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Evaluation of the cancer stem cell characteristics of acquired resistant BT549 GEM100nM breast cancer cells

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Abstract

Stem cells playing a vital role in cancer biology have drawn more attention in recent years. Many dense cancers, involving the breast, brain, colon, liver, and pancreas, have been found to be associated with cancer stem cells (CSCs). The objective of this research is to evaluate the cancer stem markers of an attained resistant BT549 GEM100nM breast cancer cell line. Fluorescence-activated cell sorting (FASC) results showed that the resistant cell lines contained higher levels of embryonic markers (Nanog, Oct 4, and Sox2) and cancer stem cell markers (ALDH and CD 133) than the parental wild-type cell (BT549). It is thought that these CSC indicators are pivotal for cancer genesis, relapse, spread and treatment resistance.

Keywords: Cancer stem cells; Resistant cell lines; Stem cell markers; Embryonic cell markers

1. Introduction

A small and unique group of undifferentiated cells called stem cells have the ability to divide through mitosis to create new stem cells as well as differentiate into specialized cells. One major characteristic of stem cells is their capacity to self-generate and yield progenitor cells, which develop into more specialized and mature cells. According to Jordan (2004), cancer stem cells (CSCs) are an uncommon subclass of tumour cells that have the ability to self-renew and differentiate into a variety of cells that promote tumorigenesis. CSCs are sometimes referred to as tumorigenic cells. Certain characteristics of tumour cells and regular cells are comparable, including the capacity to self-renew, segregate into progenitor cells and use shared pathways (Jordan, 2004). The idea of cancer stem cells were initially supported by evidence of hematological malignancies, where transplanting human acute myeloid leukemia cells into immune-compromised mice resulted in a small population of cancer cells that could grow tumors in in vivo mouse models (Bonnet & Dick, 1997). The part stem cells play in the biology of cancer has drawn more attention recently. CSCs have been found in a number of solid tumors and malignancies of the breast, brain, colon, liver, and pancreas (O'Brien et al., 2007; Prince et al., 2007). It is thought that CSCs are essential for the development, recurrence, spread, and resistance to treatment of tumors (Coker & Allan, 2008).

1.1. Breast Cancer Stem Cells

CSCs in humanoid breast cancer (BC) were first recognized in 2003 when a subpopulation of tumors from human patients with a CD44+/highCD24-/low lineage started to grow in immune-compromised mice. Within 12 weeks, injections of 100 tumorigenic cells produced palpable tumors, and serial passaging from these cells produced similar results. However, even after non-tumorigenic 10,000 cells were injected, CD44- cells were not tumorigenic (Al-Hajj et al., 2003).

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1.2. Cancer Stem Cell Markers

Several tumors, including those of the breast, brain, blood (leukemia), skin (melanoma), head and neck, thyroid, cervix, lung, and gastrointestinal and reproductive tract organs, have been found to contain cancer stem cells (CSCs) (Mimeault et al., 2007). CSCs can be identified and detected using specific markers on their cell surface. Aldehyde Dehydrogenase (ALDH), CD44, CD24, and CD133 are specific surface markers used to identify breast CSCs.

According to Ginstier et al. (2007), one functional indicator of CSCs is **Aldehyde Dehydrogenase (ALDH)**, a collection of enzymes that convert aldehydes into their analogous carboxylic acids. In humans, there are 19 different forms of ALDH that are crucial for both biological and toxicological processes. Increased ALDH activity has been identified in normal stem cells, specifically, the humanoid haemopoietic stem cells. There have also been reports of elevated ALDH activity in a number of solid cancers, including head and neck cancers, lung, pancreatic, breast, and liver cancers (Hellsten et al., 2011). In the cyclophosphamide-resistant L1210 leukemic cell line, Hilton et al. (1984) discovered the role of ALDH in chemoresistance. In early-stage colon cancer xenograft tumors, they found that more ESA+, CD44+, and ALDH-positive cell populations persisted following exposure to cyclophosphamide. Aldefluor, a BIODIPY flurochrome linked to an aminoacetaldehyde moiety (an ALDH substrate), can be used to monitor ALDH activity since it fluoresces and stays inside the cell when it is cleaved (Storms et al., 1999).

With five transmembrane domains and two sizable extracellular loops, **CD133** is a 120 kDa glycosylated protein. Since its discovery in haemopoietic stem cells (Miraglia et al., 1997), stem cells in brain tumors and CSC populations in several tumors, such as breast, liver, and prostate cancers, have been identified and isolated using its positive phenotype (Singh et al., 2004). Tumor-initiating brain tumors and CSC populations of lung, pancreas, liver, prostate, gastric, colorectal, and head and neck malignancies all express $D133^{+}$ cells (Singh et al., 2009). Zhang et al. (2009) claimed that in both *in vitro* and *in vivo* studies, increased CD133 expression indicates resistance to cisplatin, etoposide, doxorubicin, and paclitaxel therapy. Nestin, Olig2, and Nanog are stemness genes that are overexpressed in the CD133+ population of brain, lung, liver, and prostate cancers (Ma et al., 2008).

1.3. Cancer Stem Cell in Tumorigenesis

With the growth and advancement of cancer research, cancers have been associated with cancer stem cells (CSCs). The multistep process of carcinogenesis relies on the gradual accumulation of mutations in tissue cells over a prolonged period of time. But according to Al-Hajj and Clarke (2004), just a portion of cells are essential for the development of tumors. According to Pardal and Clarke (2003), these tumorigenic cells are supposed to be the primary cause of breast cancer's heterogeneity and tumor growth. Thus, CSCs offer the biological basis for genetic and epigenetic modifications that lead to the growth, spread, invasion, and metastasis of cancer.

1.4. Cancer Stem Cell and Chemoresistance

Despite recent advancements in treatment, recurrent radical breast cancer (BC) has reduced prospects and survival rate due to its tendency to be pan-resistant to several anticancer medications. Developing novel therapeutic strategies for the management of BC requires an understanding of the mechanisms underlying resistance. According to Dalerba et al. (2007), BC has a minor segment (1%) of the CSC population that possesses stem cell properties such self-regeneration, progression into the original tumors in immune-compromised animals, and the capability to differentiate into numerous lineages of progenies. Furthermore, breast cancer cells with elevated expression of ALDH facilitate the metabolism of cytotoxic drugs (Moreb, 2008).

1.5. Rationale and Aims of the Study

The purpose of this research was to ascertain whether grown cells BT 549GEM 100nM produced from the parent cell lines (BT 549) by continually cultivating them in medium containing Gemcitabine (dFdC) would exhibit more stem cell markers than the parent wild-type cells (BT 549 $_{\text{GEM 100nM}}$).

2. Methodology

2.1. Cell Lines and Reagents

The resistant cell line BT 549 $_{\text{GEM100nM}}$ was produced from the parental cell lines by continually cultivating them in medium containing Gemcitabine (dFdC) (Sigma, Dorset, UK) in a stepwise concentration-increasing technique. The parental cell line BT 549 was acquired from ATCC, Middlesex, UK.

2.2. Detection of ALDH Positive Population

Steps were followed according to the supplier's instructions using the ALDEFLUOR kit (StemCell Tech., Durham, NC, USA) to identify the population that was positive for aldehyde dehydrogenase (ALDH). After being stained for 30 minutes at 37°C in an ALDH substrate with an assay buffer, the cells (2.5 × 105) were examined. A particular ALDH inhibitor called diethylaminobenzaldehyde (DEAB) was used to treat the negative control.

2.3. Flow Cytometric Analysis of CD 133

After being trypsinized, the adherent was inserted into a 25G needle. The cells (2.5 × 105) were incubated at 4°C for 20 minutes with a CD 133 antibody (BD Pharmingen, Oxford, UK). Two percent (2%) fetal calf serum (FCS) HBSS (Sigma) was used to wash away unbound antibodies, and the cells (10,000 events) were analyzed on a BD Facscalibur no more than an hour after staining.

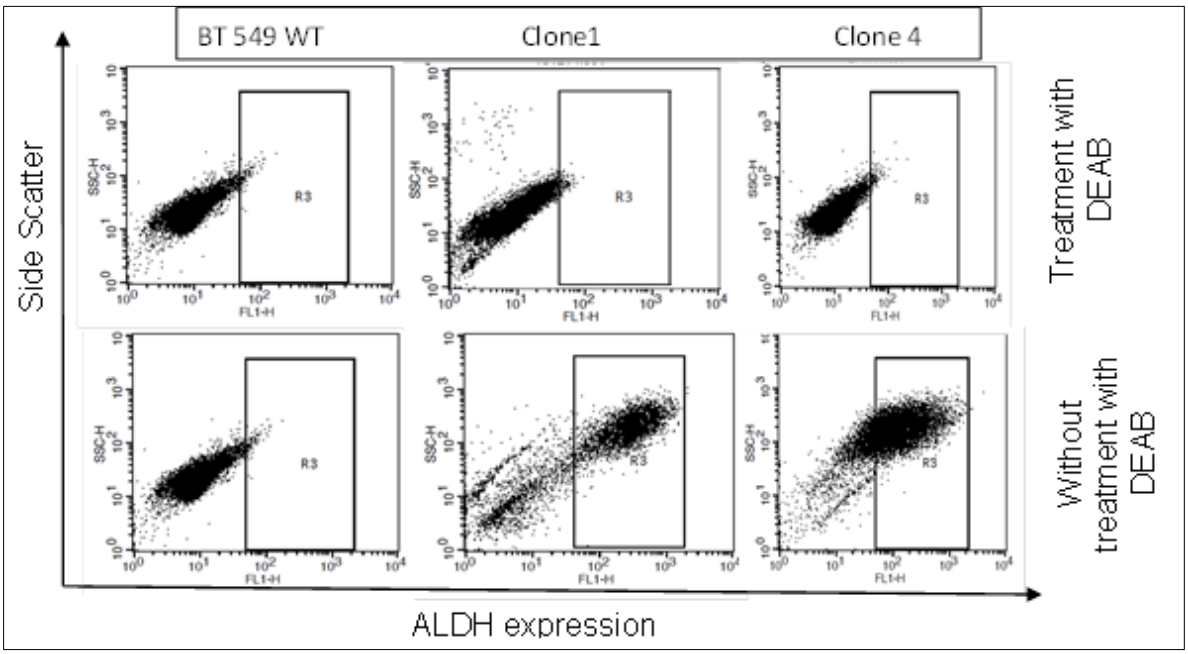
2.4. Immunofluorescent Flow Cytometric Analysis of Embryonic Stem Cell Markers

To ascertain Nanog, Oct4, and Sox2 expression, immunofluoresent flow cytometry was used. Through trypsinization, the cultivated cells were gathered. Acetone/methanol fixed the cells, and 0.1% triton-X100 permeabilized them. The cells were blocked with 3% BSA for an hour, and then they were stained for an hour at room temperature with primary (1:50 dilution) and FITC-conjugated secondary antibodies, respectively. Using a FACS Calibur flow cytometer equipped with a standard FITC 530/30 nm band pass filter and a 488-nm blue laser, the positively stained population was identified.

3. Results

3.1. CSC markers increased in resistant cell lines

The parental cell line (BT 549) resistant cell lines (BT 549 $_{\text{GEM 100nM}}$) with and without treatment with DEAB (30μM) exhibited aldehyde dehydrogenase (ALDH) activity, according to FASC findings (Figure 1). Prior to being treated with Diethylaminobenzaldehyde (DEAB), the resistant cell lines had a higher percentage of $ALDH^+$ than the parent cells. When comparing resistant cell lines to parent cell lines, the histogram (median ± interquartile range) shows a substantial increase in $ALDH^+$ activity.



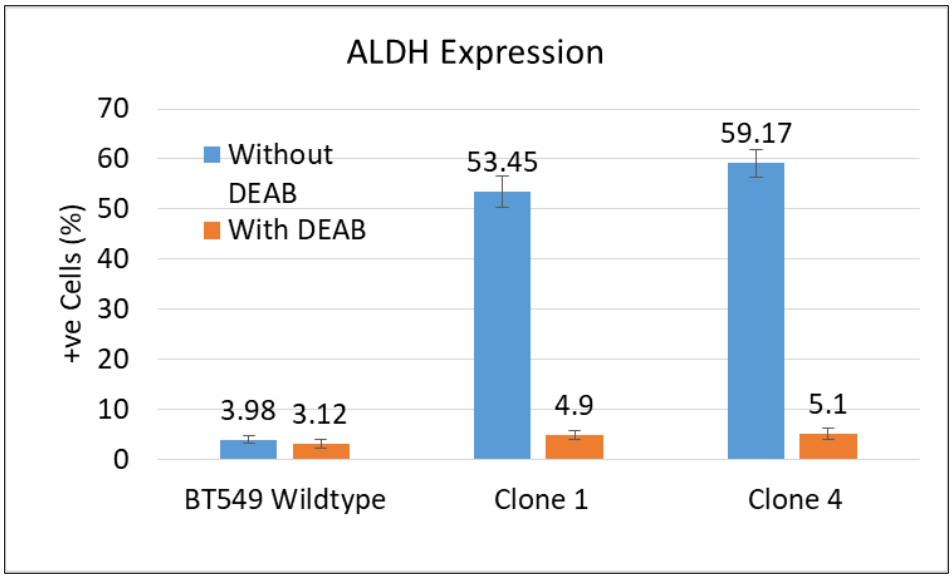
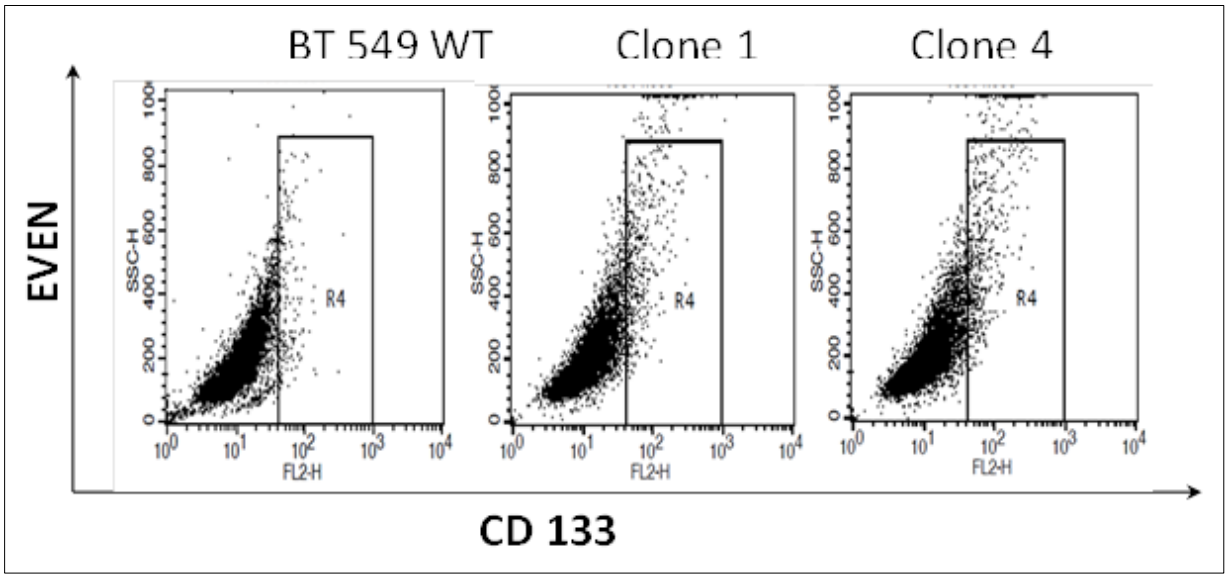


Figure 1 BT 549 WT and BT 549 $_{GEM\ 100nM}$ cell lines' representative FASCS plots and histograms of ALDH expression as determined by the ALDEFLUOR test

According to FASC findings (Figure 2), both the resistant cell lines (BT 549GEM 100nM) and the parent cell line (BT 549) expressed CD133. The expression of CD133 was higher in the resistant cell lines than in the parent cell lines. A noteworthy increase in CD133 expression in resistant cells compared to sensitive cell lines is shown by histograms (median ± interquartile range).



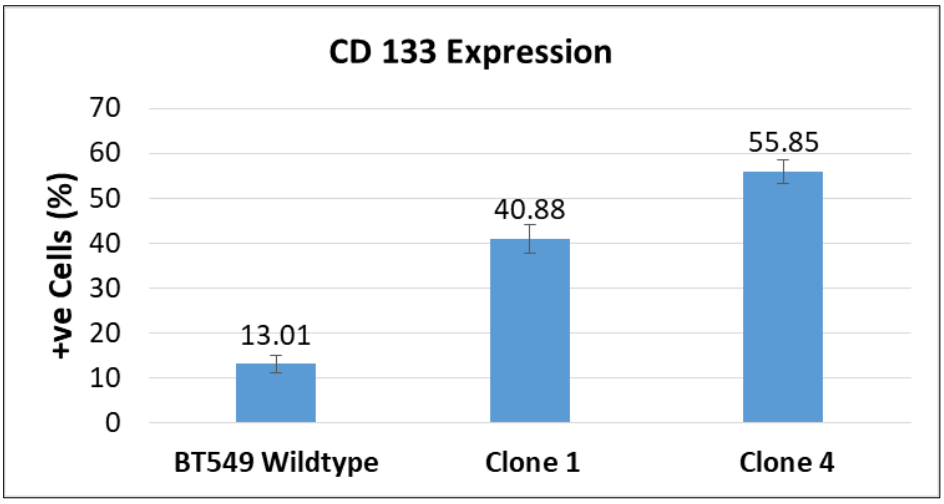


Figure 2 BT 549 WT and BT 549 GEM 100nM cell lines' representative FASCS plots and histograms of CD 133 expression

There was a significant increase in embryonic stem cell markers in resistant cell lines (BT 549 GEM 100nM) compared to the parental cell (BT 549). The fluorescence activated cell sorting (FASC) data (Figure 3) indicates that the resistant cell lines (BT 549 GEM 100nM) had higher manifestation of embryonic stem cell markers (Nanog, Sox2, and Oct4), vital for sustaining stem cell, renewal, and pluripotency.

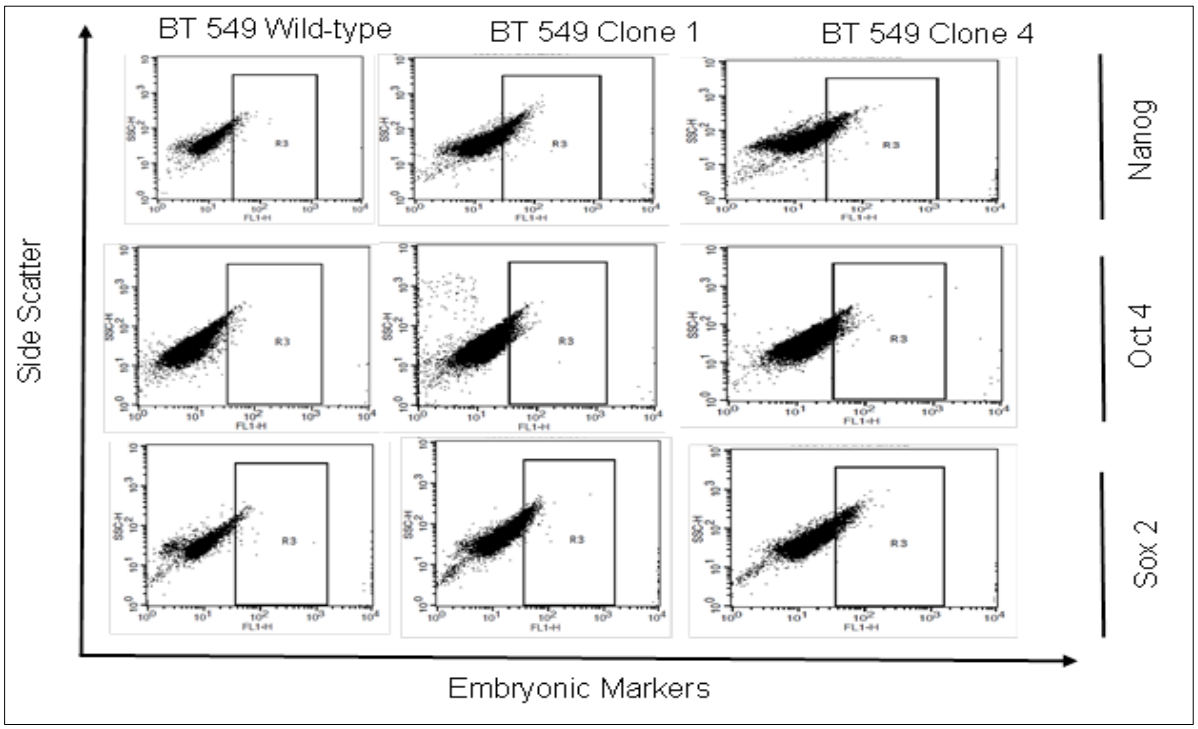


Figure 3 BT 549 WT and BT 549 GEM 100nM cell lines' representative FASCS plots and histograms of Embryonic Stem Cell markers (Nanog, Sox2 and Oct4)

4. Discussion

One characteristic that cancer stem cells (CSCs)—which are cancer cells located within the tumors and share physiognomies with regular stem cells—have in common is the capability to progress into all of the cell types in a specific cancer sample. This could make CSCs more hazardous than other cancer cells. These CSCs can develop into other types of cells and self-renew, which can lead to the growth of cancer. According to Al-Hajj and Clarke (2004), these cells are understood to exist as a divergent population within tumors and cause metastasis and recurrence by producing

fresh tumors. Consequently, the development of CSC-specific medications gives confidence in successful treatment, thereby increasing survival rate and worth of life of cancer patients, particularly for those with metastatic disease.

Data gathered in recent years has shown that CSCs are present in a wide range of solid tumors. To separate CSCs from solid and hematological malignancies, it is standard procedure to use markers unique to regular stem cells of the identical organ. Cell surface markers that have proven useful in identifying subsets that are enriched for CSC include THY1, ATP-binding cassette B5 (ABCB5), EpCAM (epithelial cell adhesion molecule, also known as epithelial specific antigen, or ESA), CD133 (also known as PROM1), CD44, and CD24 (Al-Hajj et al., 2003; Hirschmann-Jax et al. 2004; Meyer et al., 2010).

BT549 $_{GEM100nM}$ (Clone 1 and 4) Gemcitabine-acquired resistant cell lines were used in this research to characterize the cancer stem cell attribute of breast cancer cells. The outcomes of our investigation into the stem cell markers of the resistant cells were contrasted with those of the parental wild-type BT 549 cells. The parental cell line BT549 wild-type and the cell line BT549 $_{GEM100nM}$ resistant (Clone 1 and 4) were subjected to a flow cytometry experiment. Higher expression of CSC markers was found in the resistant cell line BT549 $_{GEM100nM}$ resistant (Clones 1 and 4). This suggests that the cells possessed a stem-like phenotype, which could be the reason for the chemoresistance. As shown in Figures 1 to 3, the cell line BT549 $_{GEM100nM}$ resistant (Clone 1 and 4) cells had considerably higher levels of the stem cell markers $ALDH^+$, CD133, and embryonic markers (Nanog, Sox2, and Oct4) than the parental BT549 wild-type cells. Our findings concurred with those of a number of earlier investigations that also revealed elevated stem cell markers (Tawari-Ikeh & Kasia, 2020; Anido et al., 2010; Singh et al., 2004).

5. Conclusion

These acquired resistant cell lines may be an appropriate model for examining BC cell resistance mechanisms. The study's findings showed that, in comparison to the parental wild-type cells, the resistant cell lines displayed more CSC and embryonic markers.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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