Chapter 1

Introduction

1.1. Background

Breast cancer is one of the leading causes of cancer-related deaths and prevalent malignant tumours affecting women (Obeagu & Obeagu, 2024). This is due to its heterogeneity nature, which contributes to its aggressive behaviour and variable response to therapy, thus posing a challenge in research and treatment discoveries (Turashvili & Brogi, 2017). Hence, understanding the mechanisms behind this heterogeneity is crucial in order to develop more effective therapeutic strategies (Dagogo-Jack & Shaw, 2018). In order to elucidate the mechanisms, in vitro cancer models are necessary, one of which is multicellular tumour spheroids (MCTS), a model generally used to study tumour biology (Riffle & Hegde, 2017).

MCTS are spherical aggregates of cancer cells that are able to mimic the three dimensional (3D) cell organisation of solid tumours (Weiswald et al., 2015). This is because MCTS recapitulates key features of the tumour microenvironment (TME), including nutrient and oxygen gradients, cell-cell and cell-matrix interactions, and cellular heterogeneity (Mitrakas et al., 2023). This model reflects how cancer cells can survive in floating state as they are cultured in non-adherent conditions, allowing them to have a higher chance to migrate and invade distant organs, which is a phenomenon also known as metastasis (Khan et al., 2022; Riffle, 2017).

As MCTS adopt a compact shape with extensive cell–cell contacts and reduced cell-substrate adhesion, these structural changes may affect the key cellular behaviours (Lu & Stenzel, 2018). For instance, their nutrient availability, mechanical stress, and spatial organisation may affect cell proliferation capacity (Jarett et al., 2018; Rosenbauer et al., 2023). Moreover, the change in morphology and adhesion can also influence the factors contributing to cellular migratory capacity, such as the epithelial-mesenchymal transition (EMT), a process where epithelial cells gain

mesenchymal traits that enable increased motility and invasiveness (Gonzales & Medici, 2014). This process is associated with metastasis, stemness, and resistance to therapy, which are known to contribute to cancer progression (Felipe Lima et al., 2016).

Aside from that, the 3D organisation of MCTS can affect the secretory profile of cancer cells, particularly the production of extracellular vesicles such as exosomes (Thippabhotla et al., 2019). Exosomes are small vesicles that mediate intercellular communication by transferring bioactive molecules, such as proteins, lipids, mRNAs, and non-coding RNAs to recipient cells (Jaiswal & Sedger, 2019). They serve as one of the key players in the TME, contributing to immune evasion, extracellular matrix (ECM) remodeling, angiogenesis, and metastasis (Jin et al., 2022). Previous studies have shown that exosomes derived from MCTS may differ compared to those secreted by cancer cells in 2D cultures, highlighting the role of tumor architecture in shaping tumor-derived signals (Khan et al., 2024).

As MCTS formation could alter their key cellular features, which are closely linked to potential tumour aggressiveness and therapy resistance, uncovering these changes may provide great insights on tumour progression as well as development of more effective therapeutic strategies (Weiswald et al., 2015). However, despite this possibility, the effect of morphological change from epithelial cells to MCTS on their cellular behaviour remains poorly understood. Therefore, it is crucial to characterise the MCTS formed from breast cancer epithelial cells and its influence on cellular behaviour such as proliferation, EMT potential, and exosome secretion.

1.2. Objective

This project aims to characterise MCTS from breast cancer epithelial cells and its effect on proliferation, EMT marker expression, and exosome secretion compared to non-MCTS form.

1.3. Hypothesis

H1: Expression of proliferation and EMT markers are increased in MCTS.

Exosomal characteristics are different between MCTS and non-MCTS.

H0: Expression of proliferation and EMT markers are decreased in MCTS.

Exosomal characteristics are the same between MCTS and non-MCTS.