Stem cell targeted therapeutic approaches for molecular subtypes of clinical breast cancer (Review)

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Abstract. The global profiling of differentially expressed genes in subtypes of clinical breast cancer identifies predictive and prognostic biomarkers for disease progression and rationalizes breast cancer subtype-based treatment options. The expression status of hormone and growth factor receptors dictates the options for chemo-endocrine and/or pathway selective small molecule inhibitor-based treatments. Overall, these treatment options are associated with long-term systemic toxicity and acquired tumor resistance, predominantly due to the emergence of drug-resistant cancer stem cell population and due to therapy-resistant disease progression. These limitations emphasize the identification of non-toxic testable therapeutic alternatives for the efficacious targeting of breast cancer stem cells. The present review summarizes published evidence focused on i) developing cellular models for molecular subtypes of breast cancer; ii) isolating and characterizing drug-resistant cancer stem cells from the developed models; and iii) identifying mechanistic leads for potential stem cell-targeting lead compounds. Cellular models for Luminal A, human epidermal growth factor receptor-2 (HER-2) enriched and triple-negative breast cancer subtypes represented the experimental models. Prototypic chemo-endocrine therapeutic agents were used to select the drug-resistant stem cell phenotype. The vitamin A derivative, all-*trans* retinoic acid, and the rosemary terpenoid, carnosol, respectively representing a mechanistically distinct natural product and a potential bio-active constituent of a nutritional herb provided stem cell-selective lead compounds. The cellular models for Luminal A, HER-2-enriched and triple-negative breast cancer subtypes exhibited growth inhibitory effects in response to treatment with prototypic chemo-endocrine therapeutics, natural products and nutritional herbs. Drug-resistant phenotypes exhibited an upregulated expression of stem cell-specific cellular and molecular markers. Lead compounds induced the downregulated expression of the stem cell markers in drug-resistant phenotypes. These data validate an experimental approach with which to identify potential non-toxic natural products and nutritional herbs that may represent testable alternatives for the stem cell targeted therapy of breast cancer.

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Contents

1. Introduction

Metastatic breast cancer represents a major cause of mortality in women in the USA. A recent report from the American Cancer Society estimated 266,120 newly diagnosed cases of breast cancer and 41,400 breast cancer-related deaths in 2019 (1). The global gene expression profiling of clinical breast cancers has provided a molecular classification based on hormone receptor and growth factor receptor expression (2). The identification of molecular subtypes dictates specific chemo-endocrine therapy and pathway selective small molecule inhibitor-based treatment options. Thus, selective estrogen receptor modulators, selective estrogen receptor degraders, aromatase inhibitors with or without CDK 4/6 inhibitors (3-5), human epidermal growth factor receptor-2 (HER-2)-targeted (6), PI3K/AKT-targeted or m-TOR-targeted (7-10) therapeutic options represent the treatment of choice. Long-term therapy is frequently associated with systemic toxicity and acquired tumor resistance due to the emergence of drug-resistant cancer stem cell populations that collectively compromise patient compliance and favor therapy-resistant cancer progression (11). These limitations emphasize the identification of non-toxic testable alternatives for the efficacious stem cell targeted treatment of breast cancer.

Naturally occurring phytochemicals and nutritional herbs are extensively used in alternative medicine and traditional Chinese medicine for general health issues, as well as for

the palliative treatment for breast cancer. These agents have documented growth inhibitory efficacy via distinct mechanisms in cellular models of Luminal A, HER-2-enriched and triple-negative molecular subtypes of clinical breast cancer (12,13). These relatively non-toxic agents may provide testable alternatives for chemo-endocrine therapy-resistant breast cancer by targeting drug-resistant cancer stem cells.

The present review summarizes experimental data on optimizing cellular models for select breast cancer subtypes, isolating and characterizing drug-resistant stem cell phenotypes and providing mechanistic leads for potential lead compounds for the stem cell targeted therapy of clinical breast cancer.

2. Cellular models

Human breast carcinoma-derived cell lines provide valuable cellular models for molecular subtypes (14,15). Table I summarizes the status of hormone and growth factor receptor expression in cellular models for select breast cancer subtypes. The hormone receptor-positive, HER-2-negative Luminal A subtype responds to endocrine therapy comprising of selective estrogen receptor modulators, aromatase inhibitors and CDK 4/6 inhibitors. The hormone receptor-positive, HER-2-positive Luminal B subtype responds to endocrine therapy and to HER-2 targeted therapy. The hormone receptor-negative, HER-2-positive HER-2 enriched subtype responds to cytotoxic chemotherapy and HER-2 targeted therapy. The hormone receptor-negative HER-2-negative triple-negative subtype responds to cytotoxic chemotherapy and select small molecule inhibitors. Thus, the molecular classification has provided valuable leads for breast cancer subtype-selective therapeutic interventions (2-10).

Table I. Cellular models for the molecular subtypes of clinical breast cancer.

Model	Receptor status	Molecular subtype
MCF-7	ER^+, PR^+, $HER\text{-}2^-$	Luminal A
T47D	ER^+, PR^+, $HER\text{-}2^-$	Luminal A
BT474	ER^+, PR^+, $HER\text{-}2^+$	Luminal B
MDA-MB-361	ER^+, PR^+, $HER\text{-}2^+$	Luminal B
SKBr-3	ER^-, PR^-, $HER\text{-}2^+$	HER-2-enriched
184-B5/HER	ER^-, PR^-, $HER\text{-}2^+$	HER-2-enriched
MDA-MB-231	ER^-, PR^-, $HER\text{-}2^-$	Triple-negative

ER, estrogen receptor-α; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2. [Data are summarized from previous studies (14,15,21)].

3. Test agents

Mechanistically distinct clinically relevant therapeutic agents, natural products and select nutritional herbs represented the test agents in the cellular models for Luminal A, HER-2-enriched and triple-negative molecular subtypes of clinical breast cancer. Table II summarizes the maximum cytostatic concentrations and clinical applications of the test agents. Tamoxifen (TAM), Lapatinib (LAP) and Doxorubicin (DOX) represented the positive controls for Luminal A, HER-2-enriched and triple-negative models, respectively (16,17). The maximum cytostatic concentrations of these agents, identified by dose response experiments were used to select the drug-resistant phenotype. The vitamin A derivative, all-*trans* retinoic acid (ATRA) and the rosemary terpenoid, carnosol (CSOL), were utilized to examine their efficacy on the cancer stem cell phenotype.

Mechanistic evidence for the growth inhibitory efficacy of relatively non-toxic natural products, including gucosinolates, polyphenols, isoflavones and terpenoids, as well as nutritional herbs suggest the potential applicability of these agents as testable alternatives for conventional chemo-endocrine therapy (12,18-21).

4. Mechanistic efficacy

Published evidence summarized in Table III has identified susceptible mechanistic pathways and potential molecular targets for the growth inhibitory efficacy of test agents in cellular models for breast cancer subtypes. Thus, in the Luminal A model represented by MCF-7 cells, TAM, *Cornus officinalis* (CO) and *Epimedium grandiflorum* (EG) inhibit anchorage-independent colony formation, induce G_1 phase arrest and apoptosis (12,13,18). In the HER-2-enriched model represented by 184-B5/HER cells, ATRA and CSOL inhibit colony formation, induce G_2/M phase arrest and inhibit cyclooxygenase (COX)-2 expression (21-23). In the triple-negative model represented by MDA-MB-231 cells, the anti-proliferative effects of CO and DA involve the RB and Ras signaling pathways, respectively (19,20). With regard to the nutritional herbs, it is conceivable that individual herbs may contain multiple bioactive agents. Thus, CO represents a major source for biologically active anthocyanins (24,25), EG contains Icariin and icaritin (26,27), and several Chinese nutritional herbs that contain flavonoids, including DA and EG may be effective in the prevention/therapy of metastatic breast cancer (28,29). These bio-active agents may in part be responsible for the growth inhibitory efficacy of the nutritional herbs.

5. Drug-resistant stem cell models

Drug-resistant stem cell phenotypes were selected from MCF-7, 184-B5/HER and MDA-MB-231 parental cells, based on their progressive growth in the presence of cytotoxic concentrations of TAM, LAP and DOX, respectively (30). Cellular markers, including tumor spheroid formation and the expression of CD44 have documented selectivity for cancer stem cells. In addition, nuclear transcription factors, including octamer-binding transcription factor-4 (Oct-4), Kruppel-like factor-4 (Klf-4), sex determining region Y-box-2 (SOX-2), c-Myc and NANOG exhibit stem cell selectivity, and these nuclear factors are also critical for the maintenance of induced pluripotent stem cells (31-33). The TAM-R, LAP-R and DOX-R phenotypes were characterized for their stem cell properties by examining the status of the expression of select stem cell-specific cellular and molecular markers. The stem cell-specific cellular markers included tumor spheroid formation and CD44 expression. The stem-cell specific molecular markers included the nuclear

Table II. Test agents.

Agent	Identity	Maximum cytostatic concentration (IC_{90})[a]	Clinical application
TAM	SERM	1.5 μM	Anti-estrogen therapy
LAP	EGFR/HER-2 inhibitor	10 μM	Anti-HER-2 therapy
DOX	Anthracyclin	0.5 μM	Chemotherapy
ATRA	Vitamin A derivative	3 μM	Retinoid therapy
CSOL	Rosemary terpenoid	5 μM	Nutritional herb
CO		0.07-0.5%	Nutritional herb
EG		0.44%	Nutritional herb
DA		0.03%	Nutritional herb

[a]Determined from day 7 post-seeding of $1.0x10^5$ cells. TAM, Tamoxifen; LAP, Lapatinib; DOX, Doxorubicin; ATRA, all-*trans* retinoic acid; CSOL, carnosol; CO, *Cornus officinalis*; EG, *Epimedium grandiflorum*; DA, *Dipsacus asperoides*. [Data are summarized from previous studies (16,17,19-22)].

Table III. Mechanistic efficacy of test agents in breast cancer models.

Model	Clinical subtype	Agent	Molecular targets
MCF-7	Luminal A	TAM, CO, EG	AIC, G_1 arrest, apoptosis, BCL-2/BAX
184-B5/HER	HER-2 enriched	ATRA, CSOL	AIC, RAR-β, COX-2, G_2/M arrest, cyclin B1
MDA-MB-231	TNBC	CO	G_1-S transition, cyclin D1, RB
		DA	RB, cyclin D1, CDK4/6, RAF/MEK/ERK, CDKI p21

TAM, Tamoxifen; CO, *Cornus officinalis*; EG, *Epimedium grandiflorum*; ATRA, all-*trans* retinoic acid; CSOL, carnosol; DA, *Dipsacus asperoides*; AIC, anchorage-independent growth; BCL-2, B-cell lymphoma-2; BAX, BCL-2-associated X protein; RAR-β, retinoic acid receptor-β; COX-2, cyclooxygenase-2; RB, retinoblastoma; CDK, cyclin-dependent kinase; RAF/MEK/ERK, RAS-mediated down-stream signaling protein molecules; CDKI, cyclin-dependent kinase inhibitor. [Data are summarized from previous studies (18-23)].

Table IV. Drug-resistant stem cell models.

Resistant phenotype	Drug concentration (IC_{90})	Stem cell marker expression (relative to sensitive phenotype)			
		Tumor spheroids	CD44	Oct-4	NANOG
TAM-R	TAM 1.5 μM	+1.7X	+3.8X	+1.8X	+1.9X
LAP-R	LAP 10 μM	+2.3X	+4.2X	+1.9X	+4.4X
DOX-R	DOX 0.5 μM	+2.8X	+4.4X	+2.3X	+1.5X

Tumor spheroid: ISD: 100 cells; spheroid count: day 14 post-seeding. ISD, initial seeding density; TAM-R, Tamoxifen-resistant; LAP-R, Lapatinib-resistant; DOX-R, Doxorubicin-resistant; CD44, cluster of differentiation 44; Oct-4, octamer binding transcription factor-4; NANOG, homeobox transcription factor, X, fold. [Data are summarized from previous studies; for TAM-R and DOX-R (16,17,30); Data are summarized from unpublished results for LAP-R].

transcription factors, Oct-4 and NANOG. The primary data for the status of these cellular and molecular markers were obtained as incidence of tumor spheroids and as log mean immunofluorescence for the expression of CD44, Oct-4 and NANOG (30). These primary data were expressed as marker modulation (%). The data presented in Table IV are summarized as the extent of modulation in marker expression relative to the drug sensitive phenotype. These data clearly demonstrated that the three drug-resistant stem cell phenotypes exhibited a substantial increase in the expression of stem cell-specific cellular and molecular markers.

6. Stem cell-selective lead compound efficacy

The primary data from the experiment designed to examine the stem cell-targeted efficacy of natural products were obtained as the incidence of tumor spheroid and as log mean immuno-fluorescence (17,21,30). The data presented in Table V summarize the marker modulation (%) induced by the natural products, relative to the solvent treated control. These data demonstrate that in response to treatment with the vitamin A derivative, ATRA, and with the rosemary terpenoid, CSOL, the expression of select stem cell markers was substantially downregulated. In this context, it is noteworthy

Table V. Effects of retinoid and terpenoids on LAP-R 184-B5/HER stem cells.

Treatment	Concentration (IC_{90} μM)	Stem cell marker expression (relative to solvent control)			
		Tumor spheroids	CD44	Oct-4	NANOG
ATRA	3 μM	-69.1%	-80.9%	-81.9%	-71.6%
CSOL	5 μM	-80.3%	-84.4%	-69.0%	-74.3%

ATRA, all-*trans* retinoic acid; CSOL, carnosol; CD44, cluster of differentiation 44; Oct-4, octamer binding transcription factor; NANOG, homeobox transcription factor. (Data are summarized from unpublished results).

that these agents exhibit anti-proliferative and pro-apoptotic effects via distinct mechanisms on parental 184-B5/HER cells that represent a cellular model for the HER-2-enriched breast cancer subtype (21-23). Additionally, ATRA targets gastric cancer stem cells and inhibits patient-derived gastric carcinoma tumor growth (34).

7. Conclusions and future prospects

Human tissue-derived preclinical models provide valuable approaches to reduce the extrapolation for the potential clinical translation of the data. The present review summarized the application of cellular models for select molecular subtypes of clinical breast cancer that is targeted towards developing drug-resistant cancer stem cell models. Collectively, the present review has validated approaches that identify potential testable alternatives for the stem cell targeted therapy of breast cancer. Additionally, the present review provides a rational basis for future experiments on breast cancer explant models for lead compound screening (35), patient-derived tumor xenograft models for Luminal B and triple-negative breast cancer subtypes (36), and *ex vivo* breast cancer organoids from chemo-endocrine therapy-resistant breast cancer (37).

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

The author contributed towards study conception, experimental design and data interpretation, and prepared the manuscript for publication.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The author declares that there are no competing interests.

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