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Characterization of a drug resistant MCF-7 breast cancer cell line developed by repeated cycles of 5-Fluorouracil (5-FU) therapy

Erebi Patricia Tawari * and Melvin Peremobowei Ere

Department of Chemical Pathology, Faculty of Basic Clinical Sciences, College of Health Science, Niger Delta University, Bayelsa State, Nigeria.

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Abstract

Breast cancer accounts for 14% of cancer fatalities and 23% of all cancer diagnoses, making it the most common cause of cancer death among women and one of the most frequently diagnosed cancers. This disease is a life-threatening public health concern because of its significant influence on the general population. Therefore, further molecular research is required to determine its prognosis and create tailored treatments. Since it closely resembles mammary epithelium, the human breast cancer cell line MCF-7, which expresses genes for oestrogen, progesterone, and glucocorticoid receptors, is the most appropriate in vitro model for cancer research. Resistant cell lines were created by repeatedly cultivating MCF-7 wild-type cells in media containing 5-fluorouracil, a first-line treatment for breast cancer. The resistant cell lines were found to be cross-resistance to other anticancer medications in addition to being resistant to 5-fluorouracil, according to the *in vitro* MTT cytotoxicity assay.

Keywords: MCF-7 cells; 5-Fluorouracil; Acquired resistance; Breast cancer cells

1. Introduction

A group of breast diseases that affect the same anatomical organ and originate from the same anatomical structure make up breast cancer (BC), a heterogeneous malignant disease with varying risk factors, clinical manifestations, histopathologies, genetic and genomic variations, and therapeutic and clinical outcomes (Weigelt and Reis-Filho, 2009). In both the developed and developing worlds, breast cancer is the most deadly cancer and the second most frequent cancer worldwide. It is a leading cause of cancer-related mortality among women globally (Kamangar et al., 2006).

Since cell lines can be employed widely in many aspects of laboratory research, especially as in vitro models in cancer research, they serve important roles in the molecular diagnosis of breast cancer (Burdall et al., 2003). The human breast cancer cell line MCF-7 expresses genes for glucocorticoid receptors, progesterone, and estrogen. Dr. Soule of the Michigan Cancer Foundation in Detroit, Michigan, initially extracted it from the pleural effusion of a 69-year-old Caucasian patient who had metastatic breast cancer (adenocarcinoma) in 1970 (Soule et al., 1973). The first hormone-responsive breast cancer cell line, MCF-7 cells are useful for in vitro breast studies because they have maintained a number of ideal features unique to the mammary epithelium, such as the ability to process oestrogen via cytoplasmic oestrogen receptors (OR) to produce estradiol. They are also a good model cell line for breast cancer research around the world (Levenson and Jordan, 1997).

An antimetabolite medication called 5-fluorouracil (5-FU) has been widely utilized to treat a variety of cancers, including breast and colorectal cancer. By disrupting the function of thymidylate synthase (TS) or by incorrectly integrating its metabolites into RNA and DNA, it carries out its cytotoxic action by interfering with vital biosynthesis

* Corresponding author: Tawari Erebi Patricia

(Longey et al., 2003). Despite the many benefits of 5-fluorouracil, the development of drug resistance following chemotherapy makes its therapeutic uses insufficient.

1.1. Rationale and aims of the study

The purpose of this work was to create a resistant MCF-7 breast cancer cell line that would not respond to 5-fluorouracil despite being exposed to dosages of the drug repeatedly. These cell lines can be used as a model to study breast cancer positive to oestrogen, progesterone and glucocorticoid receptors.

2. Methodology

2.1. Cell lines and reagents

To create the resistant cell line MCF-$7_{5FU10\mu M}$, the MCF-7 breast cancer cell line was acquired from ATCC, Middlesex, UK. It was then repeatedly and continuously cultivated in media containing 5-fluorouracil (5-FU) (Sigma, Dorset, UK) in a stepwise concentration increasing approach.

2.2. Construction of Sequential 5-Fluorouracil -Resistance Cancer Cell Lines

The MCF-7 breast cancer cell line was cultivated in 1.0 μM 5-Fluorouracil culture medium for 72 hours in order to create the sequential 5-Fluorouracil-resistance MCF-$7_{5FU10\mu M}$ breast cancer cell lines (Eli Lilly and company, IN, USA). Viability was checked every two to three days when the cells reached a confluence of about 90% and were replaced with DMEM containing 5-fluorouracil. A number of clones were chosen and cultivated in DMEM after the newly generated resistant cell lines multiplied to form colonies. To create cell stocks, the newly created clones were cultivated and kept in 5-fluorouracil media. To create the subsequent phase, some of the cells were transferred to a medium containing 5-fluorouracil (10μM). For additional research, cells from the first (1^{st}) and fifth (5^{th}) generations of acquired resistant 5-fluorouracil were chosen.

2.3. Cell culture and cytotoxicity analysis

Dulbecco's modified Eagle's medium (DMEM) (Lonza, Wokingham, UK) was used to cultivate the cell lines. It was enhanced with 10% FCS, 50 unitsml_1 penicillin, and 50 mgml_1 streptomycin. The medium containing 10μM of 5-fluorouracil was used to sustain the resistant cell line MCF-$7_{5FU10\mu M}$ from the first and fifth generations.

2.4. For *In vitro* Cytotoxicity Assay

For 48 hours or 72 hours, the 96-well flat-bottomed microtiter plates containing 5000 overnight-cultured cells per well were exposed to medications. The cells were subjected to a standard MTT assay after receiving paclitaxel (PAC) for 48 hours, cisplatin (CDDP) for 72 hours, vincristine (VCR) for 48 hours, and doxorubicin (DOX) for 72 hours (Plumb et al, 1989).

3. Results

3.1. Morphological features

The phenotypic of the drug-resistant cells MCF-$7_{5FU10\mu M}$ was found to be different from that of the wild-type (WT) parental cells MCF-7. Comparing the resistant cells to the original cell lines, Figure 1 demonstrates that they were smaller and had fewer distinct irregular multiple nuclei.

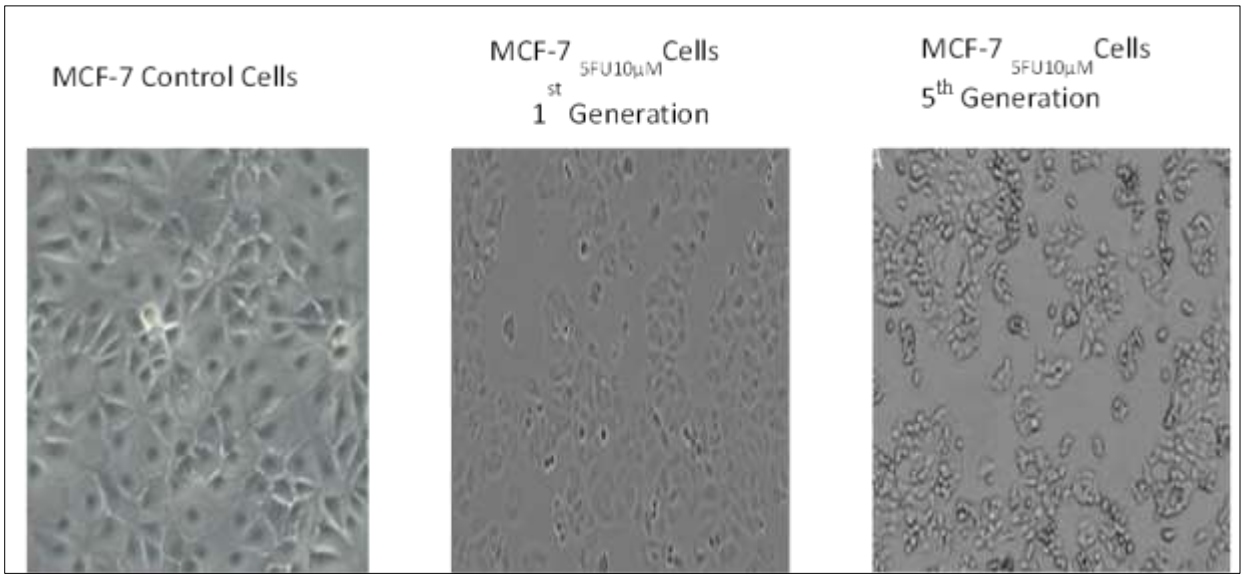

Figure 1 Representative morphologic Images of MCF-7 Control Cells and MCF-7 $_{5FU10\mu M}$Cells of the 1st and 5th Generations

3.2. Cell Viability of 5-Fluorouracil-resistance cancer cell lines

The resistant cells had a larger IC50 than the control cells and showed resistance to 5-fluorouracil, according to data from MTT cytotoxicity study of MCF-7 control cells and MCF-$7_{5FU10\mu M}$ of the first (1st) and fifth (5th) generations (Figure 2). Compared to the first generation and control cells, the fifth generation of cells' IC50 was shown to be substantially more resistant to 5-fluorouracil.

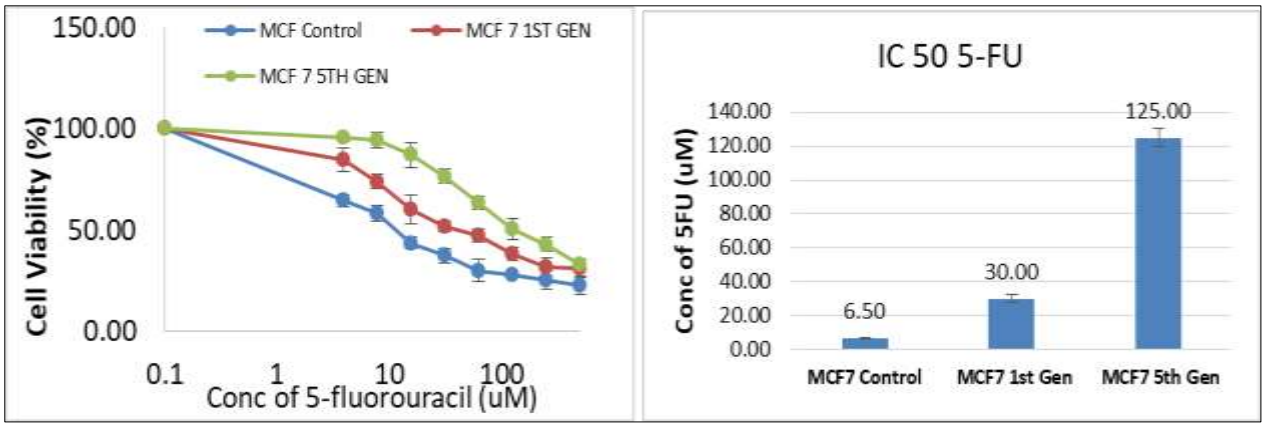

Figure 2 Representative Drug Concentration Response Curves and Histogram of MCF-7 control cells and MCF-$7_{5FU10\mu M}$ of the 1st and 5th Generations to 5-fluorouracil. (5-FU – 5-fluorouracil)

3.3. Resistant Cell Lines showed Cross and Pan-Resistance to Anticancer Drugs

The resistant cell lines MCF-$7_{5FU10\mu M}$ of the first and fifth generations, according to data from MTT cytotoxicity study, shown cross-resistance to several anticancer medications, such as vincristine (VCR) Figure 3a, doxorubicin (Dox) Figure 3b paclitaxel (PAC) Figure 3c, and cisplatin (CDDP) Figure 3d. Compared to the control cells, the resistant cells' IC 50s were noticeably greater.

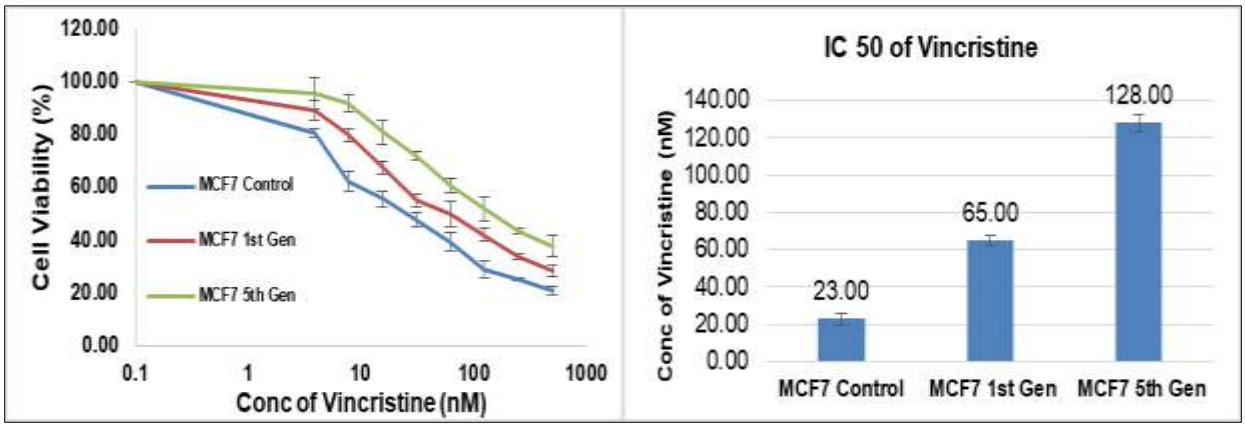

Figure 3a Representative Drug Concentration Response Curves and Histogram of MCF-7 control cells and MCF-$7_{5FU10\mu M}$ of the 1st and 5th Generations to Vincristine. (VCR- Vincristine)

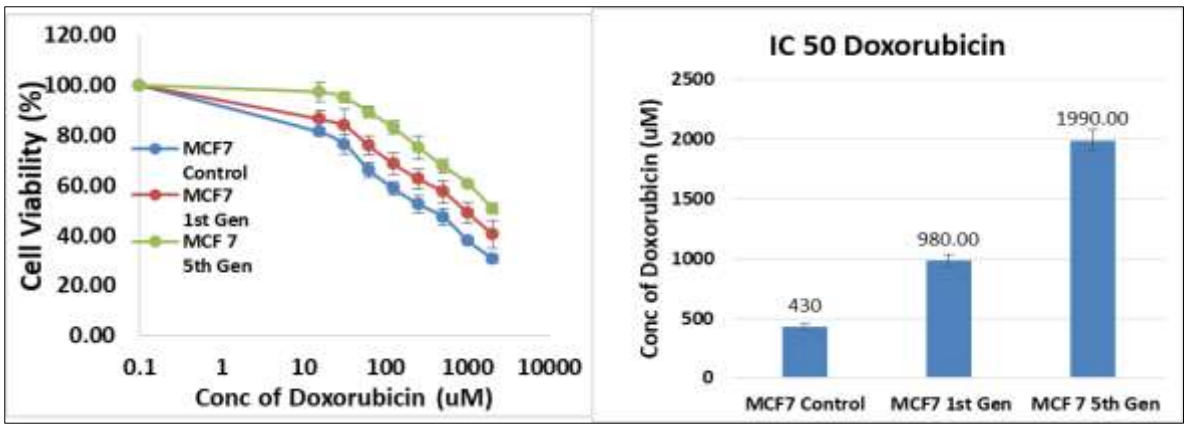

Figure 3b Representative Drug Concentration Response Curves and Histogram of MCF-7 control cells and MCF-$7_{5FU10\mu M}$ of the 1st and 5th Generations to Doxorubicin. (DOX- Doxorubicin)

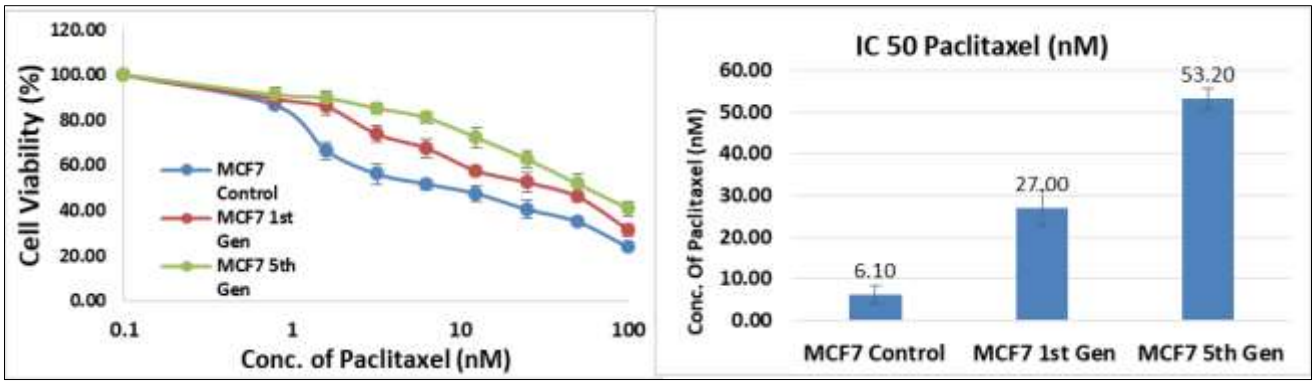

Figure 3c Representative Drug Concentration Response Curves and Histogram of MCF-7 control cells and MCF-$7_{5FU10\mu M}$ of the 1st and 5th Generations to Paclitaxel. (PAC- Paclitaxel)

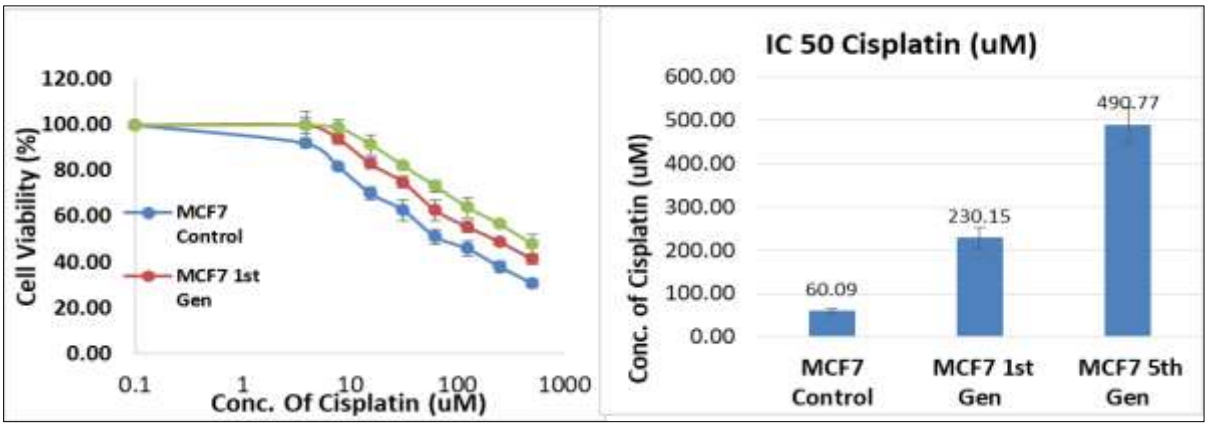

Figure 3d Representative Drug Concentration Response Curves and Histogram of MCF-7 control cells and MCF-$7_{5FU10\mu M}$ of the 1st and 5th Generations to Cisplatin. (CDDP- Cisplatin)

4. Discussion

The mortality rate has decreased as a result of improvements in cancer treatment and management over the past few years, including the use of targeted therapies, chemotherapy, and radiation (Barrera-Rodríguez and Fuentes, 2015). However, the disease still appears to be alive and well. One of the primary causes of the high death rate from advanced breast cancer is the frequent emergence of resistance to several chemotherapeutic medications during and after therapy.

Genes for oestrogen, progesterone, and glucocorticoid receptors are present in MCF-7 breast cancer cells. It belongs to the luminal A molecular subtype (Done, 2011) and is oestrogen (OR) and progesterone receptor (PR) positive (Shirazi et al., 2013). MCF-7 is a noninvasive, poorly aggressive cell line (Gest et al., 2013), and it is typically thought to have a minimal potential for metastasis (Shirazi et al., 2013).

Although 5-fluorouracil is one of the first-line medications used to treat breast cancer, resistance to it is likely to develop with time. According to data from this investigation, Figure 1 showed that after multiple treatment cycles, the resistance cells MCF-$7_{5FU10\mu M}$ of the first and fifth generations developed resistance against 5-fluorouracil, exhibiting a larger IC50 in comparison to the parent cells used as reference. Furthermore, as shown in Figures 3a and 3d, the MCF-$7_{5FU10\mu M}$ of the first and fifth generations was discovered to be resistant to further anticancer medications such vincristine, doxorubicin, paclitaxel, and cisplatin respectively. This suggests that both pan and cross resistance to anticancer medications are induced by acquired resistance. These outcomes are comparable to those of multiple studies that shown chemotherapy resistance (Videira et al., 2014; Wu et al., 2014; Tawari and Kasia, 2020; Tawari, 2024).

5. Conclusion

Since cell survival during and after chemotherapy has been associated with multiple drug resistance, it is imperative that novel treatment approaches be created to address this problem. Cross and pan resistance in breast cancer cells that contain genes for oestrogen, progesterone, and glucocorticoid receptors can be studied using the resistant cell lines that were created as appropriate *in vitro* models.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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