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# The Possible Potential of Isolation Cancer Stem Cells from MCF7 Cell Line

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**Abstract:** Breast cancer represent the second cause of death in women after lung cancer. Scientists studied breast cancer in main fields genetically and epigenetically in order to understand and defeat it. Anticancer drugs were not efficient enough due to cancer recurrence which was discovered the reason beyond it which is a small population of cells that have the ability to self-renewal and cause tumorgenicity called cancer stem cells (CSCs). Understanding CSCs and its mechanisms allow us discovering the mechanism of recurrence Particularly, mammospheres that are responsible of cancer recurrence. Aim of the work is Studying the possible potential of isolation these mammosphers and percentage that would provide a great model to reveal CSCs mechanisms. In this study we cultured MCF7 cell line and exposed it into specific conditions that only CSC stem cell will survive in. Cell line was cultured in serum-free DMEM-F12 supplemented with 0.4% bovine serum albumin, 20 ng/ml epidermal growth factor (EGF), 5 mg/ml insulin, 10 ng/ml basic fibroblast growth factor (bFGF) Then incubated for one week without any disturbances. After one week, cells were checked and mammospheres appeared clearly, pictured and characterized by accurate measurement flow cytometry CD44+/CD24- which indicated that the purity of MCF-7 cancer stem cells in the mammospheres is above 98% after being cultured in serum-free medium for 1 week. The data suggest that culture of mammospheres of these metastatic cells would provide a very suitable model to investigate how responsive tumorigenic stem cells are to therapeutic drugs.

**keywords**: Cancer, MCF7 Cell Line, Cancer Stem Cells, Mammospheres, Flow Cytometry

## 1.Introduction

Cancer is a disease characterized by uncontrolled proliferation of transformed cells, driven by genetic and epigenetic alterations in somatic cells, leading to abnormal cell growth and, in many cases, metastasize to other body parts. The multi-step process of tumor metastasis primarily consists of three stages: invasion, circulation, and colonization [1], [2], [3]. Breast cancer, a heterogeneous disease, typically originates in the breast ducts or lobules and can progress into invasive cancer through various pathways. Approximately 80% of breast cancer diagnoses are ductal in nature. A well-established progression model begins with normal cells transitioning into atypical ductal hyperplasia (ADH), followed by ductal carcinoma in situ (DCIS), then invasive ductal carcinoma (IDC), and finally metastasis. In the metastatic phase, cancer cells breach the basement membrane, enter the vasculature, and spread to distant organs, often leading to fatal outcomes [4].

A subset of tumor cells known as cancer stem cells (CSCs) possess the ability for self-renewal and can differentiate into non-stem cancer cells, which constitute the bulk of the tumor. CSCs are not only responsible for tumor initiation but also play a critical role in metastasis, which is linked to lower survival rates in patients. The relationship between CSCs and metastasis has made them a key target for cancer therapy [5].

Cancer cell lines are essential *in vitro* models used in cancer research and drug discovery [ 6]. The most commonly utilized breast cancer cell line is MCF7, which is known for its hormone sensitivity due to its high expression of estrogen receptors (ER) [7].

MCF7-derived mammospheres contain cells that exhibit the CD44+/CD24- phenotype, a characteristic of breast cancer stem cells (BCSCs) [8]. The isolation and enrichment of CSCs are vital to understanding CSC biology and developing CSC-targeted therapies [9].

Several methodologies are employed for the isolation and identification of CSCs. The most common method is based on cell surface biomarkers such as CD44 and CD24. Additionally, the side population (SP) technique utilizes the ability of CSCs to efflux dyes like Hoechst 33342, and the ALDEFLUOR assay isolates CSCs based on high ALDH1 activity. Another widely used method is the tumorsphere culture, where CSCs are grown in serum-free conditions to form multicellular spheres, reflecting their ability to self-renew [10].

The first identification of BCSCs using CD44 and CD24 markers was conducted by Al-Hajj and colleagues, who demonstrated that cells with the CD44+/CD24-/lin- phenotype could initiate tumors in immunodeficient mice and exhibit cancer stemness traits, such as self-renewal and resistance to chemotherapy [11]. CD44, a transmembrane glycoprotein, plays a crucial role in maintaining the multipotency of BCSCs, while CD24 is involved in cellular adhesion, proliferation, and metastasis [29], [30].

The goal of this study is to isolate mammospheres and analyze their characteristics, including the CD44+/CD24- phenotype, to assess their potential in cancer research and therapy. Understanding the isolation efficiency and functional capabilities of these mammospheres could unlock new insights into the behavior of CSCs and inform the development of targeted therapies.

## 2. Materials and methods

MCF7 cell line was cultured and exposed into specific conditions that only CSC stem cell will survive in. cell line was cultured in serum-free DMEM-F12 supplemented with 0.4% bovine serum albumin, 10 ng/ml basic fibroblast growth factor (bFGF), 5 mg/ml insulin and 20 ng/ml epidermal growth factor (EGF) and left incubated for a week without any disturbance. After a week, cells were checked and mammospheres appeared clearly, pictured and characterized by accurate measurement flow cytometry which indicated purity of isolation of MCF-7 CSCs.

### 1) Preparation of MCF-7 involved:

### Preparation of High Glucose Media:

DMEM high glucose (500ml) was supplied with 50 ml fetal bovine serum (10%) which was deactivated before use by heating at $56^{o}$c for 20 mins and 7 ml anti-anti then preserved at 2-8 ℃.

### Feeding and Passage:

After 2 days old culture media were removed and a new complete medium was added to the flask. When cultured cells reach confluency (70%-80%), cells were then washed with PBS and trypsinized with trypsin-EDTA ( 0.25% ) as follow: old media was discarded and cells washed with 2 ml PBS. 2.5 ml trypsin was added to flask then to incubator for 2 min. cells were examined under microscope where cells begin to be rounded, separate and detach from feeder layer of the flask. 15 ml complete media was added to flask and pipetted several times then 10 ml media was transferred into 2 new flasks ( 1:3 ) then all were incubated in humidified incubator at 37 ℃, 5% CO2 and examined after 2 days.

### Cell Counting:

After passage, cells were resuspended in 1:1 media, trypsin respectively, 10 µl of cell suspension added to 1.5 ml Eppendorf then10 µl of trypan blue (0.4% ) added too then mix gently. Hemocytometer was cleaned and both sides were filled with suspension and checked under microscope using 10x magnification. Both viable and non-viable cells were counted.

### Calculation:

Average of viable cell line cells = total number of viable cells / total number of squares.

Concentration of viable cells per ml= dilution factor$\times 10^4 \times$ average of viable cells

### 2) Preparation of Cancer Stem Cells (CSCs)

### Preparation of Media:

serum-free DMEM-F12 supplemented with 0.4% bovine serum albumin, 20 ng/ml epidermal growth factor (EGF), 5 mg/ml insulin, 10 ng/ml basic fibroblast growth factor (bFGF).

**Separation of CSCs:**

After cell culture flasks reach confluence 80%, complete media was discarded and cells were washed by PBS then serum-free DMEM-F12 supplemented with 0.4% bovine serum albumin, 20 ng/ml epidermal growth factor (EGF), 5 mg/ml insulin, 10 ng/ml basic fibroblast growth factor (bFGF) was added to each flask then left for a week without any disturbance" day 1". Under these conditions, the MCF-7 cancer cells died whereas MCF-7 cancer stem cells lived and grew as nonadherent spherical clusters of cells, known as mammospheres. Feeding was done each three days by Centrifugation to gather the mammospheres and change media at a speed of 1000 rpm for five minutes.

**Microscopic Investigation:**

Cells were examined daily of the experiment and photographed specially at days 1,4,5&6.

**Flow Cytometric Investigation:**

passaging cells till measuring fluorescence intensity of the cells was performed in the following manner: 1) $1\times10^6$ cells were resuspended in 1 ml media, 2) 100 µl of cells were stained with CD44, CD24 then incubated for 30 mis in dark, 3) at room temp, each tube were added to it a 2 ml stain buffer (FBS) then centrifuged at 2000 rpm for 10 mins, 4) remove supernatant and add ½ ml stain buffer (FBS) to each Eppendorf. Then fluorescence of cells was evaluated by accuri C6 Becton Dickinson Flow Cytometry.

**3. Results and Discussion**

**Cancer Stem Cell Isolation and Characterization:**

**1.Isolation of Cancer Stem Cells (CSCs)**

MCF-7 breast cell line was cultured in 25 $cm^2$ tissue culture flask in incubator supported with 5% co2 and 37 ℃ till reached the confluence 80% (Fig,1&2). MCF-7 cells were then exposed to cancer stem cell media DMEM-Ham's F12 culture medium supplemented with bovine serum albumin (BSA), epidermal growth factor (EGF), insulin and basic fibroblast growth factor (bFGF). After 4 days, MCF-7 CSC mammospheres cultured in serum free medium started to appear (Fig. 3). While after 5 days, cancer cells begin to die and only CSCs survive and aggregate as mammospheres (Fig. 4). In addition, after 6 days CSCs aggregates, increased in size and became more clearer as mammospheres (Fig. 5).

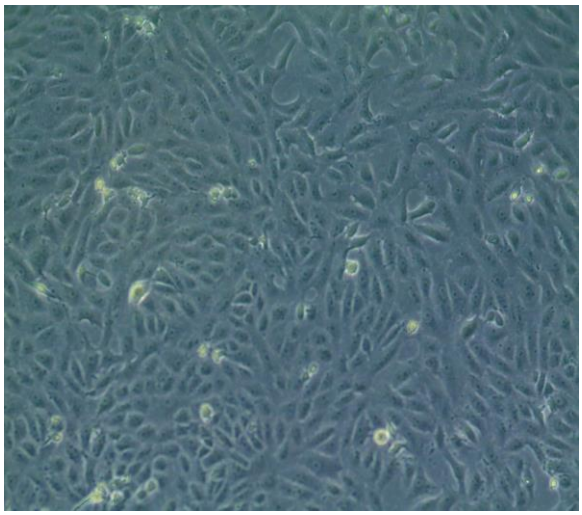


**Fig.1** Adherent MCF7 cell line cultured in serum-containing medium (X:100 )

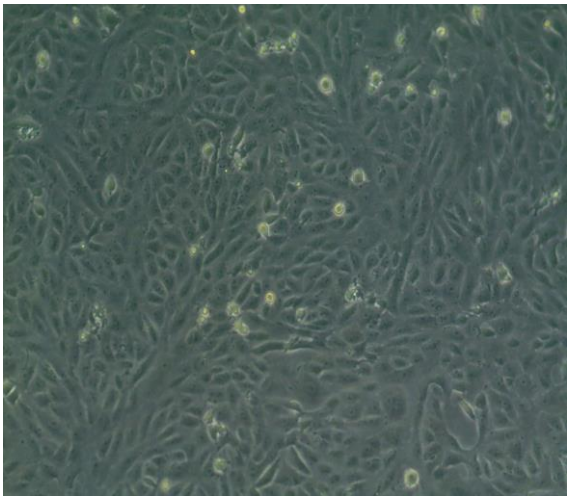


**Fig.2** Adherent MCF7 cell line cultured in serum-containing medium (X:100)

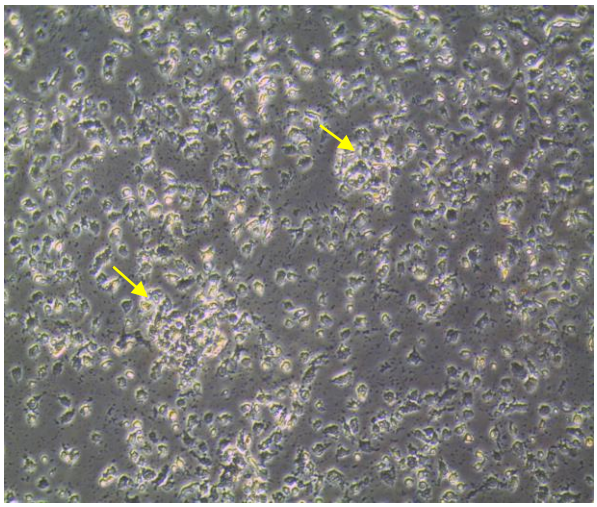


**Fig.3** MCF-7 CSCs mammospheres cultured in serum free medium at day 4 (X:100)

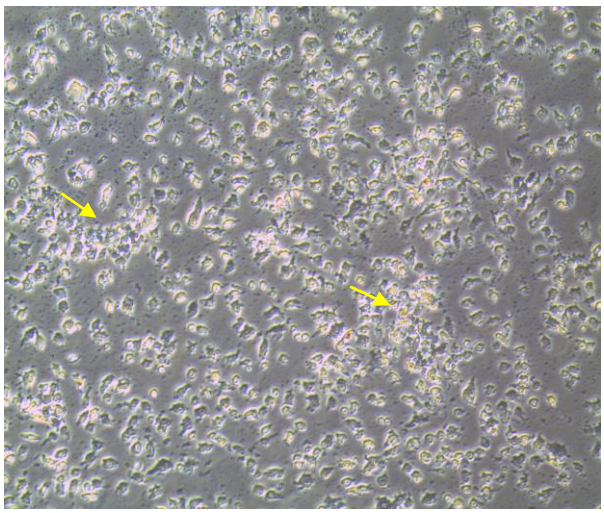


**Fig.4** MCF-7 CSCs mammospheres cultured in serum free medium at day 5 (X:100)

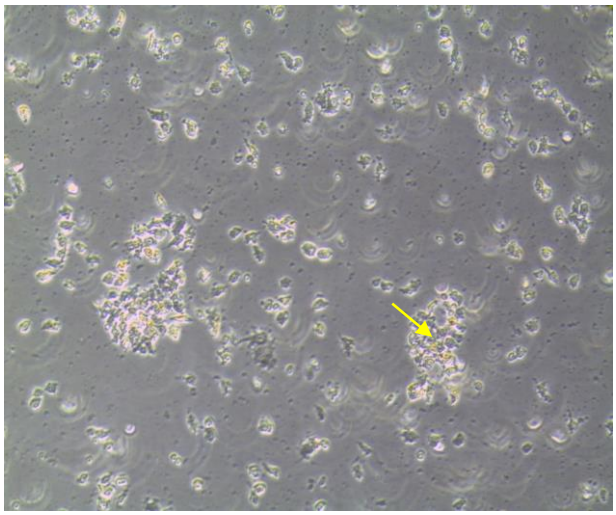
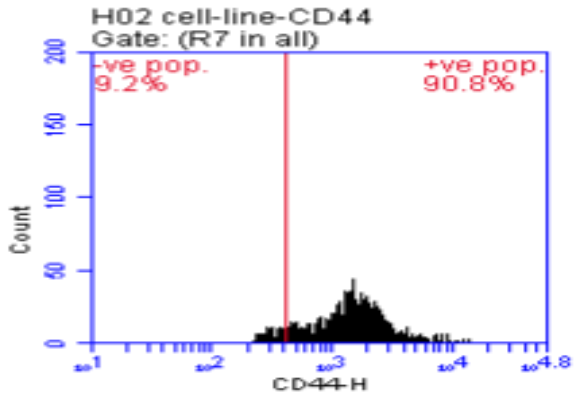


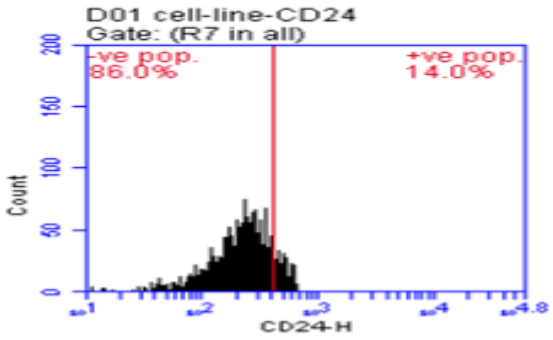
**Fig.5** MCF-7 CSCs mammospheres cultured in serum free medium at day 6, (X:100)

## 2. Characterization of Cancer Stem Cells (CSCs):

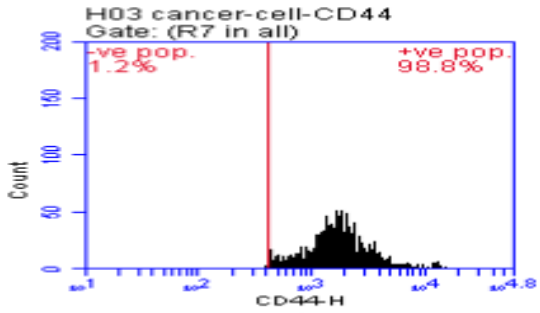
To further identify the phenotypes of the CSCs mammospheres, we analyzed the protein expressions on the surface of the cells by flow cytometry for cell line (Fig.6). The present results found that the expressions of CD44+/CD24- were [90.8%] for CD44 (Fig. 6A) and [ 14%] for CD24 (Fig. 6 C) in cell line group. whereas expressions of $CD^{44+}/CD^{24-}$ in the MCF-7 CSCs group was elevated to [98.8%] for CD44 (Fig. 6 B) and [ 5.6% ] for CD24 ( Fig. 6 D) after being treated with DMEM-F12 supplemented with bovine serum albumin, basic fibroblast growth factor (bFGF), insulin, and epidermal growth factor (EGF) (Fig. 6). These results illustrated that the purity of MCF-7 cancer stem cells in the mammospheres is above 98% after being cultured in serum-free medium for 1 week.



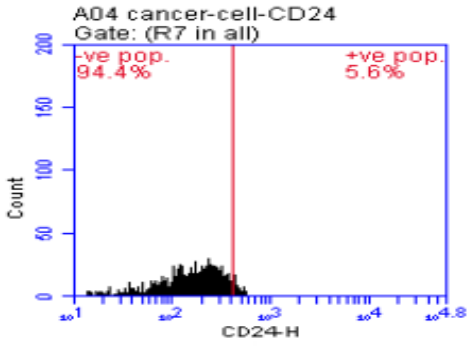

a)




b)




c)




d)

**Fig. 6**: Flow cytometry of phenotypes showing: (a) MCF-7 cell line stained with anti-CD44-FITC antibody, (b) MCF-7 cancer stem cells mammospheres stained with anti-CD44-FITC antibody, c) MCF-7 cell line stained with anti-

CD24-FITC antibody, and d) MCF-7 cancer stem cells mammospheres stained with anti-CD24-FITC antibody.

**Discussion:**

In this study MCF7 cells were cultured and exposed to specific conditions that only cancer stem cells will survive in. cell line was cultured

in serum-free DMEM-F12 supplemented with 0.4% bovine serum albumin,10 ng/ml basic fibroblast growth factor (bFGF), 5 mg/ml insulin, 20 ng/ml epidermal growth factor (EGF) and left incubated for a week without any disturbance [14].

Some studies added different conditions plus to those as B27 believing in its role as a mammosphere formation promotor and prevention of adherences [15]. While in the present work we didn't use it as mammospheres were created easily and once mammospheres were created at day 6, were collected, photographed and measured. Cells were checked at day 1 after adding media that appeared as normal clear cell line and same for the first 3 days. Some changes begin to appear at day 4, small 2,3 cells together appeared clearly as small clusters of cells gathered around itself, non-adherent and then begin to increase in number and size with time. At day 6 good mammospheres appeared clearly and were ready to be collected and examined [16].

The mostly Identifying CSC way mainly depend on expression of cell surface markers as CD44 and CD24 as that combination of these transmembrane proteins have been widely used in cancer cells stemness characterization long time ago. It was reported that $CD^{44+}$ , $CD^{24-}$ are are more tumorigenic than $CD^{44+}$, $CD^{24+}$, Al-Hajj *et al* said . Moreover, $CD^{44+}$, $CD^{24-}$ Were accepted as CSC marker and a prognosis predictor for BC [17]

Flow cytometry was used as an accurate detector. We evaluated the CD44/CD24 phenotypes in breast cancer mammospheres and in cell line and compared them together in order to make sure CSCs were isolated successfully [18]. After comparing cell line CD44+/CD24- and CSCs CD44+/CD24- together, results indicated that the purity of MCF-7 cancer stem cells in the mammospheres is above 98% after being cultured in serum-free medium for 1 week [19].

These mammospheres fulfilled with CD44+/CD24- cells which are prelevant in tumors play an important role in metastasis invasive step. studies suggested that CD44+/CD24− cells are much more rigorous in developing mammosphers. Therefore, breast cancer stem cells isolated from MCF7 cell line involve high tumorigenic potential [20]. Paying more attention to these isolated CSCs mammospheres allows us characterize the regulatory pathways of CSCs and understanding more about mechanisms of cancer relapse.

**Conclusion**

This study successfully cultured MCF7 cell line mammospheres with a CSC purity above 98% after one week in serum-free conditions. These mammospheres offer a promising model for investigating the responsiveness of cancer stem cells to therapeutic drugs, providing a useful tool for future cancer research.

**4. References**


1 Brown, J. S.; Amend, S. R.; Austin, R. H.; Gatenby, R. A.; Hammarlund, E. U.; & Pienta, K. J. (2023). Updating the definition of cancer. Molecular Cancer Research, **21(11)**: 1142-1147.

2 Saini , A.; Kumar, M.; Bhatt, S.; Saini, V.; & Malik, A. (2020). Cancer causes and treatments. *Int. J. Pharm. Sci. Res*, **11:** 3121-3134.

3 Ma, S.; Hao, R.; Lu, Y. W.; Wang, H. P.; Hu, J.; & Qi, Y. X. (2024). Identification and Validation of Novel Metastasis-Related Immune Gene Signature in Breast Cancer. Breast Cancer: Targets and Therapy: 199-219.

4 McSherry, E. A.; Donatello, S.; Hopkins, A. M.; & McDonnell, S. (2007). Common Molecular Mechanisms of Mammary Gland Development and Breast Cancer: Molecular basis of invasion in breast cancer. Cellular and Molecular Life Sciences, **64**: 3201-3218.

5 Quan, Q.; Wang, X.; Lu, C.; Ma, W.; Wang, Y.; Xia, G.; & Yang, G. (2020). Cancer stem-like cells with hybrid epithelial/mesenchymal phenotype leading the collective invasion. Cancer science, **111(2)**: 467-476.

6 Mirabelli, P.; Coppola, L.; & Salvatore, M. (2019). Cancer Cell Lines Are Useful Model Systems for Medical Research. Cancers, 11*:* 1098. https://doi.org/10.3390/cancers11081098

7 Rezano, A.; Ridhayanti, F.; Rangkuti, A. R.; Gunawan, T.; Winarno, G. N. A.; & Wijaya, I. (2021). Cytotoxicity of simvastatin in human breast cancer MCF-7 and MDA-MB-231 cell lines. *Asian Pacific Journal of Cancer Prevention*, **22(S1)**: 33-42.

8 Sukocheva, O. A.; Hu, D. G.; Meech, R.; & Bishayee, A. (2021). Divergence of intracellular trafficking of sphingosine kinase 1 and sphingosine-1-phosphate receptor 3 in MCF-7 breast cancer cells and MCF-7-derived stem cell-enriched mammospheres. *International journal of molecular sciences*, **22(9)**: 4314.

9 Duan, J. J.; Qiu, W.; Xu, S. L.; Wang, B.; Ye, X. Z.; Ping, Y. F& Yu, S. C. (2013). Strategies for isolating and enriching cancer stem cells: well begun is half done. Stem cells and development, **22(16)**: 2221-2239.

10 Zhang, X.; Powell, K.; & Li, L. (2020). Breast cancer stem cells: biomarkers, identification and isolation methods, regulating mechanisms, cellular origin, and beyond. Cancers, **12(12):** 3765.

11 Al-Hajj, M.; Wicha, M.S.; Benito-Hernandez, A.; Morrison, S.J.; & Clarke, M.F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. USA, **100**: 3983–3988. [CrossRef]

12 Pham, P. V.; Phan, N. L.; Nguyen, N. T.; Truong, N. H.; Duong, T. T.; Le, D. V.; ... & Phan, N. K. (2011). Differentiation of breast cancer stem cells by knockdown of CD44: promising differentiation therapy. *Journal of translational medicine*, **9**: 1-13.

13 Crabtree, J.S.; & Miele, L. (2018). Breast cancer stem cells. Biomedicines, **6**: 77. [CrossRef]

14 Chang, H. W.; Wang, H. C.; Chen, C. Y.; Hung, T. W.; Hou, M. F.; Yuan, S. S. F.; & Tseng, C. N. (2014). 5-azacytidine induces anoikis, inhibits mammosphere formation and reduces metalloproteinase 9 activity in MCF-7 human breast cancer cells. Molecules, **19(3)**: 3149-3159.

15 Wang, R.; Lv, Q.; Meng, W.; Tan, Q.; Zhang, S.; Mo, X.; & Yang, X. (2014). Comparison of mammosphere formation from breast cancer cell lines and primary breast tumors. *Journal of thoracic disease*, **6(6):** 829.

16 Fani, S.; Dehghan, F.; Karimian, H.; Mun Lo, K.; Ebrahimi Nigjeh, S.; Swee Keong, Y., & Mohd Hashim, N. (2016). Monobenzyltin complex C1 induces apoptosis in MCF-7 breast cancer cells through the intrinsic signaling pathway and through the targeting of MCF-7-derived breast cancer stem cells via the Wnt/β-catenin signaling pathway. PLoS One, **11(8):** e0160836.

17 Li, W.; Ma, H.; Zhang, J.; Zhu, L.; Wang, C.; & Yang, Y. (2017). Unraveling the roles of CD44/CD24 and ALDH1 as cancer stem cell markers in tumorigenesis and metastasis. Scientific reports, **7(1)**: 13856.

18 Yousefnia, S.; Ghaedi, K.; Seyed Forootan, F.; & Nasr Esfahani, M. H. (2019). Characterization of the stemness potency of mammospheres isolated from the breast cancer cell lines. Tumor Biology, **41(8)**: 1010428319869101.

19 Zhang, L.; Yao, H. J.; Yu, Y.; Zhang, Y.; Li, R. J.; Ju, R. J.; ... & Lu, W. L. (2012). Mitochondrial targeting liposomes incorporating daunorubicin and quinacrine for treatment of relapsed breast cancer arising from cancer stem cells. Biomaterials, **33(2)**: 565-582.

20 Ambrose, J. M.; Veeraraghavan, V. P.; Vennila, R.; Rupert, S.; Sathyanesan, J.; Meenakshisundaram, R.; ... & Surapaneni, K. M. (2022). Comparison of mammosphere formation from stem-like cells of normal breast, malignant primary breast tumors, and MCF-7 cell line. *Journal of the Egyptian National Cancer Institute*, **34(1)**: 51.