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Techniques in study of Cell Biology: A Review

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Abstract **Review Article**

Cell Biology has been a branch of prime importance in aiding diagnosis and management of chiefly immunological, infectious and genetic diseases. Cell culture techniques and Recombinant DNA Technology helped in designing and manufacturing viral vaccines, synthetic hormones, monoclonal antibodies, lymphokines and interleukins. The advancement of knowledge in field of molecular biology is a boon and is implicated in DNA sequencing and genomics which has been immensely helpful in understanding genetic diseases. A brief review to give a bird eye view of some of the important techniques of cell and molecular biology has been discussed here. The basics of techniques such as Cell fractionation, Cytometry, Cell Culture, Microscopy, DNA sequencing and Genomics, Polymerase Chain Reaction, Blotting Methods, Micro-array and Cytochemistry and its implication and application have been explored through literature.

Keywords: Cell fractionation: Cytometry: Cell Culture: Microscop: DNA sequencing and Genomics: Polymerase Chain Reaction: Blotting Methods: Micro-array and Cytochemistry.

INTRODUCTION

Cell biology is branch of science associated with studies of stricture and functions & cell which includes both prokaryotic and eukaryotic cells. The exploration in field of cell biology includes studying of cell morphology and that of its product, cell cycle, cell communication and metabolic activity of the cells [1-3].

The techniques used in study of structure and functions are

1- Cell fractionation, Cytometry, Cell Culture and Microscopy [1, 3, 4].
2- Cell to cell communication: The cell to cell communication can be studied by directed transmission of acyl-homoserine– Lactone (AHLs) [1, 4-6].

(a) The Acyl – Homoserine – Lactones are freely diffusing small molecules in bacterial natural quorum sensing systems which participate in homeostasis.

(b) Cell communications are also investigated through cell signalling. The target cells which hove receptor protein identify and acknowledge the signal, via binding of signalling molecule with receptor protein; the receptor protein then undergoes conformational changes thus commencing the process of photo transduction. A special type of cellular response is the result of the transduced signal in the terminal steps of cell- signalling. The varied signalling process may lead to different responses such as cell differentiation, proliferation of cells, metabolism signals etc [5-7]

Modern Molecular Biology Methods: DNA sequencing and Genomics, Polymerase Chain Reaction, Blotting Methods, Micro-array and Cytochemistry [1, 8-11].

DISCUSSION

We would further study the important techniques involved in study of cell functions

(a) Cell fractionization (b) Cytometry (c) Cell culture (d) Microscopy

1. **Cell Fractionation**: The functions of organelles can be studied by cell fractionization. The Cell fractionization is a technique by which cell organelles are separated by fractionization so that sub cellular structure morphology remains intact for further exploration of its functions. The process for sub cellular fractionization includes tissue or cell homogenization and differential centrifugation [3].

(a) **Tissue/Cell Homogenization**: Homogenization is a mechanical procedure by which tissue or cell are mechanized for isolation into intact cell contents by either freeze thawing, induction of osmotic shock, grinding, homogenizing at high pressure by passing through homogenizing valve, ultrasonic homogenization etc[3].

(b) **Differential Centrifugation:** The cell homogenate is exposed to repeated centrifugation so that the cell components are fractionated and isolated. The cell components are separated by centrifugation depending upon its size and density.

The centrifugation moves the denser and large size component to the bottom of the tube forming pellet while small size and lesser denser components remain in the supernant suspension [3].

2. Cytometry: The molecular properties of cells can be studied by cytometry. The functional properties of cell which can be investigated using cytometry includes cell cycle (cell division) phase, DNA content, identifying proteins on cell membrane surface or in its cytoplasm apart from cell morphology analysis and cell count. The technique commonly employed in detection of antigens by use of antibodies is by fluorescent labelling especially by utilizing nucleic acid specific probe and fluorescent reporter molecule [5, 6, 11, 12]. The commonly used technique of cytometry in routine diagnostic practice is Flow Cytometry.

Flow Cytometry: The physical & chemical characteristics morphology of cells is studied using flow Cytometry. Principle of Flow Cytometry: The cells are made to pass as a single file through flow cytometers and these cells are exposed to laser and this helps in detection, counting and sorting of cells. The cells organelles which are labelled fluorescently on exposure to laser emit light at a broader wavelength as compared to the light source. The scattered and fluorescent light are detected by the detectors and analysed digitally by computer software which is attached to the flow cytometers.

Application of Flow Cytometry: The Flow Cytometry helps in identifying the cell size, the quantative amount of the component of cell especially DNA, gene expression, cell signalling events, immunophenotyping, and so also helps in demarcation of amount of intracellular protein and amount of specific surface receptors.

Key Note: The benign and malignant haematological diseases especially can be assessed by Flow Cytometry and thus it aids in diagnosis and in planning treatment strategies for better prognosis. The bone marrow aspirate, cerebrospinal fluids and peripheral blood are commonly employed samples in flow Cytometry [5, 11,12].

Histology and Histopathology: It is the study of the microscopic structure of the organic. Tissues and it helps in understanding how the individual components are morphologically and functionally related. Histopathology which is branch of histology helps in staging of tumours & in evaluation of completeness of resection conducted surgically and this is immensely helpful in ascertaining prognosis and thus facilitate in planning management strategies.

The common techniques employed in preparation of histological samples include fixation (10% neutral buffered formalin for light microscopy and glutaraldehyde in 2.5 % of phosphate buffered saline for electron microscopy), selection and trimming, embedding sectioning and staining. The most commonly used stain in haematology are haematoxylin & eosin stain [1, 4].

Cell Culture: In simple words the cell culture involves removal of cell from a plant or animal and is made to grow under created controlled conditions outside their natural environment. As the cell grows in the cell culture medium; the cell characteristics during mitosis and cell differentiation are studied and these cells are designed to mature into types which will be a protype of functional analogue of the similar cells in tissue or organ in an intact animal [11].

The cell culture media are nutrients and growth substances required for cell culture growth and survival in newly intubated environment. The nutrient broth and agar plates are commonly used growth media for microorganism. The cell culture techniques are employed in molecular biology to study and understand the physiology and biochemistry of cells, exploring the drug toxicity effects on cells and events assonated in carcinogenesis. The cell culture also helps in developing and designing newer vaccines and therapeutic proteins [11,12].

Microscopy: Microscope has been very vital equipment for exploring and understanding the structures of cells. Though light microscopes were first to be utilized for cell visualization but with advancement in technology; the electron microscopes were boon towards studying & exploring functional aspects of sub cellular structures. The Scanning Electron Microscopes (providing 3 dimensional images) and Transmission Electron Microscopes which utilized technique of focussing beam electron through a specimen, helps to analyse morphological characteristics in greater percept.

Fluorescence microscopy is immensely helpful in immunohistochemistry for understanding immunological association of a cell. In fluorescence microscopy a specific component of cell is labelled with fluorescent markers & a light of specific wave length is

utilized to illuminate the marker thus enabling the labelled cell component to be visualised [7-10].

Confocal Microscopy: This provided a very clear & sharp image of three dimensional view of tissue or cell [7, 12]. Thus microscopy shall always be a important tool in understanding the morphology and physiology of cells.

Others

Methods employed in Molecular Biology

DNA Sequencing and Amplification

Key Characteristics

a- The DNA can be cleaved into fragments and can be comprehended into predictable fragments using restrictive enzymes such as restriction endonucleases. The available fragments can be overlapped using various restriction enzymes and DNA map can be made available for Genome assembly. The DNA fragments needs to be cloned in specific engineered vector plasmids [5, 12,13].

b- Recombinant DNA: This can be created by connecting two or more DNA fragments from DNA source using ligation enzymes [13,14].

c- Probe: The probe used for identification of a complementary fragments is a radioactive nuclei acid, DNA or RNA. The density gradient centrifugation also helps in is oratory.

d- Amplification of DNA using Polymerase Chain Reaction (PCR): The desired sequence of DNA can be exponentially amplified by utilizing primer which anneal to the desired sequence. [15,16,17]
RT-PCR: The reverse transcriptase enzyme helps in conversion of RNA to DNA for utilization in a PCR reaction [13, 14, 18].
Key note: The Real- time PCR in better method then conventional PCR as it helps in detection of the PCR amplified product during their synthesis.

e- Blotting Methods: They aid in transferring the DNA from gel to the membrane and Southern Blotting is most common technique used for it and is followed by specific sequence detection with help of labelled probe by hybridization. The Northern Blotting is identical to Southern Blotting but it transfers RNA from a gel membrane.

f- Microarrays: The known sequences of DNA are spotted and synthesized on Small chip. To conclude the molecular biology techniques helps to study the genes of sequence of human genome thus help in isolating & identifying the molecular sequences associated with various genetic diseases [13,18,19].

g- Cytochemistry: The cell components can be detected and assessed by biochemical analysis and visualization techniques by Cytochemistry. The chemical component of organelle of cells is demarcated by thin histological sections using procedures such as radio autography, cryo-election microscopy immunohistochemistry, X-ray-spectroscopy etc[18,19].

(III) Allied cell biology branches which also contribute towards cell study are genetics, biochemistry, medical microbiology and immunology [19, 20].

Application of cell biology in clinical care & Research

1- Cell culture techniques have been immensely helpful in manufacturing of viral vaccines, synthetic hormones, monoclonal antibodies, lymphokines and interleukins with the help of Recombinant DNA Technology [1, 11, 12].

2- Cell biology techniques of cell culture also have been immensely helpful in manufacturing anti-cancer drugs [9].

3- Cell communication studies help in understanding the role of gap junction (connexion) in cancer suppression as well as tumour progression[9,12, 19],

4- Microscopic techniques using immunofluorescence has been extensively helpful in immunohematology apart from studying the cell morphology. Conofocal microscopy is employed in assessment of eye diseases, quantification of cornea endothelial cells, and management of keratomycosis apart from its use in endoscopic procedures [7, 20].

5- Flow cytometers has been helpful in exploring gene expression, cell signalling events and immunophenotyping[5].

6- Molecular biology which is a branch of cell biology also is implicated in DNA sequencing and genomics. The genome assembly especially human genome is key towards understanding genetic diseases and its further exploration is helpful in stem cell studies too [20].

CONCLUSION

Cell Biology and Molecular Biology studies have helped understanding cellular concepts of diseases especially immunological, infectious and molecular diseases. The diagnostic techniques which are being modified and further being explored for updation will help discover the pathology of newer disease challenges and viral infections which we are facing today.

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