Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: February 28, 2023

Citation: Telang N., 2023. Drug-resistant Stem Cell Models for the Hormone-responsive Luminal A Breast Cancer, Medical Research Archives, [online] 11(2). https://doi.org/10.18103/mra.v11i2.3556

DOI: https://doi.org/10.18103/mra.v11i2.3556

ISSN: 2375-1924

REVIEW ARTICLE

Drug-resistant Stem Cell Models for the Hormone-responsive Luminal A Breast Cancer

Nitin Telang

Director, Cancer Research, Pre-clinical Oncology
Palindrome Liaisons Consultants, Montvale, New Jersey, USA

entitytoo@gmail.com

Abstract

Background: Progression of early stage breast cancer to advanced stage metastatic disease represents a major cause of death in women. The Luminal A breast cancer subtype exhibits acceptable response to Chemo-endocrine and targeted therapy. However, these treatment options are associated with intrinsic/acquired therapy resistance and emergence chemo-resistant cancer initiating stem cell population, and resultant progression to advanced stage metastatic disease. These limitations emphasize an unmet need for the development of reliable models for cancer stem cells that facilitate identification of efficacious therapeutic alternatives. Documented human consumption, low systemic toxicity, preclinical cancer growth inhibitory efficacy and stem cell targeting efficacy of natural products, such as dietary phytochemicals and nutritional herbs, provide mechanistic leads for these agents as testable therapeutic alternatives.

Objectives: The objectives of the present review are to i.) Provide a systematic discussion of published evidence relevant conceptual background of conventional/targeted therapy and nutritional herbs as testable alternatives, ii.) Growth inhibitory efficacy of nutritional herbs in a cellular model for the Luminal A breast cancer, iii.) Breast cancer stem cell biology and stem cell models for therapy-resistant breast cancer, and iv.) Future research directions.

Conclusions: Collectively, all the elements discussed in the present review validate mechanism-based experimental approaches to identify and prioritize potential therapeutic alternatives.

Future Research: This review provides a rationale for investigations on patient-derived tumor samples that may minimize extrapolation of the preclinical data for their clinical relevance and translatability.

Introduction:

Progression of metastatic breast cancer represents a major cause of death in women. The incidence of breast cancer, 287,850 newly diagnosed cases and 43,250 cancer related deaths have been projected for 2023[1]. Global gene expression profiling of breast cancer has classified the breast cancer as Luminal A, Luminal B, HER-2-enriched and triple-negative subtypes[2]. Expression status of hormone and growth factor receptors determines the treatment options. Conventional chemotherapy with anthracycline, carboplatin and paclitaxel represent the mainstream treatment options. Molecularly targeted pathway selective pharmacological inhibitors such as selective estrogen receptor modulators, selective estrogen receptor degraders, aromatase inhibitors and cyclin-dependent kinase inhibitors represent agents for mainstream targeted breast cancer therapy[3]. The Luminal A breast cancer subtype represents a predominant subtype with a positive response to therapy. However, these long-term treatment options are associated with systemic toxicity, intrinsic/acquired therapy resistance and emergence of chemo-resistant cancer initiating stem cell population and resultant metastatic disease progression.

The limitations of conventional/targeted therapy emphasize an unmet need to identify less toxic, efficacious therapeutic alternatives. Natural products such as dietary phytochemicals, nutritional herbs and naturally-occurring bioactive agents have documented human consumption, exhibit cancer growth inhibitory efficacy in preclinical models[4-9], and cancer stem cell targeted efficacy[10-14]. The advantages of natural products provide a strong rationale to evaluate these agents as testable alternatives for therapy-resistant breast cancer. In this context it is notable that drug-resistant stem cell model developed from the HER-2-epressing tumorigenic cells exhibit stem cell targeted inhibition in response to treatment with natural products such as an endogenous derivative of vitamin A and a terpene present in Rosemary[14].

Present review discusses published evidence that is directly relevant to i.) Growth inhibitory efficacy of nutritional herbs in a cellular model for Luminal A and aromatase expressing post-menopausal breast cancer subtypes, ii.) Conceptual and technical aspects of cancer stem cell biology, iii.) Development and characterization of drug-resistant stem cell model for the Luminal A subtype and iv.) Future research directions that minimize extrapolation for clinical relevance and translatability of preclinical data.

Experimental Models: Cellular models for Luminal A and aromatase expressing Luminal A subtypes represent clinically relevant systems because of similarities of hormone receptor expression. Non-tumorigenic 184-B5 model represents a relevant control to tumorigenic MCF-7 and MCF-7^{AROM} models. Molecular characterization and clinical applicability of the models is illustrated in Table 1.

Table 1: Experimental Models

Model	Characteristics				Clinical Subtype
	ER	PR	HER-2	AROM	
184-B5	-	-	-	-	Normal breast, non-tumorigenic
MCF-7	+	+	-	-	Breast carcinoma derived
MCF-7^{AROM}	+	+	-	+	Aromatase positive

ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2; AROM, aromatase.

In comparison with the non-tumorigenic 184-B5 cells, MCF-7 and MCF-7^{AROM} cells exhibit hyper-proliferation as evidenced by accelerated cell cycle progression and down-regulated cellular apoptosis. Furthermore, increased AI colony formation of the tumorigenic cells represents an *in vitro* surrogate end point for *in vivo* tumor development[4-9].

Nutritional Herbs: Nutritional herbs are routinely used in traditional Chinese medicine for general health purposes, estrogen-related health issues and palliative treatment of cancer patients[15, 16]. Traditionally, the herbal formulations are boiled in water and consumed by the patients. To simulate patient consumption, non-fractionated aqueous extracts of the herbs are used for the experiments.

Information regarding nutritional herbs, source for herbal extract and major bioactive agents is provided in Table 2.

Table 2: Nutritional Herbs

Botanical Name	Source of Extract	Bioactive Agent
Cornus officinalis (CO)	Fruit	Anthocyanin
Epimedium grandiflorum (EG)	Leaf, Stem	Prenylflavone
Lycium barabrum (LB)	Fruit, Bark	Flavone, Terpene
Taheebo NFD Marugoto (TNM)	*Tabebuia avellanedae* Inner bark	β-lapachone, Napthofuran Dione

Lead compound efficacy: Collectively, isolation and/or synthesis of potential bioactive agents, structure-activity assays for growth inhibitory effects and biomarker modulation represent essential preliminary investigations for screening of potential drug candidates as therapeutic alternatives. Confirmatory experiments using high-throughput screening and molecular biomarker assays for gene expression

profiling provide mechanistic evidence for efficacy. Rank order sequence for efficacy varies depending on individual test compound and on specific end point that is being measured. This sequence provides evidence for relative potency and mechanistic leads for individual test agent.

Mechanistic assays and quantitative end points optimized for the parental tumorigenic cells as well as the drug-resistant cancer stem cells facilitate the identification of potential mechanistic leads for growth inhibitory efficacy of nutritional herbs. The assays and their respective quantitative end points are illustrated in Table 3.

Table 3: Mechanistic End points

Assay	Quantitative End point
Parental cells	
Anchorage independent colony formation	Colony number
Cell cycle progression	G_1: S+G_2/M ratio
Cellular apoptosis	% Sub G_0, caspase 3/7 activity, BCL-2, BAX expression
E_2 metabolism	2-OHE_1: 16α-OHE_1 ratio
Aromatase activity	E_1 formation
Gene expression	ESR-1, AROM, PR, PS2, GRB2, Cyclin D1. δδ Cq (PCR)
Stem cells	
Tumor spheroid formation	Tumor spheroid number
CD44	Cellular uptake, RFU
NANOG	Cellular uptake, RFU
OCT-4	Cellular uptake, RFU

2-OHE_1, 2-hydroxyestrone; 16α-OHE_1, 16α-hydroxyestrone; E_1, estrone; ESR-1, estrogen receptor-1; AROM, aromatase; PR, progesterone receptor; PS2, estrogen responsive gene; GBRB2, growth factor receptor binding protein2; δδ Cq, relative gene expression; PCR, polymerase chain reaction; CD44, cluster of differentiation44; NANOG, DNA-binding transcription factor; OCT-4, octamer-binding transcription factor4, RFU, relative fluorescent unit.

Cell cycle progression is determined by monitoring the cell population in distinct phases of the cell cycle using flow cytometry. Cellular apoptosis is determined by the cell population in the subG_0 phase of the cell cycle, and quantified by the expression status of apoptosis specific proteins. Cellular metabolism of E2 is examined by the formation of individual metabolites. Gene expression is examined by polymerase chain

reaction assay. Stem cells are characterized by tumor spheroid formation and expression of stem cell specific molecular markers that are quantified by relative fluorescent units (RFU) due to cellular uptake of fluorescently labelled antibody [7-9, 22].

Cellular and molecular functions of 17β-estradiol (E_2):

ER-mediated signal transduction: 17β-estradiol (E_2) represents a major positive growth regulator for estrogen receptor (ER-α) expressing target tissues. This hormone functions as a physiological ligand ER-mediated multiple-step process of signal transduction that culminates by binding of ER to estrogen responsive element on DNA and upregulated expression of downstream estrogen responsive target genes such as ESR-1 (gene for ER-α), progesterone receptor (PR) and aromatase (AROM). Furthermore, the signal transduction process results in upregulated expression of estrogen-regulated genes such as PS2, GRB2 and cyclin D1[17, 18].

Cellular metabolism of E_2: Growth modulatory function of E_2 is also dependent on cellular metabolism of E_2 wherein E_2 metabolites exhibit distinct functions in breast carcinogenesis[19,20]. During oxidative metabolism of E_2, estrone (E_1) is formed that functions as a common precursor for the formation of 2-hydroxyestrone (2-OHE_1) or 16α-hydroxyestrone (16α-OHE_1). The metabolites formed from E_2 such as 2-hydroxyestradiol (2-OHE_2) and 4-hydroxylated estradiol (4-OHE_2) have opposite growth modulatory functions.

MCF-7 model: The MCF-7 cells express hormone receptors and represent a model for the Luminal a subtype of breast cancer. Experiments on MCF-7 cells have shown that treatment with E_2 and 16α-OHE_1 increase AICF *in vitro* and tumor growth *in vivo*, while treatment with 2-OHE_1 inhibits the growth of cells *in vitro* and *in vivo* in vitro[21].

Treatment of MCF-7 cells with the nutritional herbs CO, EG and LB inhibit AICF with a rank order sequence of LB > CO > EG. At the mechanistic levels these herbs inhibit cell cycle progression via G_1 and/or G_2/M phase arrest with a rank order sequence of LB > CO > EG, and increase 2-OHE_1: 16α-OHE_1 ratio in favor of the anti-proliferative metabolite 2-OHE_1 with a rank order sequence of EG < LB < CO[7, 22].

MCF-7 ^{AROM} model: During the menopause peripheral estrogen synthesis proceeds via the action of aromatase that converts androstene dione to E_1 and testosterone to E_2. Aromatase represents a target for pharmacological inhibitors that are used as treatment option for post-menopausal aromatase expressing breast cancer[3].

The MCF-7^{AROM} cells are transfected with the aromatase gene, and represent a model for post-menopausal breast cancer. Treatment of MCF-7 ^{AROM} cells with the natural product TNM from the inner bark of *Tabebuia avellanedae* tree leads to inhibition of AICF. At the mechanistic level TNM treatment downregulates the expression of E_2 responsive genes ESR1, AROM and PR and of E_2 regulated genes PS2, GRB2 and cyclin D1.

The induction of cellular apoptosis by TNM is associated with increased activity of caspase 3/7 and reciprocal modulation of anti-apoptotic BCL-2 and pro-apoptotic BAX genes[9]. It is also notable that in a comparison of aromatase inhibitory activity TNM is effective at substantially lower concentration than that of pharmacological aromatase inhibitors letrozole and exemestane. These data suggest a superior efficacy for aromatase inhibition by TNM.

Cancer stem cells: All the epithelial organ sites including upper aero-digestive tract, breast, prostate and intestines are lined by an epithelial layer where homeostatic growth control and tissue regeneration is preserved by a gradient of cell proliferation differentiation and apoptosis. Multiple signaling pathways including Wnt/β-catenin, NOTCH and Hedgehog are responsible for growth regulation of normal adult stem cells. Evolution, maintenance and biological functions of normal adult stem cells is the culmination of complex interplay of these signal transduction pathways[23].

Cancer stem cells represent a minor subpopulation in the primary tumor. These hyper-proliferative stem cells exhibit chemo-resistance and cancer initiating properties. The Wnt/β-catenin, NOTCH and Hedgehog are disrupted in therapy-resistant cancer stem cells[24-25].

The chemo-resistant cancer stem cells also exhibit activation of RAS/ BRAF/ MEK/ERK signaling pathway leading to growth advantage of stem cell population. Small molecule inhibitors of MEK have documented clinical efficacy[26].

Activation of PI3K, AKT, and mTOR signaling pathways are essential for growth advantage and survival of cancer stem cell population where pathway selective pharmacological inhibitors are in clinical use[27, 28].

Cellular plasticity in cancer stem cells plays an essential role in epithelial-mesenchymal transition (EMT), leading to metastatic progression of the disease. Activation of the NFkB/STAT-3 signaling pathway is associated with cellular plasticity. Modulation of epithelial specific and mesenchymal specific protein expression and of select nuclear transcription factors SLUG, SNAIL and ZEB represent sensitive markers for EMT and represent molecular targets for therapeutic efficacy of pharmacological inhibitors[29-31].

MCF-7/TAM-R Model: The selective estrogen receptor modulator tamoxifen (TAM) represents a mainstream agent for targeted therapy of the Luminal A and Luminal B breast cancer subtypes. Long-term TAM treatment is associated with intrinsic/acquired therapy resistance. Long-term treatment of MCF-7 cells eliminates TAM-sensitive phenotype and facilitates the growth of TAM resistant (TAM-R) phenotype.

The isolation of TAM-resistant phenotype is achieved by long-term maintenance of cells in the presences of cytotoxic concentration of TAM. This selective pressure promotes progressive growth of the resistant phenotype. The characterization of putative

stem cells is achieved by monitoring the status of select stem cell specific biological and molecular markers. The MCF-7/TAM-R cells exhibit increased viable cell number and increased tumor spheroid formation. The latter being a biological stem cell marker. Additionally, MCF-7/TAM-R cells exhibit increased expression of stem specific cell surface marker CD44, and of the nuclear transcription factors NANOG and OCT-4[14]. The nuclear transcription factors OCT-4, Klf-4, Sox-2 and c-Myc are known to play essential role in maintenance of induced pluripotent stem cells[32, 33]. The expression status of biological and molecular stem cell markers provide evidence for cellular and molecular characterization of the present breast cancer stem cell model. The specificity and sensitivity of biological and molecular stem cell markers and their applicability as modifiable end points is evident by stem cell targeting growth inhibitory efficacy natural products in the 184-B5/HER-LAP-R stem cell model[14]. In this model cells resistant to the EGFR/HER-2 inhibitor lapatinib (LAP) are isolated and characterized to represent the HER-2-enrched breast cancer subtype[14].

Conclusions: This review has discussed published evidence for growth inhibitory efficacy of Chinese nutritional herbs in cellular models for hormone receptor expressing and aromatase expressing breast cancer and development of drug-resistant cancer stem cell model. Collectively, these aspects suggest that the validated experimental approaches may provide mechanistic leads for natural products as testable alternatives for potential drug candidates in treatment of therapy resistant breast cancer.

Future research: Intrinsic or acquired resistance to targeted endocrine therapy for hormone receptor positive luminal A and Luminal B breast cancer subtypes represents a formidable challenge. For example, studies focused on intrinsic/acquired resistance to aromatase inhibitors using the MCF-7 ^{AROM} model resistance to letrozole resulted in downregulated expression of ER-α and hyper-activation of HER-2/MAPK signaling pathway. Although activation of the HER-2 signaling in this model suggests acquired susceptibility to HER-2 selective therapeutic efficacy[34]. In addition, susceptibility to the ER-α degrader fulvestrant and acquired cross- resistance to individual aromatase inhibitors such as letrozole and exemestane has been documented[35]. Collectively, this evidence emphasizes investigations to identify therapeutic alternatives that may function independent of therapy resistance.

The human telomerase reverse transcriptase (hTERT) represents a specific universal marker for immortalized cancer-initiating stem cells. This enzyme may represent a therapeutic target for pharmacological agent and natural products[36].

Epigenetic modifications impact cell plasticity and gene transcriptional activity via nuclear histone modifications, DNA methyl transferase activity and gene promoter methylation. Small molecule pharmacological inhibitors and natural products functioning as epigenetic modifiers may be effective in therapy-resistant cancer stem cells[37].

Epithelial-mesenchymal transition (EMT) characterizes cellular plasticity in cancer stem cells. This process is associated with reciprocal modulation in expression of epithelial and mesenchymal specific cellular proteins cadherin and vimentin, respectively and of the transcription factors SLUG, SNAIL and ZEB[24,25,27,28]. The NFkB/JAK-STAT-3 signaling pathway plays a critical role in EMT. Specific inhibitors and modifiers this pathway may represent testable drug candidates[29-31].

Preclinical investigations are dependent on extrapolation for clinical relevance and translatability. This limitation may be reduced by using the models developed from patient-derived tumor samples such as patient-derived tumor explants (PDTX) and tumor organoids (PDTO)[38, 39].

Corresponding Author:
Nitin Telang
Director, Cancer Research
Pre-clinical Oncology
Palindrome Liaisons Consultants,
Montvale, New Jersey, USA
Email: entitytoo@gmail.com

Conflicts of Interest Statement:
The author declares that there are no conflicts of interest.

Funding Statement:
Current research does not have extra-mural funding.

Acknowledgements:
The research program "Cellular models for molecular subtypes of clinical breast cancer: Molecular approaches for lead compound efficacy" has received past extra-mural funding from US National Cancer Institute FIRST Award CA 44741, US Department of Defense Breast Cancer Research Program IDEA Award DAMD-17-94-J-4208.

References:

1. American Cancer Society Facts and Figures-2022. American Cancer Society, Inc. Atlanta, GA, 2022.

2. Sorlie T, Perou CM, Tibshirani R, et al: Gene expression patterns of breast carcinoma distinguish tumor subclasses with clinical implications. *Proc. Natl. Acad. Sci.* 2001, 98 (19): 10869-10874.
DOI: 10.1073/pnas.191367098.

3. National Comprehensive Cancer Network 2022. http://www.nccn.org.

4. Mukherjee B, Telang N, Wong GYC: Growth inhibition of estrogen receptor positive human breast cancer cells by Taheebo from the inner bark of *Tabebuia avellanedae* tree. *Int. J. Mol. Med.* 2009, 24 (2): 253-260. DOI: 10.3892/ijmm_00000228.

5. Telang NT, Li G, Sepkovic DW, et al: Anti-proliferative effects of Chinese herb *Cornus officinalis* in a cell culture model for estrogen receptor positive clinical breast cancer. *Mol. Med. Rep.* 2012, 5 (1): 22-28.
DOI: 10.3892/mmr.2011.617.

6. Telang NT, Li G, Sepkovic D, et al: Comparative efficacy of extracts from *Lycium barbarum* bark and fruit on estrogen receptor positive human mammary carcinoma MCF-7 cells. *Nutr. Cancer* 2014, 66 (2): 278-284. DOI: 10.1080/01635581.2014.864776.

7. Telang N, Li G, Katdare M, et al: Inhibitory effects of Chinese nutritional herbs in isogenic breast carcinoma cells with modulated estrogen receptor function. Oncol. Lett. 2016, 12 (5): 3949-3957.
DOI: 10.3892/0l.2016.5197.

8. Telang NT, Li G, Katdare M, et al: The nutritional herb *Epimedium grandiflorum* inhibits the growth in a model for Luminal A molecular subtype of breast cancer. Oncol. Lett. 2017, 13 (4): 2477-2482.
DOI: 10.3892/ol.2017.5720.

9. Telang N, Nair HB, Wong GYC: Growth inhibitory efficacy and anti-aromatase activity of *Tabebuia avellanedae* in a model for post-menopausal breast cancer. Biomed. Rep. 2019, 11 (5): 222-229.
DOI: 10.3892/br.2019.1244.

10. Naujokat C, Mc Kee DI: The 'Big Five' phytochemicals targeting cancer stem cells: Curcumin, EGCG, sulforaphane, reserveratrol and genistein. Cur. Med. Chem. 2012, 28 (22): 4321-4342.
DOI: 10.2174/092986732766620022811073 8.

11. Hong M, Tan HY, Li S, et al: Cancer stem cells: Potential targets of Chinese medicines and their active components. Int. J. Mol. Sci. 2016, 17: 893.

12. Manogaran P, Umapathy D, Karthikeyan M, et al: Dietary phytochemicals as a potential source of targeting cancer stem cells. Cancer Invest. 2021, 39 (4): 349-368.
DOI: 10.1080/07357907.2021.1894569.

13. Meerson A, Khatib S, Mahjna J: Natural products targeting cancer stem cells for augmenting cancer therapeutics. Int. J. Mol. Sci. 2021, 22: 13044.

14. Telang N: Stem cell models for cancer therapy. Int. J. Mol. Sci. 2022, 23: 7055. DOI: 10.3390/ijms23137055.

15. Ye L, Jia Y, Ji KE, et al: Traditional Chinese medicines in prevention and treatment of

breast cancer and metastasis. *Oncol. Lett.* 2015, 10 (3): 1240-1250. DOI: 10.3892/ol.2015.3459.

16. Yang Z, Zhang Q, Yu L, et al: The signaling pathways and targets of traditional Chinese medicine and natural medicine in triple-negative breast cancer. *J. Ethno-pharmacology* 2021, 264: 113249. DOI: 10.1016/jep.2020.113249.

17. Moy B, Goss PE: Estrogen receptor pathway: Resistance to endocrine therapy and new therapeutic approaches. *Clin. Cancer Res.* 2006, 12 (16): 4790-4793. DOI: 10.1158/1078-0432.CCR-06-1535.

18. O'Hara J, Vareslija D, Mc Brian J, et al: A1B1: ER-α transcriptional activity is selectively enhanced in aromatase inhibitor-resistant breast cancer cells. *Clin Cancer Res.* 2012, 18 (12): 3305-3315. DOI: 10.1158/1078-0423.CCR-11-3300.

19. Gupta M, McDugal A, Safe S: Estrogenic and anti-estrogenic activities of 16α- and 2-hydroxy metabolites of 17β-estradiol in MCF-7 and T47D human breast cancer cells. *J. Steroid Biochem. Mol. Biol.* 1998, 67 (5-6): 413-419. DOI: 10.1016/s0960-0760(98)00135-6.

20. Santen RJ, Yue W, Wang J-P: Estrogen metabolites and breast cancer. *Steroids* 2015, 99 (Pt A): 61-66. DOI: 10.1016/j.steroids.2014.08.003.

21. Suto A, Telang NT, Tanino H, et al: *In vitro* and *in vivo* modulation of growth regulation in the human breast cancer cell line MCF-7 by estradiol metabolites. *Breast Cancer* 1999, 6 (2): 87-92. DOI: 10.1007/BFO2966913.

22. Telang NT: The divergent effects of ovarian steroid hormones in the MCF-7 model for Luminal A breast cancer: Mechanistic leads for therapy. Int. J. Mol. Sci. 2022, 23: 4800. DOI: 10.3390/ijms23094800.

23. Barker N: Adult intestinal stem cells: Critical drivers of epithelial homeostasis and regeneration. *Nat. Rev. Mol. Cell. Biol.* 2014, 15 (1): 19-33. DOI: 10.1038/nrm3721.

24. Soteriou D, Fuchs Y: A matter of life and death: Stem cell survival in tissue regeneration and tumor formation. *Nat. Rev. Cancer* 2018, 18 (3): 187-201. DOI: 10.1038/nrc.2017.122.

25. Lytle NK, Barber AG, Reya T: Stem cell fate in cancer growth, progression and therapy resistance. *Nat. Rev. Cancer* 2018, 18 (11): 669-680. DOI: 10.1038/s41568-018-0056-x.

26. Yaeger R, Solit DB: Overcoming adaptive resistance to KRAS inhibitors through vertical pathway targeting. *Clin. Cancer Res.* 2020, 26 (7): 1538-1540, 2020. DOI: 10.1158/1078-0432.CCR-19-4060.

27. Shibue T, Weinberg RA: EMT, CSC and drug resistance. *Nat. Rev. Clin. Oncol.* 2017, 14 (10): 611-629. DOI: 10.1038/nrclinonc.2017.44.

28. Nunes T, Hamdan D, Leboeuf C, et al: Targeting cancer stem cells to overcome chemo-resistance. Int. J. Mol. Sci. 2018, 19: 4036.

29. Wook J: Role of JAK/STAT-3 signaling in in the regulation of metastasis, the transition of cancer stem cells, and chemo-resistance of cancer by epithelial-mesenchymal transition. Cells 2020, 9: 217. DOI: 10.3390/cells9010217.

30. Gooding AJ, Scheimann WP: Epithelial-mesenchymal transition programs and cancer stem cell phenotypes: mediators of breast cancer therapy resistance. Mol. Cancer Res. 2020, 18 (9): 1257-1270.
DOI: 10.1158/1541-7786.MCR-20-0067.

31. Kanzaki H, Chatterjee A, Hosein H, et al: Disabling the nuclear trans-localization of RelA/NFkB by a small molecule inhibits triple-negative breast cancer growth. Breast Cancer (Dove Med. Press) 2021, 13: 419-430.
DOI: 10.2147/BCTT.S310231.

32. Park IH, Zhou R, West JA, et al: Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008, 451 (7175): 141-146.
DOI: 10.1038/nature06534.

33. Yu J, Hu K, Smuga-Otto K, et al: Human induced pluripotent cells free of vector and transgene sequences. *Science* 2009, 324 (5928): 797-801.
DOI: 10.1126/science.1172482.

34. Sabnis G, Brodie A: Understanding resistance to endocrine agents: Molecular mechanisms and potential for intervention. *Clin. Breast Cancer* 2010, 10 (1): E6-E15.
DOI: 10.3816/CBC.2010.n.014.

35. Hole S, Pedersen AM, Hansen SK, et al: New cell culture model for aromatase inhibitor-resistant breast cancer shows sensitivity to fulvestrant treatment and cross-resistance between letrozole and exemestane. Int. J. Oncol.2015, 46 (4): 1481-1490. DOI: 10.3892/ijo.2015.2850.

36. Fragiadaki P, Ranieri E, Kalliantasi K, et al: Telomerase inhibition and activation: A systematic review. Mol. Med. Rep. 2022, 25 (5): 158. DOI: 10.3892/mmr.2022.12674.

37. Kumar VE, Nambiar R, De Souza C, et al: Targeting epigenetic modifiers of tumor plasticity and stem cell behavior. Cells 2020, 11: 1403. DOI: 10.3390/cells11091403.

38. Bruna A, Rueda OM, Greenwood W, et al: A biobank of breast cancer explants with preserved intra-tumor heterogeneity to screen anti-cancer compounds. *Cell* 2016, 167 (1): 260-274.
DOI: 10.1016/j.cell.2016.08.041.

39. Drost J, Clevers H: Organoids in cancer research. *Nat. Rev. Cancer* 2018, 18 (7): 407-418. DOI: 10.1038/s41568-018-0007-6.