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Research Article

Phenotypic Changes During Breast Cancer Treatment: Biological Mechanisms and Clinical Reflections

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Objective

Breast cancer in women is a major public health problem throughout the world. It is the most common cancer among women both in developed and developing countries. Cancer stem cells have gained new insights in cancer treatment after it was shown that only a small proportion of cancer cells retains the ability to form new tumors and these cells show resistance to therapy. In this study we aimed to compare gene expression profile of breast cancer and their stem cells in different biologic characteristics.

Breast cancer is defined as a heterogeneous disease due to its clinical behaviour, clinical outcome, and biological nature. Careful planning of breast cancer patients' treatment should take into account factors related to the short- or long-term course of the disease and the associated systemic treatment.

The TNM tumor staging system has been a common language since 1953 because it reflects patient prognosis, guides treatment plans, and helps monitor the effects of applied treatments [1]. However, the treatment approach for breast cancer has changed over the last 20 years with the introduction of treatments targeting the Estrogen Receptor (ER) and Human Epithelial Growth Factor Receptor 2 (HER2) signalling pathways. Treatment selection now considers prognostic factors, which are related to the natural history of the disease and associated with survival in the absence of systemic adjuvant therapy, and predictive factors, which are measurements related to treatment response. However, due to the limitations of definitive prognostic and predictive factors currently in use, a portion of patients may not benefit from treatment. When stratified according to current prognostic factors, some low-risk patients may relapse, while high-risk patients may not experience recurrence. More aggressive treatments administered to younger women may lead to long-term sequelae. Older women may be more susceptible to the toxicities of aggressive treatment due to the presence of other health problems. Since the main goal of treatment is not only disease-free survival but also total survival and quality of life, studies are being conducted to discover new factors that can be used to select patients who may benefit from adjuvant systemic therapy to avoid toxicity.

In this study, we aimed to identify possible new molecular treatment targets by detecting differences in gene expression profiles in stem cells of breast cancer cells with different molecular and biological characteristics.

Keywords: Breast Cancer, Cancer Stem Cell, Gene Expression

Material and Methods

The research is an experimental research. Four breast cancer cell lines in different molecular and biologic characteristics (MDA-MB-231: ER-PR-HER2$^-$; MCF-7 (HTB-22): ER$^+$PR$^+$HER2; BT474 (HTB-20): ER$^+$PR$^+$HER2$^+$; T47D (HTB-133): ER$^+$PR$^+$HER2$^-$) were cultured. These cell s were positive controls. Magnetic isolation for CD44$^+$CD24$^-$ cells were done to separate cancer stem cells. RNA isolation, cDNA conversion were done to both group of cells. Breast cancer related 84 gene array (PAHS-131Z) expressions were evaluated by RT-PCR.

a) Working Materials - Consumables and Materials Used

b) Commercial Cell Lines

i. MCF-7 (HTB-22)

ii. HTB-133 (T47D)

iii. MDA- MB-231 (HTB-26)

iv. nHTB-20 (BT-474)

Materials

Plastic cell culture dishes, DMEM: Ham's F12 (1:1) culture medium, Fetal bovine serum, Cell scraper, Trypsin/EDTA, PBS, L-Glutamine, Penicillin/Streptomycin, RNA isolation kit, cDNA synthesis kit, Human Breast Cancer RT-PCR Array kit (PAHS-131Z), $CD44^+CD24^-$ breast cancer stem cell isolation kit.

Four types of breast cancer cell lines were cultured in this study. HTB-133 (T47D), one of the cell lines used in this study, is a metastatic cell line derived from a human ductal epithelial breast tumor. In addition to expressing mutant p53 protein, these cells are positive for progesterone and estrogen receptors under normal culture conditions and lack ERBB2 amplification. They can also be used in tumorigenic mouse experiments with estrogen supplementation. Another cell line used will be MDA-MB-231 (HTB-26). These cells are of human origin, have characteristics of invasive ductal breast carcinoma, and have metastatic origin. They are also negative for ER and PR, lack ERBB2 amplification, and express mutant p53 protein. They can also be used in tumorigenic mouse experiments. The third cell line to be used, BT-474 (HTB-20), is a primary cell line derived from a human ductal epithelial breast tumor. They are also Er and PR positive, have ERBB2 amplification, and express mutant p53 protein. They can also be used in tumorigenic mouse experiments. MCF-7 (HTB-22) is a human metastatic adenocarcinoma, is ER^+, PR^+, and has E The cultured cells were then rinsed with PBS. The cells were then treated with trypsin-EDTA solution to lift the cells. A 1:1 ratio of media was added, and the cells were centrifuged in falcon tubes. The supernatant on the resulting cell pellet was removed. The cells were resuspended with magnetic separation buffer and incubated with CD24 monoclonal antibody. For magnetic labelling, they were incubated with ferritin solution, which binds to $CD24^+$ cells. The tubes were then placed in a magnetic field, and the supernatant, which contained $CD24^-$ cells that did not adhere to the magnet, was collected. These cells were incubated with CD44 monoclonal antibody. For magnetic labelling, they were incubated with ferritin solution, which binds to $CD44^+$ cells. The tubes were then placed in a magnetic field, and magnetically labelled $CD44^+$ cells that adhered to the magnet were collected. Thus, $CD44^+CD24^-$ breast cancer stem cells were isolated. RBB amplification. All of these cells described above are adherent.

Isolated $CD44^+CD24^-$ stem cells and non-stem breast cancer cells were counted using a TOMA slide after appropriate dilutions. The procedure for obtaining approximately 15µg of RNA per 1 x 106 cells was performed as follows: Cells were resuspended in 200µl of PBS. 400µl of lysis and binding buffer were added and vortexed for 15 seconds. The high-purity filter tube was attached to the collection tube. The sample was transferred to the upper filter tube and centrifuged at 8000g for 15 seconds. The filter tube was removed from the collection tube. After removing the liquid from the collection tube, the same tubes were reinserted. 90µl of DNAase incubation buffer was added to each sample in the reaction tube. 10µl of DNAase I was added and mixed. This mixture was added to the filter tube and incubated for 15 minutes at room temperature. 500µL of Wash Buffer I was added to the filtered tube and centrifuged for 15 seconds at 8000g. After removing the liquid collected in the lower collection tube, the same tubes were reattached. 500µL of Wash Buffer II was added to the filtered tube and centrifuged for 15 seconds at 8000g. After removing the liquid collected in the lower collection tube, the same tubes were reattached. 200µL of Wash Buffer II was added to the filtered tube and centrifuged for 2 minutes at maximum speed (13000g). The collection tube was then discarded, and the filtered tube was placed in a new sterile 1.5ml microcentrifuge tube. For RNA elution, 50-100µL of elution buffer was added to the filtered tube and centrifuged for 1 minute at 8000g. The desired RNA was now located in the bottom 1.5ml microcentrifuge tube. This RNA was either used immediately in the RT-PCR run or stored at -80°C for later use.

Evaluation of Data

Fold changes (FOLD CHANGE) were calculated for each condition, indicating an increase or decrease in expression compared to the expression in control neuroblastoma cells. These analyses were performed using SA Bioscience's data analysis expression page.

The main limitations of this study are that only four cancer cell lines were studied due to financial reasons, the number of isolated stem cells was limited, and the stem cell properties were not re-evaluated after the stem cells were isolated.

Results

Gene expression of VEGFA was increased MKI67 and TFF3 was decreased in HTB-133 cancer stem cells. CTNBB1, CDKN1A, JUN, TFF3 were decreased in HTB-20 cancer stem cells. SLC39A6, TFF3, XBP1, RB1, MAPK3 were increased and ADAM23, PLAU, GSTP1, BCL2, MMP2, MMP9, MGMT were decreased in MCF-7 (HTB-22) cancer stem cells. In MDA-MB-231 cancer stem cells ABCB1 and SLC39A6 were increased and MMP9 and MKI67 were decreased. We determined differences in gene expression between breast cancer cells and their stem cells, especially in angiogenesis, migration, proliferation and DNA repair genes. Besides these differences we found increase in AKT1, CTNNB1, THBS1, Tp53 and XBP1 genes in all breast cancer cells and their stem cells.

Significant increases and decreases were detected when comparing the gene expression of 84 genes associated with breast cancer carcinogenesis, coded PAHS-131Z, in four breast cancer stem cells with different molecular and biological characteristics with their own normal control cells.

Discussion

The use of genomic technology in the analysis of tumor samples aims to improve clinical decision-making and prognosis. Studies aimed at understanding the molecular structure of breast cancer initiation and progression and, using this knowledge, developing

molecular-based targeted therapies to reduce breast cancer mortality have gained significant importance in recent years. Breast cancer is not a single disease but a collection of biologically distinct conditions. Uncontrolled cell proliferation associated with breast cancer often exhibits changes such as signs of genomic instability and the disappearance of certain epithelial features. Therefore, it is important to understand the molecular mechanisms that drive cancer development and the characteristics of each patient's tumor, and to determine the appropriate treatment method accordingly. Every molecular analytical method applicable to human cancer tissue has the potential to be prognostic/predictive. Despite all these advances in diagnosis, treatment, and prognosis, understanding the properties of breast cancer stem cells is also necessary to explain conditions such as recurrence and metastasis in breast cancer.

In another study on stem cell cells, which are thought to originate from tumor cells with different characteristics according to the molecular classification of breast cancer, ER+ was also found in stem cell cells of ER+ breast cancer cells [2]. It has been shown that ER^+ cells, which form in the terminal phase when the cells that form breast tissue initially begin to differentiate from ER^- cells, transform into this form under the influence of BRCA1 [3]. In our study, three of the cell lines used showed ER^+ expression, while one did not. When gene expression was examined in both the cancer cell lines and the stem cells of these cell lines, changes were observed in different genes in each group. These changes were also observed in the HTB 22 and HTB 133 cell lines, which have identical ER, PR, and HER2 expression levels. For example, TFF3 expression increased in the stem cells of HTB 22 cells, while this expression decreased in the HTB 133 line. When evaluated together with the previously mentioned study, it can be said that tumors with the same ER, PR and HER expression contain stem cell-like cells with different genetic properties.

Cyclin-dependent kinase inhibitor 1 (CDKI1), which functions as a regulator of the cell cycle, is encoded by the CDKN1A gene located on chromosome 6 in humans [4,5]. Studies on metastatic mouse breast cancers have found that CDKI1 is increased in both the primary tumor and metastasis [6-8]. Expression of this gene was found to be significantly increased in stem cell-like cells isolated from the HTB 20 cancer cell line derived from human primary tumors compared to cancer cells. In three other breast cancer cell lines, all of which had metastatic tumors, expression of this gene in stem cell-like cells was similar to that in cancer cells.

Conclusion

Our data suggest that there is not a common cancer stem cell for breast cancer. There are different breast cancer stem cells with different genetic characterization and phenotypes, so it is suggested that the usage of "breast cancer initiating cells" term can be more convenient than the term of breast cancer stem cell. The novel therapeutical targets might be planned including these genetic changes.

Acknowledgement

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Conflict of Interest

None.

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