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FORENSIC ASPECTS IN CELL BIOLOGY

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ABSTRACT

Forensic science and cell biology play an essential role in crime investigation. All cells are important for the functioning of organisms and every single detail is important for the cases. Basic knowledge of cell biology in forensic science is needed. The relation between cell biology and toxicology is known as cell toxicology. Cell toxicology is the branch of toxicology which is the field of science that helps us to understand the harmful effects of chemicals, heavy metals, Drugs, Gases, and Poisons on human, animal cells, and the environment.

Cell damage, cell death (apoptosis, necrosis), and organ dysfunction due to ROS production in cells by xenobiotics and exogenous toxicants are known as cell toxicity. Mitochondria dysfunction caused by oxidative stress in the human cell by the overdose of chemicals and drugs, lead to damage of cell DNA and cell death. For example, if someone takes potassium cyanide immediate death to occur due to the stop of the respiratory system where ROS (reactive oxygen species) is generated and oxidative stress takes place and the mitochondrial respiration cycle stops. These all effects of a chemical /toxicant are dose-dependent and species-specific. In forensic toxicology knowledge about every single cell, chemical OD and drug effects on cells are important. The understanding of these phenomenons may enable us to clarify the cell toxicity mechanism and the importance of every single cell which play important role in the investigation of cases. Like blood samples, urine samples, or any saliva samples all these samples are contains many types of cells which are important in the cases of investigation of Drugs, Alcohol, OD of any medicine or pesticides, heavy metals toxicity or gaseous toxicity, etc.

Keywords: Forensic toxicology, Cell biology, Oxidative stress, Apoptosis, Necrosis, etc.

INTRODUCTION

Cell Biology: Cell biology or cytology is the study of the structure and function of various cells in living organisms. A cell is the basic unit of life that is responsible for the living and functioning of organisms. Cell dysregulation can lead to various types of diseases, and toxicities. British scientist, Robert Hooke first coined the term 'cell' to describe a cork under a microscope in 1665. After further advancements in the new biological field 'Anton van Leeuwenhoek', invented semi-powerful microscopes and observed the cells of several organisms by the 1800s [1,2]. Three scientists Schwann, Schleiden, and Virchow then proposed the idea of cell theory, which states that:

- All organisms are made of cells.
- All cells come from existing cells.
- All biological life functions of living organisms occur within cells.

In the 20th century Cell-biology improved, such as

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observing organelles within cells after the invention of the electron microscope, the fluorescence microscope, etc. Cell biology is now one of the main branches of biological sciences that focuses on cellular structure and function; including a cell's ability to form, divide, specialize, differentiate, and Signaling. There are numerous subfields of study within cell biology such as cell signaling, cell metabolism, cell adhesion, and cell toxicology. Many of these subfields are heavily researched in diseases such as cancer, tumor, and xenobiotic agents effects [3,4].

Forensic science is the use of scientific methods or expertise to investigate crimes and civil cases or examine evidence that might be presented in a court of law; like fingerprint and DNA analysis blood alcohol level reports, drug analysis, etc. Forensic science remains a powerful force in support of justice and public safety. The forensic sciences have many Specializations. Among the most common are: Forensic Chemistry, Forensic toxicology, Forensic pathology, Forensic Molecular biology, Forensic cell biology or cell toxicology, Firearms, Ballistics, Forensic DNA, Cyber Security, Photography, Forensic Documentation, and some specific choices in human anatomy and physiology.[5,6]

Forensic toxicology and cell toxicology or cytotoxicity are relatable to each other. A forensic toxicologist must know cell biology and toxicology. Forensic toxicology focuses on medico-legal aspects of different chemical, biological and miscellaneous exposures and toxic substances on humans, animals, and the environment [4]. Toxins could be anything, it might be a chemical, animal poison, plant poison, any medicine, drug of abuse, any pesticides, any kind of noxious gas, agrochemicals, doping substances like steroids, or any synthetic chemicals. A forensic toxicologist determines the cause of death through extraction of blood, pesticides, and isolation or detection of poison from the dead body remains (blood, urine, vitreous, viscera, hair, saliva, discoloration of body, etc.).[7] All these dead remains or suspected criminal samples were investigated by a forensic toxicologist. Every evidence is important from the medico-legal aspect and all the techniques which were used in the Forensic Toxicology laboratory are based on cellular and molecular techniques that's why the knowledge of cellular mechanism, and cell toxicology is important in forensic toxicology.[3,5]

FORENSIC ASPECTS IN CELL BIOLOGY

Forensic science is a broad field and diverges into many branches like forensic anthropology, forensic serology, forensic pathology, forensic toxicology, forensic entomology, clinical medicine forensic science [8]. All branches are directly related to cell biology because all samples are from tissues, organs, and fluids.

- Life-altering diseases such as cancer, Alzheimer's disease, blood cell disorders, mitochondrial diseases, malaria, and countless others are all down to defects within the cellular mechanisms of eukaryotic cells.[8]
- Many types of research in molecular and cell biology improved treatments through clinical trials, drug discovery, and toxicology; and ultimately enhance the quality of life of those suffering from these diseases.[6]

Example: Drug-Induced Liver Injury (DILI) [9] is a disease characterized by hepatic cell injury that can lead to various pathological conditions, such as acute hepatitis, cirrhosis, and cholestasis, all cells may undergo necrosis/apoptosis. Toxicological studies have shown several drug classes causing DILI, such as antibiotics, NSAIDs, etc. Cellular and toxicological level studies help to understand the DILI disease in the early stages of drug discovery using *in-vitro*, *in-vivo*, and *in-silico* methods. Forensic toxicology is a branch of pure toxicology and also forensics important part deals with the legal aspects of substance abuse, such as the use of performance-enhancing drugs or driving a vehicle while under the influence of alcohol.[10]

- Cell biology is not just about the disease; DNA testing has been used in archaeology to provide evidence that a living person is related to a long-dead ancestor.
- It has been used to show that two plants that look different have the same genetic origins.
- Forensic medicine uses cell biology and DNA fingerprinting to help solve murders and assaults. Neither the courts of law nor the criminals can escape the importance of cell biology.

CELL TYPES IN FORENSIC CASES

The ability to differentiate between different cell populations present within any type of biological evidence obtained from a crime scene is crucial to any investigation[11]

Several different analytical techniques have been

used till date, which are,

1. Histological staining,
2. mRNA expression,
3. DNA methylation analysis.
4. Fluorescence-activated cell sorting.

The need for cell differentiation in forensics

- For example: Consider a sexual assault case involving a woman who has accused the defendant of using a bottle to sexually violate the victim [11].
- During the collection of evidence from the crime scene, forensic scientists obtain the weapon of interest, which in this case would be the water bottle, and confirm that the DNA profile obtained from the trace evidence found on the bottle matches that of the victim.[12]
- To defend their client, the defense may assert that the DNA found on the bottle arose due to the victim drinking from the bottle, whereas the prosecution may instead allege that the DNA was vaginal in its origin.[13]

These types of criminal cases, therefore, require thorough cell identification methods capable of differentiating the specific cellular origin [14].

Histological techniques

- The three most relevant cell types are:

1. Skin,
2. Buccal cavity
3. Vaginal epithelial cells, all three of which are classified as stratified squamous epithelium are important forensic evidence.

Although skin epithelial cells are keratinized and lack nuclei, buccal and vaginal cells are practically indistinguishable from one another, as both of these cell types contain nuclei. Histology stains are frequently used in a forensic laboratory; therefore, this method was one of the first applied to this challenging situation [14].

- **Lugo's method**

Many different techniques have been employed or developed to differentiate cell types from one another. Lugo's IODINE method/technique was developed in early 1970. [15]

- In this method, +ve staining for Lugol's iodine represented the presence of glycogen granules, which ultimately indicated vaginal cells, and was also useful to confirm the presence of gylcogenated squamous cells within the oral mucosa, male urinary tract, and the penis [15].

- **Case study**

In a 2008 study, 11 different histological stains, some of which included Dane's Csaba's and Ayoub-Shklar stains, were evaluated for their effectiveness in differentiating between these three stratified squamous epithelial tissue samples [14].

Of all of the strains tested in this study, Dane's stain, which primarily stains for pre-keratin, keratin, and mucin proteins in tissue samples, was found to provide different staining patterns for skin, buccal, and vaginal smeared cells.

mRNA sequencing

Messenger RNA (mRNA) is a single-stranded RNA molecule that is complementary to a single DNA strand in a given gene. During translation, mRNA is decoded by ribosomes to produce a specific amino acid chain, which will ultimately contribute to the formation of an active protein (central dogma) [16, 17].

The significance of studying mRNA expression is attributed to its unique ability to predict protein expression. One of the most widely used methods for mRNA expression quantization is a real-time reverse transcriptase-polymerase chain reaction (RT-PCR).

Within the field of forensic science, RT-PCR has been widely used to assess the expression of various mRNA markers in forensically relevant body fluids like saliva, blood, semen, vaginal secretions, and menstrual blood. Every tissue has specific genes that have been identified in these critical human tissues[16,17].

- Now a day RT-PCR is being used to test covid-19.

FLUORESCENCE-ACTIVATED CELL SORTING

Fluorescence-activated cell sorting (FACS) is widely used in the medical setting for diagnostic and research purposes. FACS for separating fresh blood and saliva mixture. Also useful in improving the number of detectable alleles from targeted cell types during subsequent DNA extraction and analysis [18]. Due to high-cost FACS has rarely been used for the analysis of forensic samples; like separate mixed sperm and vaginal epithelial cells and only used in preliminary, utilized markers that were specific to sperm cells, thereby limiting the validity of this method. Cell

sorting has become more sensitive to a point where single cell can be isolated and identified.

Case study

In a 2015 study, a group of Australian researchers utilized FACS for the separation of cellular mixtures before DNA extraction. To this end, 14 different ratios of blood and saliva mixtures were analyzed by FACS. To identify a saliva-based epithelial cell, anti-CD227 was used, whereas blood-derived leukocytes were targeted with an anti-CD45 probe. Forensic science has yet to take full advantage of single-cell analysis. Its greatest benefit is the ability to alleviate the challenges associated with DNA mixture analysis, which remains a significant hurdle in forensic science.

DISCUSSION

This article has discussed about the forensic and cytology function their importance. The techniques like Lugo, STR, FACS, mRNA etc. all xenobiotic agents (drugs, chemicals, carcinogens, environment pollutants, pesticides) can affect living cells in both a beneficial or harmful way. 'Toxicology'- that focuses on individual cells' reactions to foreign substances such as medicinal drugs, recreational drugs, hazardous wastes, pesticides, cosmetics etc. These chemicals could be from food/water ingestion, the air, and atmosphere, or through recreational activities. Harmful side effects of drugs intentionally administered to an individual for therapeutic intentions; for example, drug testing within clinical trials. Case study with the help of techniques which basically depends on single cell and the examination of single cell is important in many drugs, abuse, rape, murder cases also DNA fingerprinting at molecular level through cells separation processes and protein or amino acids interactions. Forensic toxicology and cell toxicology knowledge is important for the medico-legal aspects. Every single cell plays a essential role in investigation of a case and every technique is depends on cellular and molecular mechanism. Cell toxicology is based on toxicity of any substance to the living organisms which is toxic/ threat to the life and the study of these toxic substances is known as toxicology on the other hand the forensic toxicology is same as cell toxicology. In cell toxicology we found the toxicity of any chemical and cure also for the living organisms through toxicokinetics and toxicodynamics, on the other hand in Forensic toxicology we found the cause of death and determination of toxic chemicals through analytic qualitative and quantitative methods. Both studies are important for the living organisms and the environment.

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