Cisplatin induced apoptosis and cell adhesion molecule

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Abstract:
Burrows and other workers (WILLIAMS, 1946) demonstrated growth of epithelial cells, connective tissue and a variety of tumor cells. A number of additional properties of cisplatin are now emerging including activation of signal transduction pathways leading to apoptosis. The elegance with which these cells develop, grow, regenerate and indeed die has been partly elucidated by scientist studying the cells in vitro. Cells treated with 5µg Drug Cisplatin. The result was that due to the effect of drug the cells showed rounding off and apoptosis.The demonstration that human tumors could also give rise to continuous cell lines encouraged interest in human tissue.

Introduction

The vast arrays of cells that support these (and many other functions) arise from very few cell types by a process collectively known as differentiation. The elegance with which these cells develop, grow, regenerate and indeed die has been partly elucidated by scientist studying the cells in vitro.
There are three main methods of initiating a culture (Das,2014)

1. Organ Culture: Implies that the architecture characteristic of the tissue in vivo is retained, at least in the culture. Toward this end tissue is cultured at the liquid-gas interface which favours the retention of a spherical or three dimensional shape

2. In primary explant culture: A fragment of tissue is placed at a glass (or plastic) liquid interface, where, after attachment migration is promoted in the plane of the solid substrate.

3. Cell Culture: Implies that the tissue or outgrowth from the primary explant, is dispersed (mechanically or enzymatically) into a cell suspension, which may be cultured as an adherent monolayer on a solid substrate or as a suspension in the culture medium.

Cell cultures may be initiated from normal, embryonic or malignant tissue by aseptic collection from the animal host(NELSON, 1940). In some cases, physical and enzymatic methods are used to disrupt the cells to prepare suspensions of single cells.

These cell lines are often very useful because they can be stored in liquid nitrogen and revised for later use, without having to obtain another tissue sample. Cell lines may be Finite or continuous.
When cells are selected from a culture, by cloning or by some other method, the subline is known as a cell strain. HeLa cells — the first continuous cancer cell line — have been a mainstay of cancer research ever since their isolation from the aggressive glandular cervical cancer of a young woman more than 50 years ago(John R. 2002) .

Burrows established mammalian cell culture using chick embryos as the source of cells grown in the presence of plasma clots using Harrison's method. A Significant development made by his group was the demonstration of the principle of media exchange and sub culture. Burrows and other workers (WILLIAMS, 1946) demonstrated growth of epithelial cells, connective tissue and a variety of tumor cells. A number of additional properties of cisplatin are now emerging including activation of signal transduction pathways leading to apoptosis. Firing of such pathways may originate at the level of the cell membrane after damage of receptor or lipid molecules by cisplatin, in the cytoplasm by modulation of proteins via interaction of their thiol groups with cisplatin, for example involving kinases, and other enzymes or finally from DNA damage via activation of the DNA repair pathways.

Cell-cell adhesion molecules, CAMs (Ca^{2+} independent) and cadherins (Ca^{2+} dependent) are involved primarily in interactions between homologous cells. These proteins are self-interactive; that is, homologous molecules in opposing cells interact with eachother, and the cell-cell recognition that this generates has a signaling role in cell behavior. Cell substrate interactions are mediated primarily by integrins, receptors 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) sequence.

Materials and methods :

Although Harrison is normally accredited with the development of cell culture as a scientific tool, he described his own work as an extension of Wilhelm Roux.

We follow various standard microbial and tissue culture process based on this but with some modifications, as follows

1. Pre-warmed Media
2. T-75 Flask
3. Sterile disposable pipettes
4. Pipette Aid
5. Dulbecco's Phosphate Buffered Saline
6. 0.05% Trypsin

When 70-80% Confluency is observed then passage is carried out.

Procedure for Passage/subculture/transfer:

The Following steps are involved in sub culturing procedure:

1. The flask was observed and found confluent, thus subculturing was carried out.
2. The Suspension from the flask was discarded gently.
3. To the flask 7ml of Dulbecco's Phosphate Buffered Saline (DPBS) was added. It was thoroughly rinsed and then drained off.
4. 3mL Trypsin was then added into the flask mainly to detach the cells.
5. The flask was then kept in CO_2 Incubator for 3-4 minutes.
6. The flask was then tapped with hands and the cell detachment was observed under the microscope.
7. 3mL of Media was then added into the flask and was mixed properly with pipette.
8. A new T-75 Flask was labeled with succeeding passage number, cell line and culture date.
9. Add 20mL media into newly labeled flask.
10. Seed 20% cells into it.

Other steps need to be followed are as below.

- **Heat Inactivation of Serum:**
- **Media Formulation:**
- **Cell Counting using Haemocytometer:**
- **Establishment and Maintenance of Cell Cultures: Drug Dilutions:**
- **Cell count:**
- **Immunohistochemical staining:**

Results and Discussion: All results are summarised as below -

Experiment :- The effect of drug at different time intervals and at different concentrations

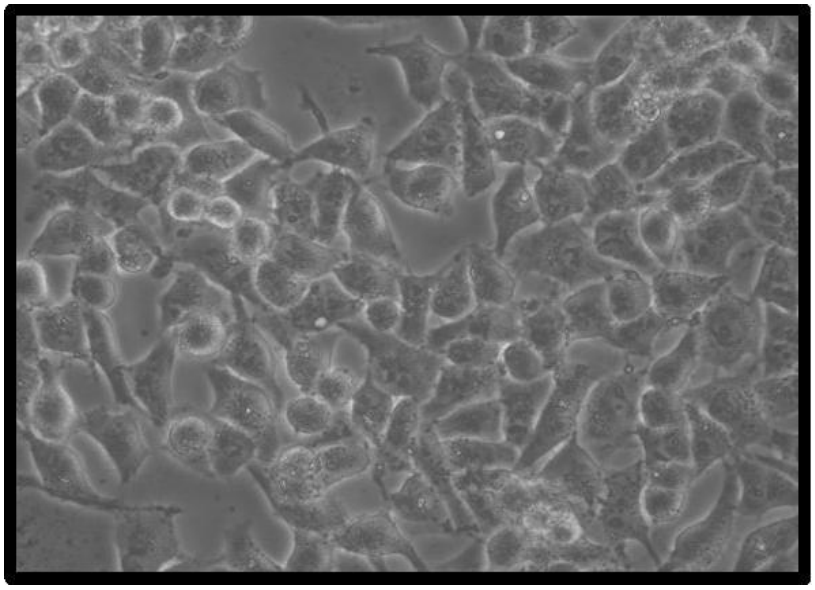

Figure 1: Pre treatment Panc-1 cells (Control)

Control depicts attached panc-1 cells.

The morphology is epithelial-like.

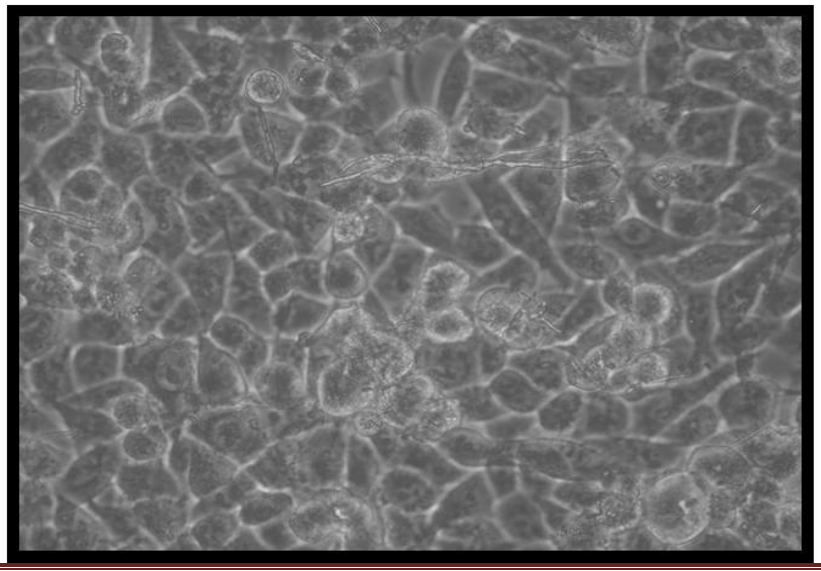

Figure 2: Post treatment Panc-1 cells Cisplatin-1µg

Panc-1 cells treated with 1µg Drug Cisplatin. The result was that most of the cells showed attachment.

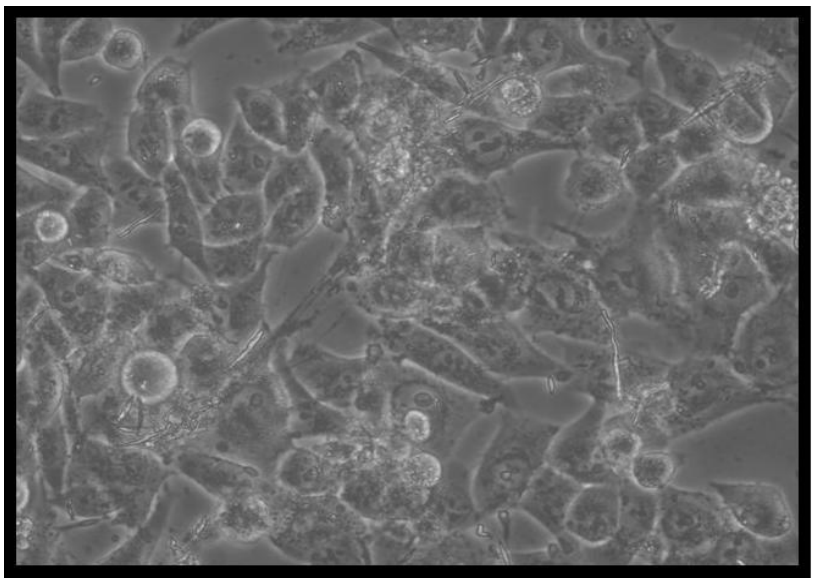

Figure 3: Post treatment Panc-1 cells Cisplatin-2.5µg

Panc-1 cells treated with 2.5µg Drug Cisplatin. The result was that due to the effect of drug most of the cells showed attachment and rounding off.

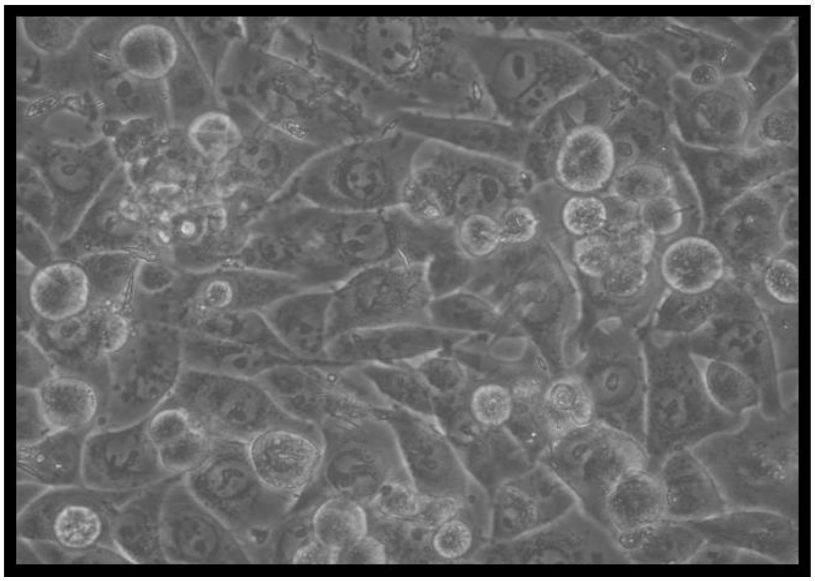

Figure 4: Post treatment Panc-1 cells Cisplatin-5µg

Panc-1 cells treated with 5µg Drug Cisplatin. The result was that due to the effect of drug the cells showed rounding off and apoptosis.

In ancient time when Cell culture was not so popular but many scientist performed it at great efforts . HeLa strain human epidermoid carcinoma cells (Scherer et al., 1953) were grown on flying cover slips in Leighton tissue culture tubes. They were cultivated in a nutrient medium consisting of 5 per cent human serum, 20 per cent filtered chick embryo extract (Bryant et al., 1953) and 75 per cent Earle's balanced salt solution (Earle, 1943).

From this primitive study we can conclusions - Control cells are attached, elongated and shows Cytoplasmic, membrane and nuclear negative for biomarker. Membrane and Cytoplasmic presence of biomarker is gained in presence of DNA damaging agent in dose dependent manner.The demonstration that human tumors could also give rise to continuous cell lines encouraged interest in human tissue, helped later by the classic studies of LeonardHay flick on the finite life span of cells in culture(Ahlert,1983)

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