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DRUG RELATED ALTERATION OF EXPRESSION OF THE CELL ADHESION MOLECULE

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ABSTRACT

Many scientists were interested in studying specific forms of cellular differentiation during embryo development. Roux however was not concerned with the multiplication of cells in culture. Pancreatic adenocarcinoma (PA) is an aggressive disease that develops in a relatively symptom-free manner and is usually advanced at the time of diagnosis. depicts the presence of expression of the Cell Adhesion Molecule on Membrane and Cytoplasm at time interval of 72 Hours due to the effect of Drug (Cisplatin-1µg). Membrane and cytoplasm are stained brown by DAB treatment. The Nucleus appears Brown. HeLa strain human epidermoid carcinoma cells (Scherer et al., 1953) were grown on flying cover slips in Leighton tissue culture tubes.

KEYWORDS: Membrane, Cytoplasm, carcinoma cells.

INTRODUCTION

Cell lines or cell strain may be propagated as an adherent monolayer or in suspension. There are three main methods of initiating a culture (Das, 2014). Monolayer culture signifies that, the cells will attach to the substrate and that normally the cells will be propagated in this mode. Anchorage dependence means that attachment to the substrate is a prerequisite for cell proliferation. Many scientists were interested in studying specific forms of cellular differentiation during embryo development. Roux however was not concerned with the multiplication of cells in culture. Harrison's "Hanging drop" experiment enabled observation of the growth of nerve cells from original explants.

The first permanent cell line was developed by Earle in 1943 from subcutaneous mouse tissue. Cell cultures were propagated continuously and were shown to be morphologically

quite different from the original tissue. Gey et al, (1952) established the first continuous human cell line, HeLa. Thus it was shown that "transformed" cell line was the HeLa cell, derived from a cervical carcinoma. It was not until the 1950s that trypsin became more generally used for subculture, following procedures described by Dulbecco to obtain passaged monolayer cultures. Other techniques that came to be important for both small scale and large scale cell culture were developed during the 1950s and 1960s. The inoculated HeLa cell cultures and controls were incubated at 37 C. Flying cover slips from each of the three series were harvested 1, 2, 3, and 4 days post inoculation. They were washed in balanced salt solution 10 min (room temperature), fixed in absolute methanol 5 min, and stained 2 hr in Giemsa diluted 1:50 in pH 7 phosphate buffer, or buffered azure B eosin (Shepard, 1956 and 1957). 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).

It was shown that cells would attach to some plastics, such as polystyrene, if the plastic was appropriately treated with an electric discharge or high-energy ionizing radiation. 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.

Each integrin comprises one α and one β subunit, the extracellular domains of which are highly polymorphic, thus generating considerable diversity among the integrin. The third group of cell adhesion molecules is the transmembrane proteoglycans, also interacting with matrix constituents, Pancreatic adenocarcinoma (PA) is an aggressive disease that develops in a relatively symptom-free manner and is usually advanced at the time of diagnosis. As is common in epithelial tumors, carcinogenesis develops through accumulation of mutations and genetic lesions leading to activation of oncogenes and inactivation of tumor suppressor genes. Since multiple combinations of mutations can lead to the development of PA 1, disease sub-classes may present different survival strategies requiring multiple targeted intervention strategies.

MATERIALS AND METHODS

Cell line was cultured as an Adherent cell line. The panc-1 cultures were established from working cell bank cryo stocks and stored in liquid nitrogen (-196˚C).

Cell harvest procedure

Steps for cell harvesting

1. The Suspension from the flask was discarded gently.
2. To the flask 7ml of Dulbecco's Phosphate Buffered Saline (DPBS) was added. It was thoroughly rinsed and then drained off.
3. 3mL Trypsin was then added into the flask mainly to detach the cells.
4. The flask was then kept in CO_2 Incubator for 3-4 minutes.
5. The flask was then tapped with hands and the cell detachment was observed under the microscope.
6. 3mL of Media was then added into the flask and was mixed properly with pipette.
7. The suspension was then taken into falcon.
8. A new T-25 Flask was labelled with succeeding passage number, cell line and culture
9. While it was centrifuging various drug dilutions were prepared as follows.
10. 10mL Media was added into newly labelled T-25 flask.
11. Remaining suspension from T-75 flask was added into T-25 flask.
12. The Falcon containing cell suspension was centrifuged at 2100 rpm for 5 minutes.

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 - Biomarker assessment assay (IHC) for Panc-1 cells:

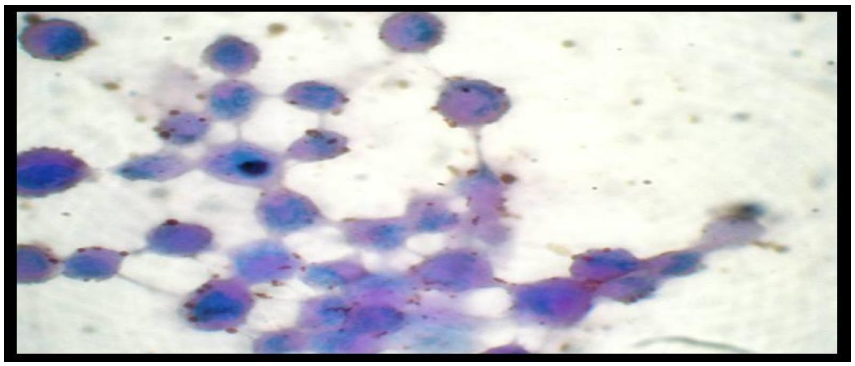

Figure 1:- Pre treatment Panc-1 cells (Control)

Figure 1 depicts the Panc-1 cells without treatment of Drug Cisplatin. Nucleu s, Cytoplasm and Membrane appears blue.

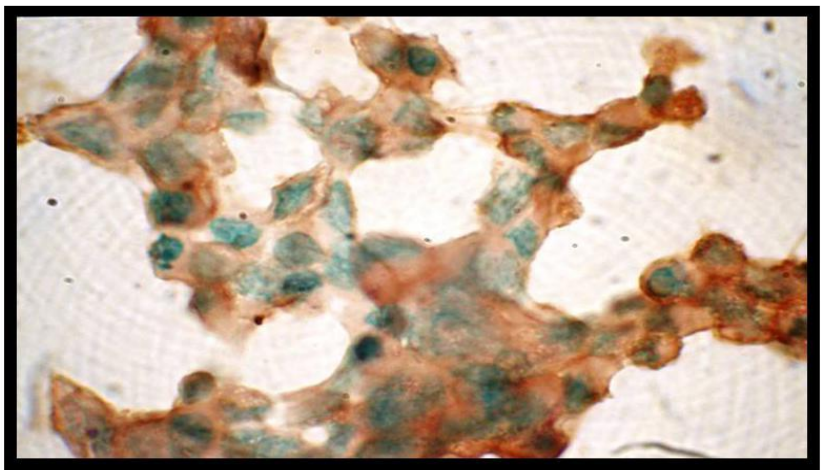

Figure 2:-Post treatment Panc-1 cells Cisplatin-1µg (48 Hours)

Figure 2 depicts the presence of expression of the Cell Adhesion Molecule on Membrane and Cytoplasm after 48 hrs due to the effect of Drug (Cisplatin-1µg). Membrane and cytoplasm are stained brown by DAB treatment. The Nucleus appears Blue because of the counter stain Methyl Green.

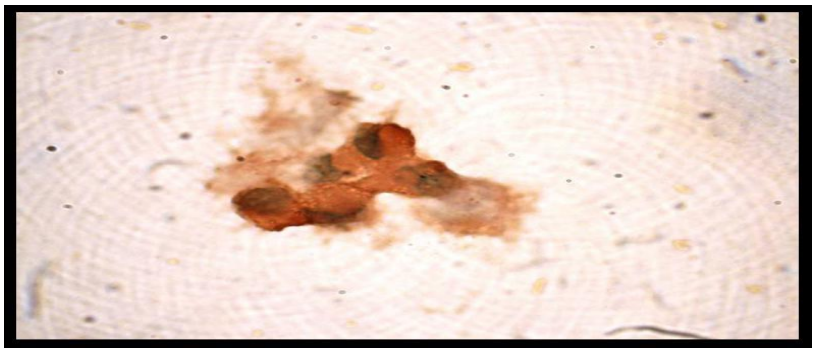

Figure 3:- Post treatment Panc-1 cells Cisplatin-2.5µg (48 Hours)

Figure 3 depicts the presence of expression of the Cell Adhesion Molecule on Membrane and Cytoplasm after 48 hrs due to the effect of Drug (Cisplatin-2.5µg). Membrane and cytoplasm are stained brown by DAB treatment. The Nucleus appears light brown.

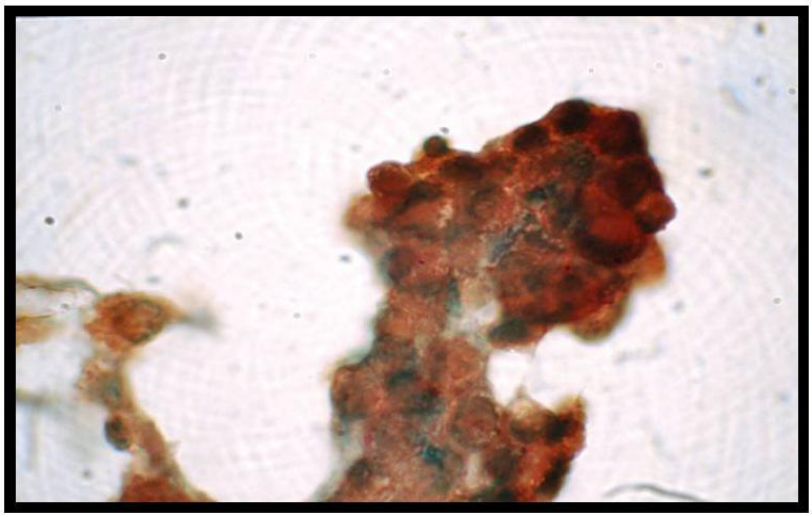

Figure 4:- Post treatment Panc-1 cells Cisplatin-1µg (72 Hours)

Figure 4 depicts the presence of expression of the Cell Adhesion Molecule on Membrane and Cytoplasm at time interval of 72 Hours due to the effect of Drug (Cisplatin-1µg). Membrane and cytoplasm are stained brown by DAB treatment. The Nucleus appears Brown.

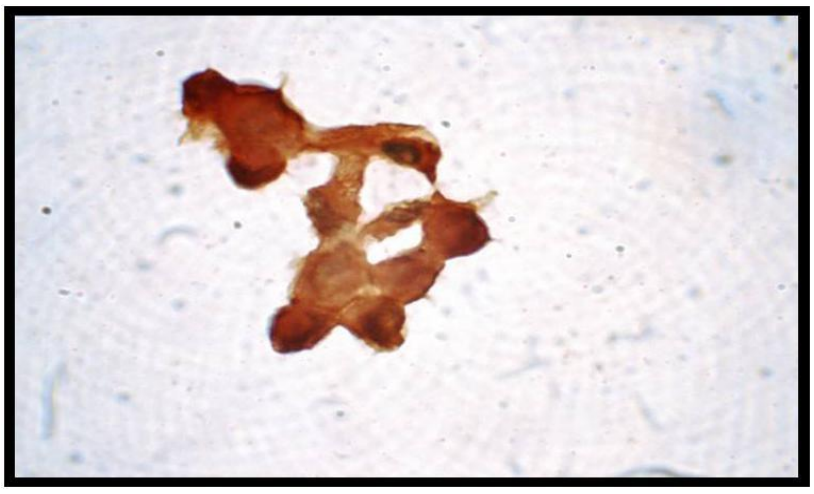

Figure 5:- Post treatment Panc-1 cells Cisplatin-2.5µg (72 Hours)

Figure 5 depicts the presence of expression of the Cell Adhesion Molecule on Membrane and Cytoplasm at time interval of 72 Hours due to the effect of Drug (Cisplatin-2.5µg). Membrane, cytoplasm and Nucleus are stained brown by DAB treatment.

Cell line	Staining	Control	Cisplatin (1 µg)		Cisplatin (2.5 µg)	
	Cytoplasmic	- ve	Cytoplasmic	+ ve	Cytoplasmic	+ ve
Panc-1 (48 hrs)	Membrane	- ve	Membrane	+ ve	Membrane	+ ve
	Nuclear	- ve	Nuclear	- ve	Nuclear	Mild + ve
	Cytoplasmic	- ve	Cytoplasmic			+ ve
Panc-1 (72 hrs)	Membrane	- ve	Membrane			+ ve
	Nuclear	- ve	Nuclear	Mild + ve	Nuclear	+ ve

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.

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