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ASSESSMENT OF THE MIGRATION/INVASION TRAITS OF ACQUIRED RESISTANT BT 549 GEM100NM BREAST CANCER CELL LINES

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ABSTRACT: *One of a cell's behavioural characteristics is migration. Cell migration is a major contributor to cancer-related mortality and plays a significant role in the spread of metastatic tumours. The purpose of this study was to evaluate the invasive characteristics and migration traits of acquired gemcitabine-resistant BT 549 Gem100nM breast cancer cell lines. According to data from the results obtained, the resistant cell lines Clones 1 and 4 exhibited higher migratory and invasive activities than the wild-type cell line BT549. This suggests that acquired resistance may cause cancer cells to become more lethal by increasing their migratory and invasive characteristics.*

KEYWORDS: Migration, Invasion, Acquired Resistance, BT 549 breast cancer cells.

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INTRODUCTION

One of the leading causes of death for many cancer patients is cancer cell invasion and migration, which are essential components of metastatic illness. According to Van Zijil et al. (2011), cancer cells can spread and wander through a variety of different methods, including collective cell migration, mesenchymal cell migration, and amoeboid cell migration. These different movement techniques exhibit clear and specific distinguishing characteristics in matrix adhesion, protease activity, actin cytoskeleton, and cell-cell junctions. Tumour cells typically develop malignant phenotypes in order to travel to different locations, which allows them to separate from the main tumour mass. Following their passage through the basal membrane, these cells enter the circulation by penetrating the extracellular matrix (ECM) (Span et al., 2012).

When cancer reaches its most deadly stage, it is not just an ailment of unchecked cell growth but also unchecked cell migration. Tumour invasion is the process by which cancer cells migrate away from the original tumour, marking the beginning of metastasis. The invading cells initially penetrate the basement membrane (BM) of solid epithelial tumours, also known as carcinomas. The stroma, a network of extracellular matrix (ECM) that envelops the tissue and is occupied by many cell types, and the epithelium is naturally separated by the BM. Metastasizing cells can be transported to different organs after passing through the stroma and arriving at blood or lymph arteries (Gentis et al., 2014).

Although cancer cells have a wide range of migration and invasion strategies, little is known about the underlying processes that allow them to move and infiltrate via the various tumour microenvironments. Numerous techniques have been used to investigate cell migration, such as the Boyden chamber assays (Woo et al., 2007), live-cell imaging for real-time observation (Kijanka et al., 2015), the in vitro wound healing (WH) assay (Martinotti and Ranzato, 2020), also known as the in vitro scratch assay; protein-protein interaction analysis to look at the molecular interactions involved in cell migration (Feng and Walsh, 2001); gene expression profiling to find important regulators (Willier et al., 2013); and single-cell tracking for quantitative analysis (Blockhuys et al., 2020); protein-protein interaction analysis to look at the molecular interactions involved in cell migration (Feng and Walsh, 2001); gene expression profiling to find important regulators (Willier et al., 2013); and single-cell tracking for quantitative analysis (Blockhuys et al., 2020).

The common, economical, and standardized in vitro WH assay method is creating a controlled scratch inside a confluent cell monolayer, usually using mechanical, thermal, or chemical damage (Jonkman et al., 2014). Cells migrate into the wound bed after injury, helping to restore the structure and functionality of the epidermal barrier (Tremel et al., 2009). In this work, the migration and invasion of the resistant BT 549 $_{Gem100nM}$ breast cancer cell lines were investigated using a wound-healing assay.

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METHODOLOGY

Cell lines and reagents:

The parental cell lines were continuously cultured in media with Gemcitabine (dFdC) (Sigma, Dorset, UK) using a stepwise concentration-increasing approach to create the resistant cell line BT 549 $_{GEM100nM}$. The ATCC in Middlesex, UK, provided the parental cell line BT 549.

Migration assay

The scratch or wound healing assay is a widely used, low-cost method for examining cell migration. For 24 hours at 37°C and 5% CO2, 0.5x106 cells of BT 549 $_{GEM100nM}$ (Clones 1 and 4) test cells and wild-type BT 548 cells control cells were sown in a 6-well plate with 500µL of 10% FBS-containing RPMI medium.

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Invasion assay

At 0 hours, 24 hours, and 48 hours, photos of the scratch area were taken. The movement of cells from the undamaged areas into the scratched areas can be observed under a microscope. Using the image J program, the percentage migration may be computed by tracking the decline of the exposed area at various intervals until the "wound" is closed. It is possible to see cell migration as sheets of cells (epithelial cells), single cells, or a loosely connected population (mesenchymal cells).

To rehydrate the basement membrane layer of the cell culture inserts, 300 µl of warm, serum-free medium was added, and the inserts were then incubated for one hour at room temperature. Once the incubation period was up, the media was carefully removed. Each insert was filled with 300 µl of BT 549 $_{GEM100nM}$ (Clone 1 and 4) test cells and a suspension of wild-type BT 548 cells control cells with 0.5–1x106 cells/ml in serum-free medium. The lower well of the plate was filled with 500 µl of medium containing 10% fetal bovine serum. The medium was gently aspirated from the implant following a 48-hour incubation period. Cells were extracted from the inserts inside using moist cotton-tipped swabs. After that, the insert was placed in a well with 400 µl of cell stain solution and allowed to sit at room temperature for 10 minutes. The inserts were given a few mild washes in a beaker of water before being let to air-dry. After that, the inserts were placed in an orbital shaker and incubated for ten minutes in a well containing 200 µl of extraction solution. Each sample's optical density was measured at 560 nm after 100 µl was put on a 96-well plate.

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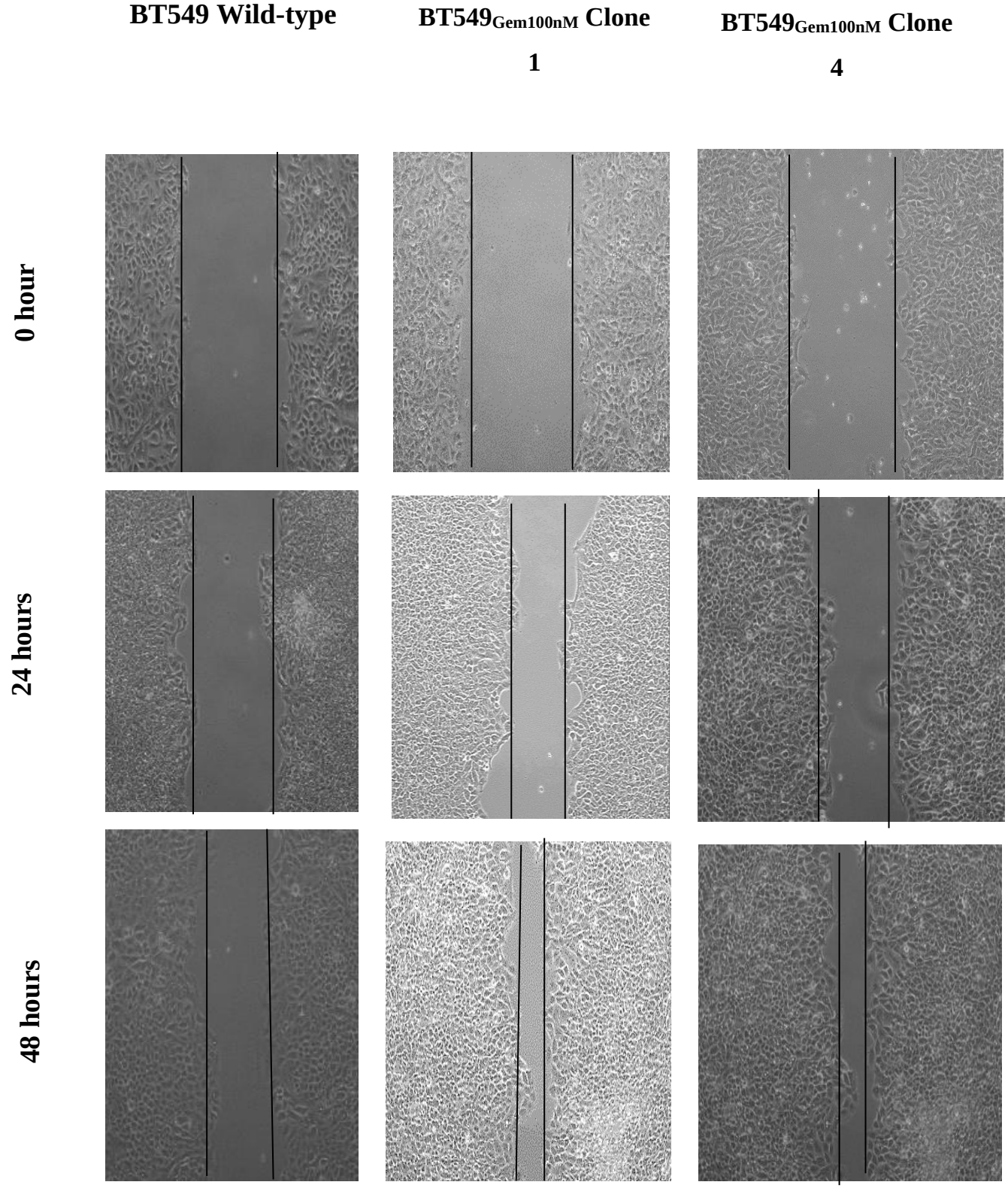

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RESULTS

The acquired resistant cells BT 549 $_{Gem100nM}$ Clones 1 and 4 exhibited greater invasive capability in comparison to the wild-type BT 549 cells, according to images obtained from the wound healing scratch assay and the matrigel invasion assay.

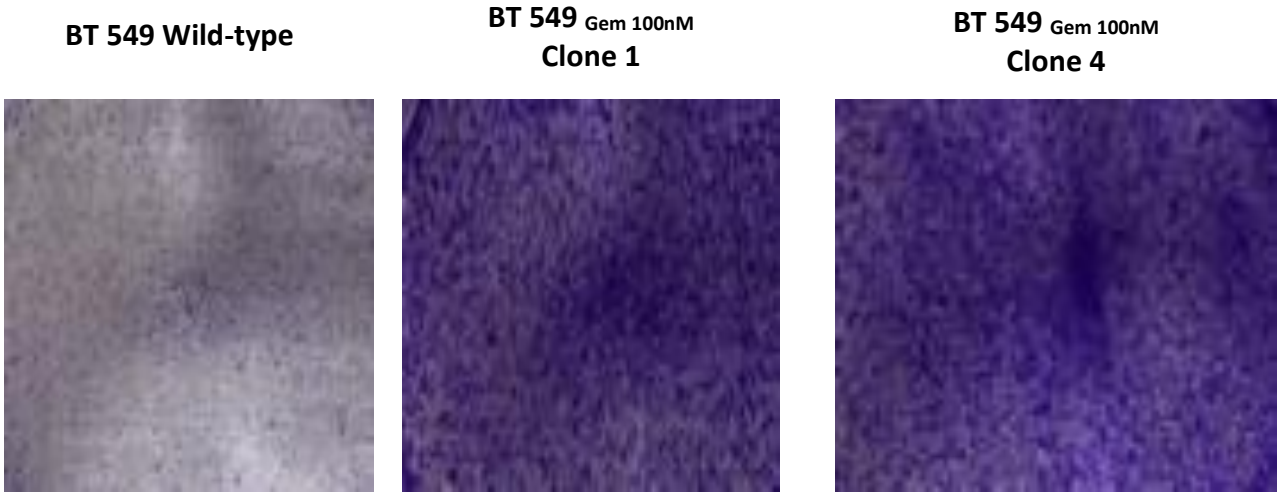

Figure 1. Representative Matrigel Invasion assay Images of BT 549 Wild-type and BT 549 $_{Gem\ 100nM}$ Clone 1 and Clone 4 Breast cancer cells.

After three days, images (magnification 20X) of acquired resistant cells BT 549 Gem100nM clones 1 and 4 revealed increased activity and improved invasive capabilities compared to wild-type BT549 cells, showing mesenchymal phenotype and EMT activation.

Figure 2. Representative Migration (wound healing) assay Images of BT 549 Wild-type and BT 549 $_{Gem\ 100nM}$ Clone 1 and Clone 4 Breast cancer cells.

Even 48 hours after EMT activation and mesenchymal activation, images (magnification 10X) revealed increased migratory activity in BT 549 Gem 100nM Clone 1 and Clone 4 breast cancer cells, suggesting an increase in migratory capabilities relative to BT 549 Wild-type cells.

DISCUSSION

Tumor cell migration, a mechanism that is still poorly understood, is the main source of metastasis, the leading cause of mortality for cancer patients. Immune monitoring, wound healing, and embryonic development all depend on cell migration. Many diseases, including cancer patients. Immune monitoring, wound healing, and embryonic development all depend on cell migration. Many diseases, including cancer metastases and chronic inflammation, are characterized by abnormal cell migration (Paluch et al., 2016). Morphological polarization, which occurs when cells extend a protrusion in the direction of movement and create a new cell-matrix adhesion between the protrusion and the cell substrate, is the first step in cell migration. After that, the cell body contracts and advances, ultimately terminating the cycle by pulling back adhesions at the back (Trepat et al., 2012).

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When examining the migratory and invasive characteristics of cells, the most economical and standardized in vitro method is the in vitro wound healing (WH) assay, sometimes referred to as the in vitro scratch assay. Through mechanical, thermal, or chemical damage, the WH entails creating a controlled scratch inside a confluent cell monolayer (Jonkman et al., 2014).

This work evaluated the migratory and invasive characteristics of an acquired gemcitabine-resistant breast cancer cell line, BT 549 Gem100nM, using an in vitro wound healing test. Clone 1 and Clone 4, two (2) clones of the resistant cell, were employed in the study. Pictures (ten times magnification) Even 48 hours after EMT activation and mesenchymal activation, Figure 2 demonstrated an increase in migratory activity in BT 549 $_{\text{Gem 100nM}}$ Clone 1 and Clone 4 breast cancer cells, suggesting an increase in migratory capabilities relative to BT 549 Wild-type cells.

The Matrigel assay, which was used to investigate cell invasion, also produced similar results. After three days, the acquired resistant cells BT 549 $_{\text{Gem100nM}}$ clones 1 and 4 exhibited increased activity and enhanced invasive properties compared to wild-type BT549 cells, indicating EMT activation and mesenchymal phenotype (Figure 1). These findings showed that the cells resistant to gemcitabine were consistent with findings from other investigations (Lee et al., 2017; Li et al., 2014).

CONCLUSION

Chemotherapy is a medical treatment that uses a chemical or combination of drugs that are cytotoxic to cells that divide and expand quickly, including cancer cells. Chemotherapy-resistant cells, like acquired gemcitabine-resistant cells, exhibited more invasive and migratory characteristics than their parent wild-type cells. This suggests that resistance to chemotherapeutic drugs may make some cancers more aggressive and fatal.

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