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Cancer Cell Lines: A Review

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ABSTRACTS

The human cancer cell lines are playing an important role in drug discovery practices. However, modeling the complexity of cancer utilizing these cell lines, does not accurately represent the tumor micro environment. Research into developing advanced cancer and tumor cell culture models in a three- dimensional (3D) architecture that more valuable characterizes the disease state have been undertaken by the number of laboratories around the world. These 3D cell culture models are particularly beneficial for investigating and mechanistic processes and drug resistance in cancer and tumor cells.

Keywords: Laboratories, cancer, cell lines, 3D cell culture

INTRODUCTION CELL LINES

Cell line have improved scientific research which is used in vaccine production ,antibody production, cytotoxicity, drug metabolism testing gene functioning, generation of artificial tissue(e.g artificial skin) and also synthesis of biological compounds.

Cell lines are also used in place of primary cell to study the biological process. The term cell line refers to the propagation of culture after the first subculture. As we can say once the primary culture sub – cultured it becomes cell line.[1]

TYPES 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.

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.[2]

Continuous Cell Lines: A few cells in culture may have a different morphology and get altered. Such cells are capable of growing faster resulting in an independent culture. The progeny derived from these altered cells has unlimited life (unlike the cell strains from which they originated).

They are designated as continuous cell lines. The continuous cell lines are transformed, immortal and tumorigenic. The transformed 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 oncogenic viruses[3]

Cell lines have an important role in scientific research and are being used in vaccine production, testing drug metabolism and cytotoxicity, antibody production, study of gene function, generation of artificial tissues (e.g., artificial skin) and synthesis of biological

compounds for example therapeutic proteins.[4] Some time these cell lines are contaminated with other cell lines and cause mycoplasma. The bitter truth of cross contamination of cell lines either inter or intra species was exposed by Walter Nelson-Rees in the early 1970s. He showed that at that time point the majority of cell lines being used worldwide and distributed by cell banks were contaminated with HeLa cells.[5]The fact that the large number of long- established cancer cell lines were originated from aggressive and metastatic tumours[6]. HeLa cell contamination is well known to cause such problems. like, mycoplasma, contamination can persist undetected in cell cultures for a long period of time and cause extensive alterations in gene expression and cell behavior. Based on submissions to cell banks,15–35% of cell lines were estimated to be contaminated with mycoplasm.[7],[8].

Therefore, great care should be taken when using cell lines and experiments where key findings are confirmed in primary cultures should always be included. Herein we share our experience using an immortalized mouse Sertoli cell line (MSC-1), that was developed in 1992 by Peschon et al. This cell line was isolated from transgenic mice containing Sertoli cells transformed by the small and large T-antigens of the SV40 virus, which were targeted to

Sertoli cells using the promoter for Mullerian inhibiting substance. MSC-1 cells were similar to primary Sertoli cells morphologically and expressed many of the same genes as primary Sertoli cells.[9,10]

VIRAL CONTAMINATION

Viruses are infectious agents that rely on host cells for their own replication. Owing to their limited size of upto 300 nm and their intracellular lifecycle, they are not visible in generic light microscopy and very difficult to detect.While some viruses may induce morphological changes in the cultured cells (cytopathic effects), other species may integrate into the cellular genome and alter the phenotype of the investigated cell line. Viruses can enter cell cultures, for example, through the use of animal-derived cell culture products such as trypsin or fetal bovine serum and are a serious health concern for laboratory workers. The presence of viral contaminants can be challenging to confirm but generally relies on PCR, ELISA, immune cytochemistry, or electron microscopy[11]

TEMPERATURE, pH, CARBANDIOXIDE, AND OXYGEN LEVELS

The temperature for cell cultures dependson the body temperature of the species and the microenvironment from which the cultured cell types were isolated. While most human and mammalian cell lines are incubated at 36–37°C, cell lines originating from cold-blooded animals can be maintained at wider temperature ranges between 15°C and 26°C. The pH level for most human and mammalian cell lines cultured in the lab should be tightly controlled and kept at a physiological pH level of 7.2–7.4. In contrast, some fibroblast cell lines favorslightly more alkaline conditions between pH 7.4 and 7.7, while transformed cell lines prefer more acidic environments between pH 7.0 and 7.4[12]

CULTURE MEDIA

The most of the culture media are based on the balanced salt solutions with a variable number of additions in the form of amino acids, and vitamins, trace minerals, a pH indicator, and eventually antibiotics.[13]. Such media are called as Basel media. Some examples are RPMI 1640 (Roswell Park Memorial Institute 1640) and DMEM (Dulbecco's Modified Eagle Medium), eventually with the Ham's F12 nutrient mixture (DMEM/ F12). In addition to the basal media special medium formulations that require less serum addition, so called as Reduced Serum Media,(RSM) and Serum-Free Media (SFM), are available for the special cell types and special applications.[14].The culture medium is most important for successful cell culture experiments. In general, recommend- ations are available for each particular cell line or cell type. However, different media may satisfy the required conditions for a particular cell type. Such personal experience may also included to observed the beneficial effects due to the addition of non-essential amino acids, like pyruvate, reducing agents etc.[15] It is very important to know that any addition to ready- made media requires components that have been tested and certified for cell culture work. Even chemicals of pro-analysis quality may contain traces of toxic compounds that can be determine the presence of cells in culture media.[16]

CLASSIFICATION OF CULTURE MEDIA

Liquid media: The liquid media are available for use in test tubes, bottles and flasks. Liquid media are referred to as ,example. The liquid media is preferred when a particular population of bacteria is desired within a short period of time for pure cultures of bacteria. [17].

Solid Media: Any liquid medium can be rendered by the addition of certain solidifying agents like agar media.Agar (simply called agar) is the mostly used to solidifying agent. It is an unbranched polysaccharide obtained from the cell wall of some species of red algae such as the genera Gelidium. It is melted at 95 degree celsius (sol) and solidifies at 42 degree celsius (gel). It is not easily hydrolyzed by the bacterial species and thus contributes only as a solidifying agent. The most common concentration used for bacteriological purpose is at concentration of 1- 3% to make a solid agar medium.[18]

The most useful advantage of solid culture over liquid culture is that it immobilizes the bacterial cell as a result of which discrete colonies are formed which help us to study the bacterial morphology in research.[19]. The solid agar medium can be various types depending upon the application. The agar medium after it is poured into the culture tubes and after sterilization is cooled and hardened in a slanted position then it is known as agar slants.[20]. It is used mainly for physiological studies or maintenance of pure cultures. Similar tubes when hardened in an upright position are called as agar deeps.[21]. Agar deeps are mainly used for physiological studies of the microorganisms and in the maintenance of anaerobic bacteria . When sterilized solid media is spread over large surface area such as petriplates it is known as agar plates and provides large surface area for the separation of pure cultures from mixed culture.[22].

MAINTANENCE OF CULTURE MEDIA

The good maintenance of thesecell lines in culture (primary culture or subculture) the examination of cell morphology and the periodic change of medium are most important.[23]

Cell Morphology:

The cells in the culture media must be examined regularly an (into the culture media) absence of contamination, and any other serious complications (toxins in medium, inadequate nutrients etc.). [24]

Replacement of Medium:

Periodic changes of the medium is required for the maintenance of cell lines in the culture media, whether the cells are proliferating or non- proliferating. For the proliferation of cells, the medium need to be changed more frequently when compared with non-proliferating cells. [25]. The time interval between medium changes depends on the rate of cell growth and metabolism. For instance, for rapidly growing transformed cells (e.g. HeLa), the medium needs to be changed twice in aweek, while for slowly growing non-transformed cells (e.g. IMR-90) the medium may be changed once in a week. Further, for rapidly proliferating cells, the sub- culturing has to be done for more frequently than for the slowly growing cells.[26]

ISOLATION OF CELLS FOR CULTURE

The first, and most important, element in the collection of tissue is the cooperation and collaboration of the clinical staff. This is best achieved if a member of the surgical team is also a member of the culture project, but even in the absence of this, time and care must be spent to ensure the sympathy and understanding of those who will provide the clinical material.[27]. It is worth preparing a short handout explaining the objectives of the project and spending some time with the person likely to be most closely involved with obtaining samples. This may be the chief surgeon (who will need to be informed anyway), or it may be a more junior member of the team willing to set up a collaboration, one of the nursing staff, or the pathologist, who may also require part of the tissue. Whoever fulfils this role should be identified and provided with labeled containers of culture medium containing antibiotics, bearing a contact name and phone number for the cell culture laboratory. [28]

A refrigerator should be identified where the containers can be stored, and the label should also state clearly DO NOT FREEZE! The next step is best carried out by someone from the laboratory collecting the sample personally, but it is also possible to leave instructions for transportation by taxi or courier.[29]. If a third party is involved, it is important to ensure that the container is well protected, preferably double wrapped in a sealed polythene bag and an outer padded envelope provided with the name, address, and phone number of the recipient at the laboratory.[30]. Refrigeration during transport is not usually necessary, as long as the sample is not allowed to get too warm, but if delivery will take more than an hour or two, then one or two refrigeration packs, such as used in picnic chillers, should be included but kept out of direct contact.[31]

EQUIPMENT FOR THE CELL CULTURE LABORATORY :

Using various techniques and assays carried out in different cell culture labs, the common theme of cell culture work is asepsis—the creation of a microenvironment free of unwanted pathogenic micro-organisms, like bacteria, viruses, fungi, and parasites. Since asepsis is a important component of successful cell culture works, a separate room or designated area should be dedicated to this work and not be utilized for other purposes. Several pieces of equipment can aid in achieving such a sterile workspace and generally lead to higher efficiency, accuracy, and consistency of the cell culture performance . Aseptic Cell Culture Practices While the previous section has explored methods aimed at decreasing the exposure of hazardous substances to the laboratory worker, this section will address the practices that should be put in place by laboratory workers to protect the cultured cells. Infectious agents such as bacteria are toxic for eukaryotic cells and ultimately lead to cell death.[32]. Further more even low levels of contamination can result in abnormal results and lead to wrong scientific interpretation. By adhering to several techniques that ensure asepsis in the cell culture lab, researchers can reduce the frequency and extent of contaminations and diminish loss of cells, resources, and time. This can be achieved by eliminating the entry of microorganisms into the cell culture through contaminated equipment, media, cell culture components, incubators, work surfaces, and defect or opened cell culture vessels.[33].

- Biosafety cabinet – To create sterile work surface; class II and III recommended.
- Humid.CO2 incubator – To provide a physiological environment for cellular growth.
- Inverted light . microscope – To assess cell morphology and count cells.
- Fridge, freezers (−20°C, −80°C)liquid nitrogen storage – To store cells, cell material, and culture components.
- Centrifuge – To condense cells.
- pH meter – To determine the correct pH of media components.
- Pipettes and pipettors – To aliquot different volumes .
- Cell media and supplementary components – To culture cells in desirable components .
- Hemacytometer – To count cells, determine growth kinetics and prepare suitable plating densities.
- Autoclave – To sterilize pipettes and other equipment in contact with cells .
- Vacuum pump – To aspirate cell culture medium.
- Water bath (with adjustable temperature) – To warm up cell culture media.

- Cell culture dishes – To culture cells in different formats (e.g., flasks, Petri dishes, 96-well plates) Containers for waste (biohazardous).[34]

APPLICATIONS OF CELLCULTURE STUDIES IN PHARMACEUTICAL TECHNOLODY

Studies conducted during the initial development of drugs such as toxicity, corrosion and drug activity were carried out on animals; however, in the past 10 to 20 years, alternatives have been sought due to the fact that animals do not effectively model human in vivo conditions and unexpected responses are observed in the studies. Cell culture studies made positive contributions to the initial development of drugs. Contrary to animal studies, the need for low drug and a short response time are the characteristics for in vitro cell culture methods1

. In 2005, more than 100 million animals were used and 10 billion dollars were spent for animal toxicity experiments . It is possible to reduce this cost and the amount of animal use for experiments with well-designed cell culture studies.[35]

CANCER CELL LINES

The utility of cell lines acquired from tumors allows the investigation of tumor cells in a simplified and controlled environment. There are specific advantages and disadvantages to exploit cancer cell lines over animal models.[36] These then dictate the nature of the experiment that can be organised. Firstly, the cost involved with sustaining them is significantly less than maintaining animal subjects. They are promptly available and research studies can be implemented relatively quickly.[37]

Large quantities and volumes of cells may be propagated to create high-throughput studies. Cell lines are exceptionally versatile in the types of studies they may be used in. [38]. Not only can they be build in vitro but can also be injected into mice to form xenograft models of prostate cancer progression.[39]. They can be transformed and reviewed over time to dispose sequential events that occur as a result of specific stimulus. As well as the productsproduced from the cells such as their secretome can be analyzed readily.[40] Disadvantages that are coupled with cell lines are that they do not represent the heterogeneity of the tumour microenvironment as well as the necessarily heterogeneous nature of tumours with a patient and between patients. [41]. As a result multiple cell lines may be required to address the full heterogeneity seen in a tumour phenotype. Cell lines are also subject to genetic alterations in culture that may alter their phenotype over the course of a long experiment. The path to the progression of the tumour is lost and does not provide insight in the pathogenic process significantly [42].

IMPORTANCE OF CELL LINES IN CANCER

Cancer is a molecularly heterogeneous disease and one of the major causes of the death worldwide. The existence of various types of tumours with different histopathologies, genetic and epigenetic variations, and clinical outcomes, difficult for the understanding to this disease, the mechanisms of action of chemotherapeutics and the creation of novel therapies.[43]. The advances in the cancer pathophysiology study has its origin on the availability of different types of experimental model systems that review the various forms of this disease, allowing the knowledge of genetics and epigenetics alterations and anticancer drugs testing. Studies of cance use for the primary tumours , paraffin- embedded samples , cancer cell lines , xenografts , tumour primary cell cultures and genetically engineered mice. Each of these diverse models are used for different studies, mainly because certain types of manipulations for the genetic and DNA methylation analysis and drug testing are ethically, and in practice, difficult to perform in animals.[44].

Cell lines are used as alternative to overcome these issues, being at the same time easy to manipulate and molecularly characterize (e.g. genetic and/or epigenetically). This cell model uses for the fundamental study of the cellular pathways and for disclose the critical genes involved in cancer. [45]. This characterization provides important insights about the complexity of the polygenetic etiology of cancer and the biological mechanisms involved in this disease reinforcing its value as models for its study and also the characterization of cancer cell lines is essential for the development of new anticancer drugs, understanding the action mechanisms and the resistance, sensitivity patterns of chemotherapeutics already in use in cancer treatment and the development of more targeted anticancer drugs.[46]

HUMAN CANCER CELL LINES

In the last few years, human immortal cancer cell lines have aggregated an accessible, easily usable set of biological models with which is used to examine the cancer biology and to analyze the inherent efficacy of anticancer. Drug resistance is one of the hefty hindrances to chemotherapy of cancer. Studies with cell lines can serve as an initial screen for agents that might regulate drug resistance.[47]

APPLICATIONS OF HUMAN CANCER CELL LINES

Cell culture is among the most widely used laboratory approaches probably because of the diverse aspects it covers and the relatively short period of time it requires. In pharmacology and therapeutic researches, cell culture represents a largely used basic approach. [48]

The aim of the modern pharmacology is to first identify active compounds from natural elements that can constitute a starting point to develop therapeutic drugs.[49]

Nowadays, the drug development lies mainly on the identification of compounds that are thought to be active or based on observation reported by biologists or physiologists for example. Those compounds are then further investigated. The first step in drug discovery is, in many cases, to test compounds in cell culture to find out how active they are in terms of pharmacological actions. [50]

The challenges faced in this step are diverse. Indeed, selecting the cell line is an important issue since the compound activity may be specific. Defining the optimum conditions for both the cell culture media and the drug solvent, in addition to the other reagents that are eventually used, remains important since a variation of each of these factors could affect the robustness of the cell-based assay by influencing the live cells and therefore the assay's results.[51]

Another important element is the choice of the positive control, which is in many cases a commonly used drug that is well studied and well known for the activity we are about to test. [52]. Importantly, the use of negative controls assures a better interpretation of the results since it allows us to distinguish the effects of the tested drugs from those due to other elements such as the reagents or the cell culture medium ingredients. For instance, many pharmacological discoveries related to one of the most important pharmacological targets, G protein coupled Receptors.[53]

THE IMPORTANCE OF THE MOLECULAR CHARACTERIZATION OF CANCER CELL LINES.

A cancer cell line is more valuable as an in vitro model for cancer research if it is properly molecularly characterized [54]. This type of analysis will allow a more detailed study of the genetic/epigenetic events e.g. disclose critical cancer genes and DNA methylationalterations) and cellular pathways associated with oncogenesis [55], in the understanding of the microevolutionary progression of the tumour (when the molecular profiling is done in different passages [56]) and unveil the molecular patterns associated with resistance/sensitivity to anticancer drugs [57]. Specifically, the tumour transcriptional profiling and the DNA methylation patterns i.e. that result in gene expression alterations) can be useful as a first approach in the development of new anticancer targeted therapeutics [58].

CONCLUSION

From this review we concluded that cell lines are exceptionally very usefull in the types of studies they may be used in. Immortalised cell lines are the in vitro equivalent of cancerous cells. Not only can they be build in vitro but can also be injected into mice to form xenograft models of prostate cancer progression. They can be transformed and reviewed over time to dispose sequential events that occur as a result of specific stimulus. It has also shown essential practices and techniques for successfully working with cell lines and explained the conditions required for creating a cellular environment that mimics their in vivo niche.

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