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(RESEARCH ARTICLE)

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Formation of a Gemcitabine (dFdC) Acquired Resistant BT 549 Triple Negative Breast Cancer Cells

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Abstract

Triple negative breast cancer (TNBC) is an aggressive variant of breast cancer (BC). TNBC can develop an acquired resistance after repeated exposure to anticancer drugs, which remains a major hurdle for the success of chemotherapy. The relapsed TNBC is commonly pan-resistant to various drugs with completely different resistant mechanisms. Gemcitabine BT549 cells were developed from white-type cells. The acquired resistant cells were observed to show high resistance to Gemcitabine, in addition they were also found to be cross resistant to 3 anticancer drugs (Vincristine, Doxorubicin and Paclitaxel). These acquired resistant cell can be used as models to further study the various mechanisms of resistance in cancer cells.

Keywords: BT 549 cells; Acquired resistance; Chemotherapy; Cancer cells

1. Introduction

In recent years, breast cancer has become the most common cancer and one of the main causes of cancer-related deaths. As per the World Health Organization's forecast, one in every eight women will experience breast cancer by 2022 (Sung, 2021). The genes encoding the oestrogen receptor (OR), progesterone receptor (PR), and Her2/neu are not expressed in triple-negative breast cancer (TNBC), an aggressive form of invasive breast cancer (Henry, 2020, Lehmann et al., 2010). TNBC is typically of the invasive ductal carcinoma subtype and is detected in 15–25% of all instances of breast cancer (Carey et al., 2006). Approximately 170,000 instances of TNBC occur worldwide.

Of the projected 1 million cases of breast cancer that are found each year, 170,000 cases worldwide have the TNBC (ER–/PR–/HER2–) phenotype (Anders and Carey, 2009). Race has an impact on TNBC prevalence and incidence. According to Carey et al. (2006), premenopausal African American and Hispanic women have the greatest incidence rate of TNBC. According to Tsang et al. (2009), the prevalence of TNBC varies between 12% to 19% for Asian women, 24% for Hispanic women, and 39% for black women who are premenopausal (Carey et al., 2006). Black women have the highest prevalence of TNBC, ranging from 26% at all ages to around 39%. In addition to premenopausal status and African ancestry, early menarche, increased parity, and earlier age at first menarche are risk factors related with the development of TNBC.

Compared to other forms of breast cancer, TNBC is more likely to grow quickly, have spread when it is discovered, and return after therapy (Nofech-Mozes *et al.,* 2009). As a result, compared to other forms of breast cancer, the survival rates for TNBC are typically not as great Mersin *et al.,* 2008. Compared to other forms of invasive breast cancer, triple-negative breast cancer offers fewer therapeutic choices. This is due to the cancer cells' lack of HER2 protein and estrogen or progesterone receptors, which are necessary for the effectiveness of hormone therapy or targeted HER2 medications.

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Chemotherapy is frequently used because women with triple-negative breast cancer do not have access to hormone treatment or anti-HER2 medications (Goldhirsch et. al., 2007).

The invasive ductal carcinoma in the breast of a 72-year-old female cancer patient with widespread lymph node metastases is the source of the BT-549 cell line. These epithelial cells, which were isolated in 1978, retain the usual tissue properties of the breast and mammary glands.

With response rates ranging from 13% to 29%, gemcitabine is used in combination therapy for non-small cell lung cancer, ovarian, bladder, breast, and head and neck squamous cell cancers (Albain et al., 2004; Mini et al., 2006). Gemcitabine is a first-line treatment for advanced pancreatic cancers (Burris et al., 1997). The phosphorylated derivatives of dFdC, which are inhibitors of DNA synthesis, facilitate the cytotoxic action of the compound. When dFdCTP is integrated into DNA and one more nucleotide is added, it causes single strand breakage and DNA polymerization termination, competing with deoxycytidine triphosphate (dCTP) as an inhibitor of DNA polymerase (Gandhi and Plunkett, 1990; Ross and Cuddy, 1994).

The additional nucleotide can stop DNA repair enzymes from recognizing dFdCTP, which causes apoptosis. By depleting the pools of deoxyribonucleotides and preventing the conversion of RNA nucleotides to DNA nucleotides, dFdCDP, a strong inhibitor of ribonucleotide reductase, indirectly inhibits DNA synthesis. Resistance to dFdC is associated with elevated ribonucleotide reductase expression, CdR kinase levels in tumors, and inhibition of nucleoside transporters, which stops dFdC from entering cells.

1.1. Rationale and aims of the study

This study was carried out to confirm if cultured cells BT $549_{GEM\ 100nM}$ generated from the parental cell lines (BT 549) by continuously cultured in medium containing Gemcitabine (dFdC) would cause acquired resistance of initially sensitive cancer cells to chemotherapeutic drugs and to evaluate the drug sensitivity of BT $549_{GEM\ 100nM}$ to a number of conventional anticancer drugs and the possible mechanisms for drug resistance.

2. Methodology

2.1. Cell lines and reagents

Parental cell line BT 549 was purchased from ATCC, Middlesex, UK and the resistant cell line BT $549_{GEM100nM}$ was generated from the parental cell lines by continuously cultured in medium containing Gemcitabine(dFdC) (Sigma, Dorset, UK) in a stepwise concentration increasing procedure.

2.2. Construction of sequential gemcitabine-resistance cancer cell lines

To establish sequential gemcitabine-resistance cancer (GRC) cell lines, parental BT 549 cells were treated with 1.0 μM gemcitabine (Eli Lilly and company, IN, USA) in a culture medium for 72 h when cells reached a confluence of ~90%. DMEM containing gemcitabine was replaced, and viability was monitored every 2–3 days. When the GRC cell lines proliferated to form colonies, they further proliferated in DMEM and were named BT Clones. The constructed BT Clones were further cultured and maintained in gemcitabine medium in to make cell stocks, and some cells were moved to a medium containing gemcitabine (100nM) to build the next phase. A total of four types of GRC cell lines were constructed and named BT Clones GRC1-4 cells.

2.3. Cell culture and cytotoxicity analysis

The cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) (Lonza, Wokingham, UK) supplemented with 10% FCS, 50 unitsml_1 penicillin and 50 mgml_1 streptomycin. The BT $549_{GEM100nM}$ cells Clone 1 and Clone 4 were maintained in the medium containing 100nM of GEM.

For in vitro cytotoxicity assay, the overnight cultured cells (5000 per well) in 96-well flat-bottomed microtiter plates were exposed to drugs for 72 hours (PAC), dFdC 72hours, Vincristine (VCR) 48hours, Doxorubicin (DOX) 72hours and subjected to a standard MTT assay (Plumb et al, 1989).

3. Results

3.1. Morphological features

The drug resistance cells (BT 549$_{GEM100nM}$) were observed to have a different phenotype than the wild type (WT) parental cells. Figure 1 shows that the resistant cells were smaller and with less defined irregular multiple nuclei when compared to the parental cell lines.

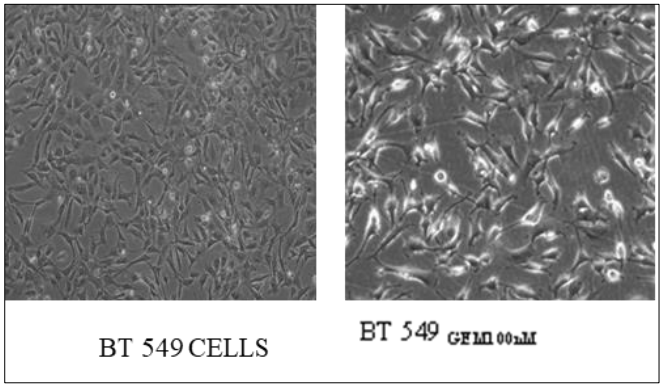

Figure 1 Representative morphologic Images of BT549 cells and BT 549$_{GEM100nM}$

3.2. Cell Viability of Gemcitabine-resistance cancer cell lines

Data from MTT cytotoxicity analysis showed the cell viability of BT549 wild-type cells and the BT Clones (1-4) Figure 2. Two (2) clones were selected for this study Clone 1 and Clone 4.

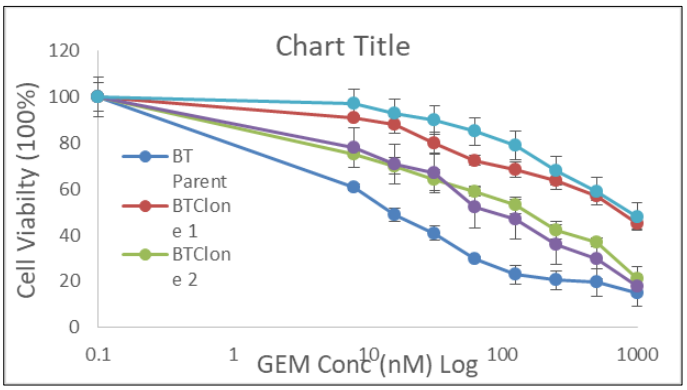

Figure 2 Representative Drug Concentration Response Curves of BT549 white-type cells and the BT Clones (1-4)

3.3. Resistant cell lines show Drug Resistance to Gemcitabine

Data from MTT cytotoxicity analysis showed that the BT 549$_{GEM100nM}$ cells were highly resistant to dFdC and had much higher IC50 compared to the BT549 white-type cells. (Figure 3).

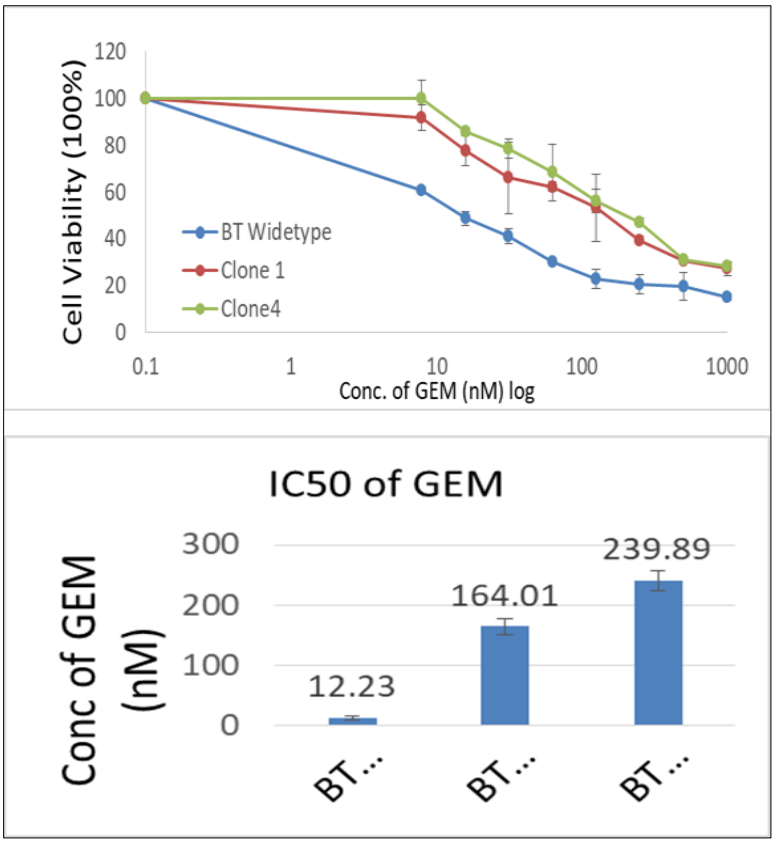

Figure 3 Representative Drug Concentration Response Curves of BT549 wild-type cells and the Gemcitabine resistant BT 549 Clones (1 and 4). (GEM- Gemcitabine)

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

Data from MTT cytotoxicity analysis showed that the BT $549_{GEM100nM}$ cell line is also cross resistant to other anticancer drugs e.g. VCR, Dox, and PAC (Figure 4a-4c respectively). The parental wide-type cell line has a much lower IC50s compared to that of the resistant cell line BT $549_{GEM100nM}$

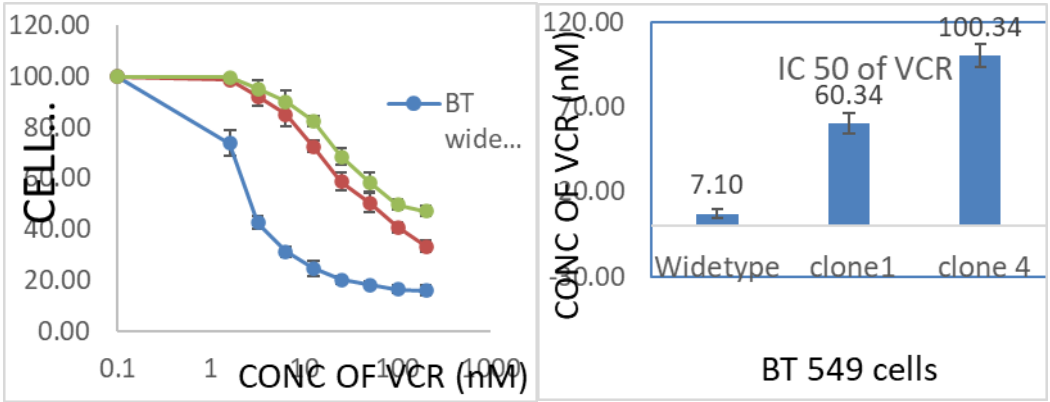

Figure 4a Representative Drug Concentration Response Curves of BT549 wild-type cells and the Gemcitabine resistant BT 549 Clones (1 and 4) to Vincristine. (VCR- Vincristine).

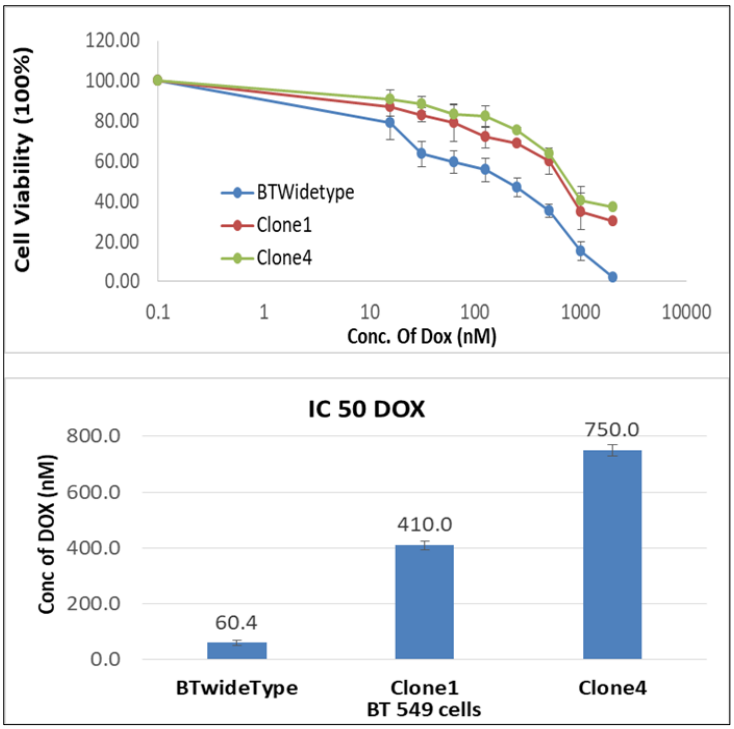

Figure 4b Representative Drug Concentration Response Curves of BT549 white-type cells and the Gemcitabine resistant BT 549 Clones (1and 4) to Doxorubicin. (DOX- Doxorubicin).

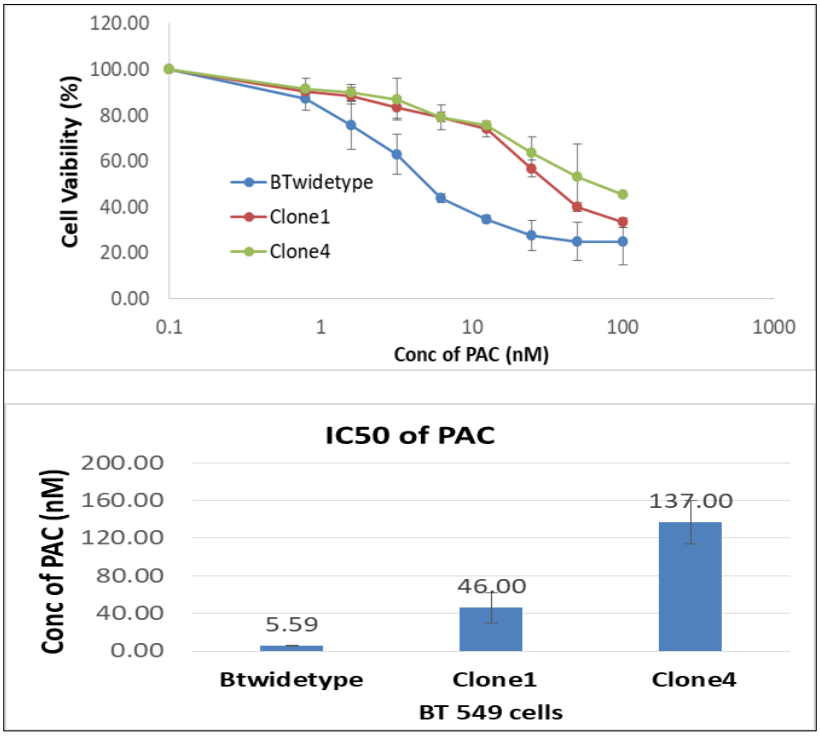

Figure 4c Representative Drug Concentration Response Curves of BT549 wild-type cells and the Gemcitabine resistant BT 549 Clones (1and 4) to Paclitaxel. (PAC- Paclitaxel)

4. Discussion

Chemotherapy is a treatment that involves the use of a drug or a combination of drugs that are cytotoxic to rapidly growing and dividing cells such as cancer cells. It is a promising treatment for improving breast cancer patients. Gemcitabine a deoxycytidine analog requiring active cell membrane transport and represents an anticancer effect against many types of solid cancer (Moore et al., 1997).

One significant barrier to cancer treatment is acquired resistance to chemotherapy; which is thought to be a main driver of resistance. Gemcitabine, a first line drug for the therapy of BC and has a high preliminary activity against tumours but is prone to cause acquired resistance in many cancer cells. In this study BT549 wide-type cells were cultured in medium containing Gemcitabine and several clones BT549 $_{GEM100nM}$ were developed (Figure 2). Data from MTT studies demonstrated that these new cells were found to be resistance Gemcitabine (Figure 3) and other anticancer drugs such as Vincristine, Paclitaxel and Doxorubicin (Figure 4a-4c) indicating cross and pan resistance to the various anticancer drugs. The resistant cells had much higher IC50 compared to the parent wide-type cells. Several studies have also reported similar findings (Gottesman 2002, Tawari and Kasia 2020).

5. Conclusion

Investigating the mechanisms of resistance in Breast cancer cells may be made easoer with the use of the resistant cell lines. Data from this study demonstrated that a variety of anticancer medications had little effect on the resistant cell lines, indicating that acquired resistance may cause pan-resistance in cells.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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