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International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Assessment of the Cancer Stemness of an Acquired 5-Fluorouracil (5-FU) Resistant MCF-7 Breast Cancer Cell line

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A B S T R A C T

A tiny subgroup of cells located in breast cancer tumors, breast cancer stem cells (BCSCs) are known to be crucial to the development of the disease. BCSC indicators are connected to poor outcomes and are associated with cells' increased ability to proliferate, adhere, migrate, and invade. This study set out to evaluate the stemness of the acquired resistant cell line MCF-75FU10µM. The resistant cell lines MCF-75FU10µM had higher amounts of cancer stem cell biomarkers (ALDH and CD 133) and embryonic biomarkers (Nanog, Oct4, and Sox2) than the original wild-type cell MCF-7 breast cancer cells, according to the results of fluorescence-activated cell sorting (FASC). Furthermore, the 5th generation of the resistant cells had greater expression of these BCSC biomarkers than the 1st generation which is suggestive that the stemness of the resistant cells increases as the expression of the BCSC biomarkers increases.

Keywords: MCF-7 cells, Breast Cancer stem cells, stem cell biomarkers, embryonic cell markers, Acquired resistance.

1.0 Introduction

After lung cancer, breast cancer (BC) is the second prominent cause of cancer-related mortality in females and one of the most frequent cancers diagnosed in females (DeSantis et al., 2019; Siegel et al., 2019). Premature menarche, advanced age at first childbirth, advanced age on menopause, long-standing use of hormonal contraception and hormone replacement therapy, unhealthy diet, lack of daily exercise, being overweight or obese, exposure to ionizing radiation, certain benign proliferative diseases of the breast, and specific gene mutations (primarily BRCA1, BRCA2, or TP53) with a family history of breast cancer (particularly in young patients) are some risk factors linked to the advancement of breast cancer (BC) (Sun et al., 2017; Jia et al., 2022).

The luminal epithelial cells and myoepithelial cells are the two primary cellular lineages that make up the mammary epithelium. These cells are descended from mutual ancestors known as mammary epithelial stem cells, according to research conducted on both humans and mice (Gudjonsson et al., 2002). Positioned in the outer basal compartment, mammary epithelial stem cells are essential for the manufacturing of either basal inner luminal progeny cells (multi/bi-potent stem cells) or basal myoepithelial progeny cells (unipotent stem cells), which can then differentiate into specialized cells (Van Amerongen et al., 2012). Stem cells, which may self-renew and specialize into a range of cells that support tumorigenesis, are created when mammary epithelial cells develop cancer.

Conventional treatments for breast cancer typically do not work on breast cancer stem cells (BCSCs), which can result in medication resistance and recurrence (Lee et al., 2019). According to Phillips et al. (2006), BCSCs are classified based on their capacity to develop tumors from their minute numbers and their resistance to chemotherapy and radiation treatments. According to Beck and Blanpain (2013), the BCSCs display a precise balance between self-renewal and differentiation, which helps to preserve tumor homeostasis. Moreover, the tumor mass can be established by these cells differentiating into a large number of diverse cancer cells (Li et al., 2017). Due to their cell latency, improved capacity for DNA damage repair, and improved drug efflux, BCSCs can withstand oxidative stress or DNA damage when exposed to environmental stimuli while non-CSCs are eliminated by conventional chemotherapy or radiation therapy (Lytle et al., 2017).

Cancer stem cells (CSCs) have been discovered in a number of malignancies, including those of the breast, brain, blood (leukemia), skin (melanoma), head and neck, thyroid, cervix, lung, and gastrointestinal and reproductive tract organs (Mimeault et al., 2007). Certain cell surface indicators can be used to identify and detect CSCs. Breast CSCs can be identified by specific surface markers like as CD44, CD24, CD133, and Aldehyde Dehydrogenase (ALDH).

1.1 Rationale and aims of the study

The aim of this study was to ascertain whether the acquired resistance cells, MCF-$7_{5FU10\mu M}$, expressed more stem cell indicators than the wild-type cells, MCF-7. This study also sought to ascertain whether the degree of expression of cancer stem cell biomarkers influences the stemness of cells.

2.0 Methodology

2.1 Cell lines and reagents:

The original cell lines were continuously cultured in media containing 5-fluorouracil (5-FU) (Sigma, Dorset, UK) in a stepwise concentration-increasing approach to produce the resistant cell line MCF-$7_{5FU10\mu M}$. ATCC, Middlesex, UK, provided the wild-type MCF-7 breast cancer cell line.

2.2 Detection of ALDH positive population

The ALDEFLUOR kit (StemCell Tech., Durham, NC, USA) was used in accordance with the supplier's instructions to determine which population tested positive for aldehyde dehydrogenase (ALDH). The cells (2.5 × 105) were analyzed after being stained for 30 minutes at 37°C in ALDH substrate with assay buffer. The negative control was treated with diethylaminobenzaldehyde (DEAB), a specific ALDH inhibitor.

2.3 CD 133 Flow Cytometric Analysis

The adherent had been trypsinized and then put into a 25G needle. The 2.5 × 105 cells were treated with a CD 133 antibody (BD Pharmingen, Oxford, UK) for 20 minutes at 4°C. A maximum of one hour after staining, the cells (10,000 events) were examined on a BD Facscalibur after unbound antibodies were removed using 2% fetal calf serum (FCS) HBSS (Sigma).

2.4 Analysis of Embryonic Stem Cell markers using Immunofluorescent Flow Cytometric

The expression of Nanog, Oct4, and Sox2 was assessed by immunofluoresent flow cytometry. Trypsinization was used to harvest the cultivated cells. Acetone/methanol was used to fix the cells, and 0.1% triton-X100 was used to permeabilize them. The cells were stained with primary (1:50 dilution) and FITC-conjugated secondary antibodies for an extra hour at room temperature following an hour of 3% BSA blocking. A FACS Calibur flow cytometer equipped with a 488-nm blue laser and a conventional FITC 530/30 nm band pass filter was used to identify the favorably stained population.

3.0 Results

3.1 Cancer Stem Cell biomarkers increased in acquired resistant cell lines.

FASC results showed that aldehyde dehydrogenase (ALDH) activity was present in both the wild-type cell line (MCF-7) and the resistant cell lines (MCF-$7_{5FU10\mu M}$) with and without treatment with DEAB (30μM) (Figure 1a and 1b). The resistant cell lines (MCF-$7_{5FU10\mu M}$) had a higher percentage of $ALDH^+$ than the parent cells (MCF-7) before they were treated with Diethylaminobenzaldehyde (DEAB). The FASC data and histogram representation showed that the resistant cells expressed higher ALDH+ activity than the control, the wild-type cells, when the ALDH+ activity of the resistant cell lines (MCF-$7_{5FU10\mu M}$) and the wild-type cell line (MCF-7) used as control were compared as demonstrated in Figure 1.

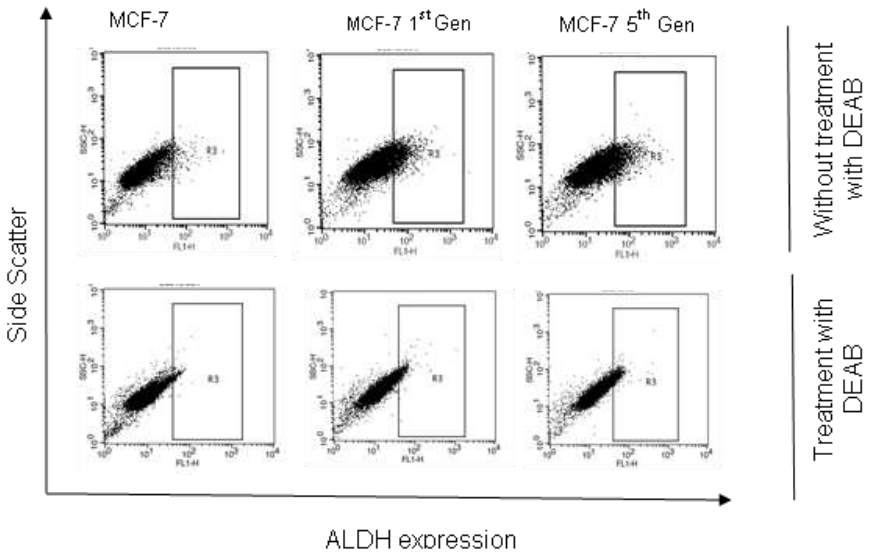

Figure 1a: MCF-7 Wild-type and MCF-$7_{5FU10\mu M}$ cell lines' representative FASCS plots and histograms of $ALDH^+$ expression with and without DEAB as determined by the ALDEFLUOR test.

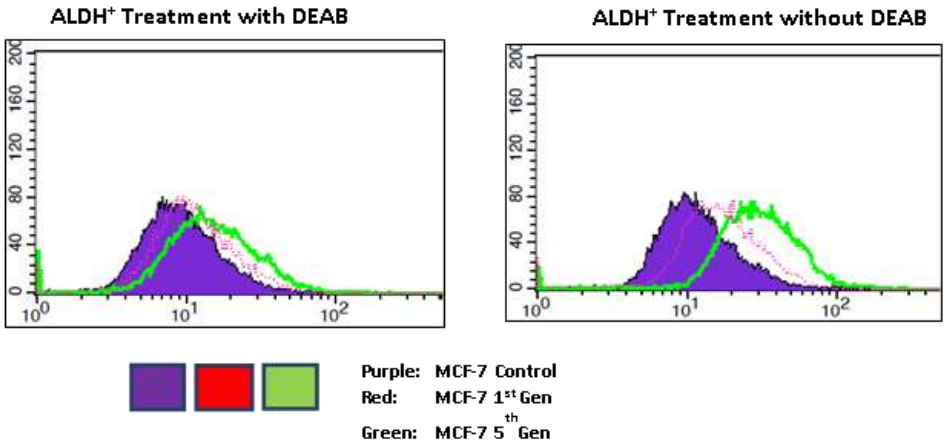

Figure 1b: Representative FASCS overlay of MCF-7 Wild-type and MCF-$7_{5FU10\mu M}$ cell lines' ALDH$^+$ expression with and without DEAB as determined by the ALDEFLUOR test.

The parent cell line (MCF-7) and the resistant cell lines (MCF-$7_{5FU10\mu M}$) both express CD133, per FASC results (Figure 2a). Nonetheless, the resistant cells' CD133 expression was noticeably greater than the wild-type cells'.

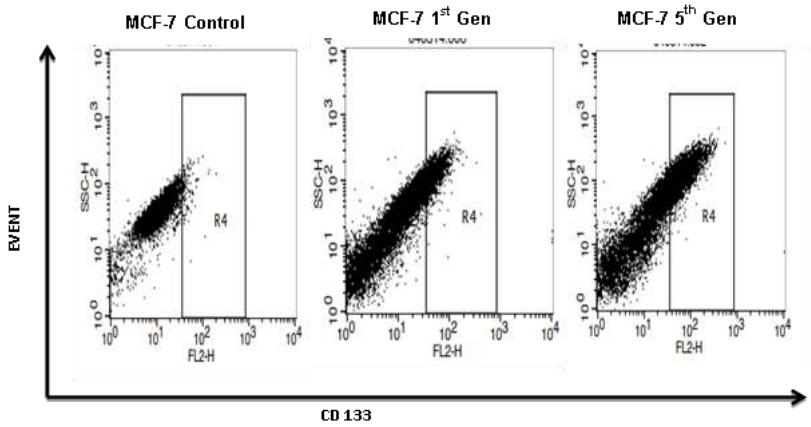

Figure 2: MCF-7 Wild-type and MCF-$7_{5FU10\mu M}$ cell lines' representative FASCS plots of CD 133 expression

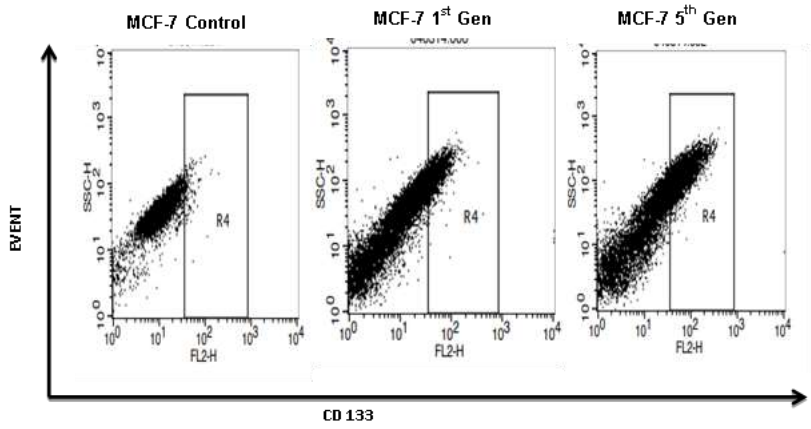

Figure 2a: MCF-7 Wild-type and MCF-$7_{5FU10\mu M}$ cell lines' representative FASCS plots of CD 133 expression

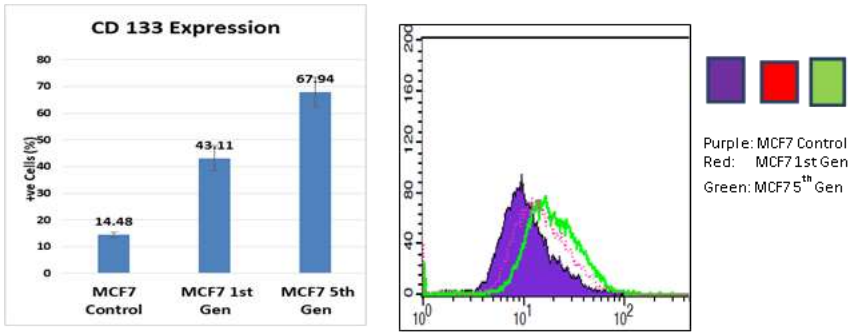

Figure 2b: Representative Histogram and FASCS overlay of MCF-7 Wild-type and MCF-$7_{5FU10\mu M}$ cell lines' CD 133 expression

When comparing resistant cell lines (MCF-$7_{5FU10\mu M}$) to the parental cell (MCF-7), there was a notable rise in embryonic stem cell markers. The resistant cell lines (MCF-$7_{5FU10\mu M}$) demonstrated higher levels of embryonic stem cell markers (Nanog, Sox2, and Oct4) than the wild-type control cells MCF-7, according to the fluorescence activated cell sorting (FASC) data (Figure 3). These markers are essential for stem cell sustenance, self-renewal, and pluripotency.

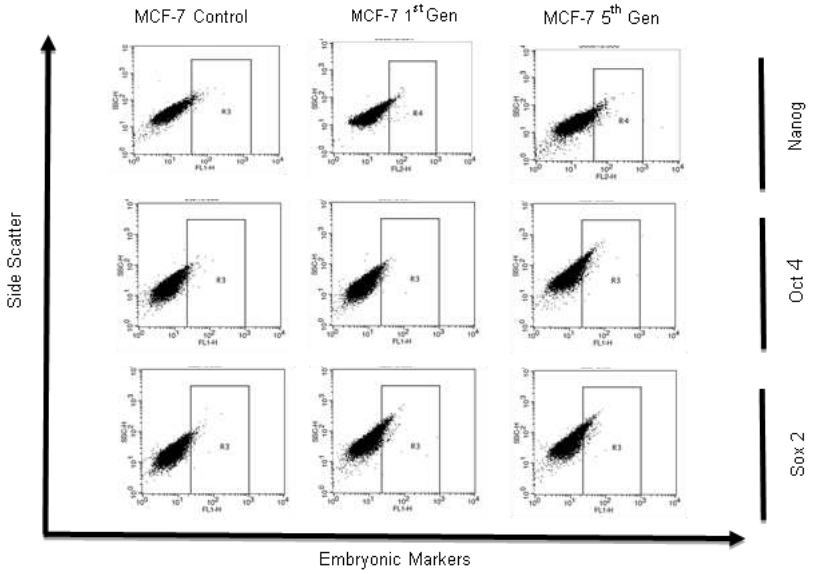

Figure 3a: MCF-7 Wild-type and MCF-$7_{5FU10\mu M}$ cell lines' representative FASCS plots and histograms of Embryonic Biomarker expression (Nanog, Oct 4 and Sox2).

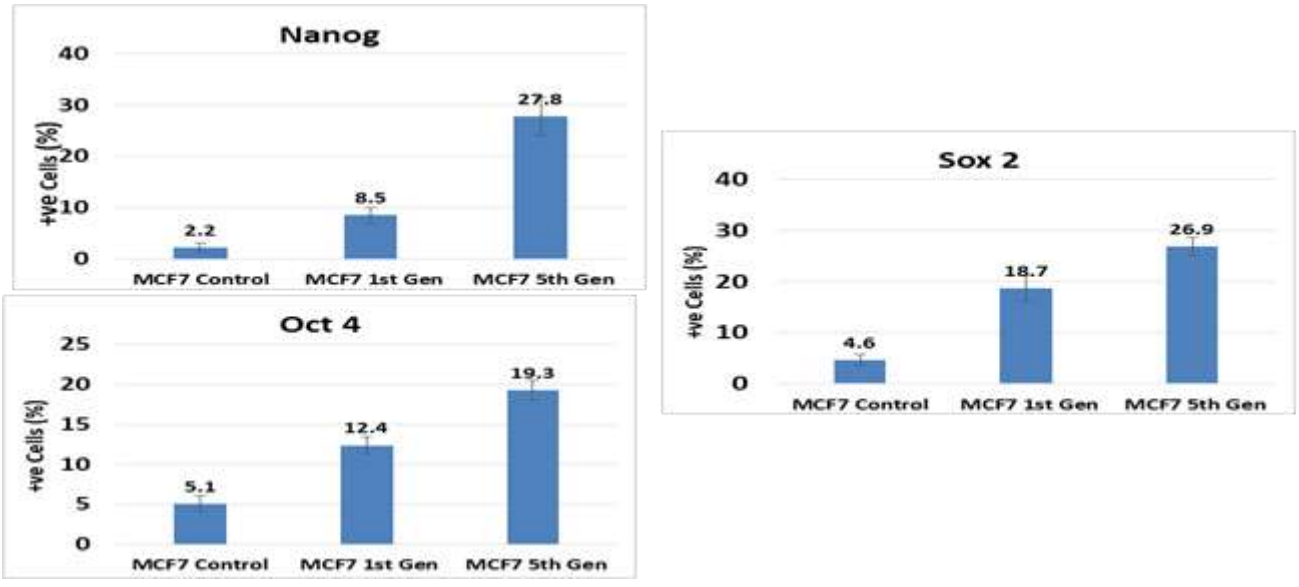

Figure 3b: MCF-7 Wild-type and MCF-$7_{5FU10\mu M}$ cell lines' histograms of Embryonic Biomarker expression (Nanog, Oct 4 and Sox2).

4.1 Discussion

In both the developed and developing worlds, breast cancer (BS) is steadily growing in importance as a public health concern. One of the most prevalent cancers in the world, BC is responsible for 6.9% of deaths and 11.6% of all fresh cancer cases in women globally each year, according to a research from the Global Cancer Observatory. Breast cancer stem cells (BCSCs), a subclass of tumor cells distinguished by their exceptional capacity for self-renewal and their capacity to segregate into non-BCSCs, have been linked to aggressiveness, development, spread, and poor response to treatment (Crabtree and Miele, 2018).

Although the breast cancer stem cells and mammary epithelial stem cells have certain physiognomies in common, such as the capacity to self-generate and produce progenitor cells, the BCSCs can be differentiated from normal stem cells by their increased expression of CSC markers and decreased expression of differentiation markers (Watson, 2021). Furthermore, because of their high potential for carcinogenesis and unique signaling pathway activity, BCSCs have been shown to be resistant to conventional therapy (Sorlie et al., 2001).

A number of surface indicators of BCSCs are used to isolate or identify BC. This study examined several stem cell markers, including $ALDH^{+}$, CD 133, and embryonic markers (Nanog, Sox2, and Oct4), using a flow cytometry method. Our study's data revealed that, in comparison to the wild-type cell line MCF-7, the resistant cell line MCF-75FU10μM expressed more ALDH+ (Figure 1), CD 133 (Figure 2), and the embryonic markers (Nanog, Sox2, and Oct4) (Figure 3). Our findings showed that, as seen in the fifth generations of cells grown in conditions containing 5-fluorouracil, greater expression of BCSC surface indicators enhanced the stemness of the cell lines. According to FASCs data, the fifth generation of cells expressed more of the BCSC surface biomarkers than the first generation did. Our results are consistent with those of prior research that found that resistant cells have greater levels of stem cell markers (Tawari-Ikeh and Kasia 2020, Anido et al., 2010, Singh et al., 2004).

4.2 Conclusion

More BCSC biomarkers were expressed by the acquired resistant cells than by the parent wild-type cells. Furthermore, compared to the first generation, the fifth generation of resistant cells expressed higher levels of these BCSC biomarkers. This suggests that the fifth generation of cells would be significantly more aggressive than the first generation, as the stemness of the resistant cells increases as the expression of the BCSC biomarkers increases.

Acknowledgment: Nil

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