly (usually 1 °C min^{-1}) in preservative media (usually growth medium with 10% DMSO). An automatic controlled-rate cooling apparatus gives the most reproducible cryopreservation that provided the freezing programme used has been optimised for that cell line's requirements but simpler devices may suffice [9].

Many cell types, for example, hESC, may be require ultra-rapid freezing or vitrification [10] where water is frozen *in situ* to form a glass and not allowed to permeate out of the cell as in slow freezing and is often used to freeze stem cells.

Every time a batch of cells is frozen down, it is recommended that one vial is resuscitated immediately to check viability. Vials removed from the bank should be thawed rapidly (by immersion in a water bath at 37 °C) and the cell suspension diluted gradually with pre-warmed medium.

III. STORAGE OF CELL STOCKS

Cell stocks should be kept below −130 °C as viability may be lost within a few months at −80 °C. Once at their final storage temperature it is also detrimental to warm them to −80 °C even for short periods. Commonly, cells can be kept at −80 °C during the freezing process either for convenience, although usually for no more than a few days before being transferred to the definitive storage vessel, or when cells need to be kept frozen in multiwell dishes while waiting for results from a screen. This is commonly provide during gene-targeting experiments with ES cells where it is necessary to screen many individual clones to find the relatively few that will be thawed for further growth and research. The multiwell petridishes need to be thoroughly sealed so that they do not dry out at −80 °C.

Greatly infectious material must be stored in the vapour phase of LN_2 to reduce the risk of transfer of contaminating organisms [11]. It also eliminates the hazard of LN_2-penetrating ampoules that may then explode on warming. Storage in vapour phase of LN_2 is increasingly the norm for safety purposes but requires careful monitoring of the level of the LN_2 as the smaller volumes used in vapour-phase storage will run out quicker.

For security point of view, important material, (e.g., MCBs) should be divided into more than one storage vessel, preferably on different sites. Deposition and removal of frozen stocks should be recorded and controlled to avoid loss of entire stocks and to indicate when re-banking of stocks should be performed. Labelling of frozen stocks should be legible and resistant to LN_2. It is recommended that the label on the frozen vial should contain the name of the cell line, batch number and freeze date as a minimum. These labels should be printed rather than handwritten, using labels that are suitable for prolonged storage in liquid nitrogen. Barcoding has proved to be not complicated method that can contain most information on a small label.

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The location of the vials should be detailed in a spreadsheet or database linked to details of the origin and characteristics and identification of the cell line and the QC measures that have been applied to it.

Cryostorage vessels should be fitted with alarms and storage temperatures checked regularly and properly. It is recommended that levels of LN_2 in the storage vessels are recorded at least once a week. Periodic audits for which tende tends to prove regular maintenance, monitoring and stock control will also help ensure safety and security of storage facilities.

IV. CONCLUSIONS

Strategies moving forward need to take into account the genetic characteristics of the patient population in which genetic risk is largely polygenic, and a mixture of many common variants of small effect, as well as few rare variants of large effect. In contrast, *a priori* we would expect to find the most robust phenotypes in cells derived from patients carrying highly genetically penetrant rare variants and cell models created using genome editing of isogenic iPSC lines. It will be important to connect the knowledge gained from single gene deficits and that gained from the accumulated effects of multiple subtle genetic risk alleles.

Both the selection of patients carrying rare variants of large effect and the selection of patients of extremely high polygenic risk require large patient populations to optimize the selection. When genetic risk in selected patients is not sufficiently causal, any experiment will require the analysis of large numbers of patient cell lines. An important step is to have robust protocols for reprogramming and differentiation of large numbers of patient samples. This will require standardization and rigorous quality control to reduce technical variation to an acceptable minimum. Given the high current reagent costs for stem cell research, the unit price per patient cell assay needs to drop substantially before this will be feasible. These processes need to integrate well with global efforts in patient recruitment and accompanying clinical phenotyping and genomic analysis.

Beyond the issues of variability and capacity lies the key question of what is the relevant cellular phenotype or phenotypes. We have discussed what is currently possible and under development, and how these might relate to function and physiology in the intact brain. However, these investigations have only just begun, and are likely to require multiple lines of converging evidence, carried out in numerous centers and with validation against clinical and animal model studies, before consensus cellular phenotypes can be established and accepted.

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