Natural products as drug candidates for breast cancer (Review)

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Received December 27, 2022; Accepted May 12, 2023

DOI: 10.3892/ol.2023.13935

Abstract. Progression of early-stage breast cancer to advanced-stage metastatic disease represents a major cause of death in women. Long-term conventional and targeted therapy for breast cancer includes multi-drug combinations of cytotoxic chemotherapeutics and pathway-selective small molecule inhibitors. These treatment options are frequently associated with systemic toxicity, intrinsic/acquired therapy resistance and emergence of a drug-resistant cancer stem cell population. This stem cell population has a chemo-resistant, cancer-initiating, premalignant phenotype that is accompanied by cellular plasticity and metastatic potential. These limitations emphasize an unmet need to identify testable alternatives against therapy-resistant metastatic breast cancer. Natural products such as dietary phytochemicals, nutritional herbs and their constitutive bioactive agents have documented human consumption, and lack detectable systemic toxicity and resultant off-target unfavorable side effects. Because of these advantages, natural products may represent testable alternatives for therapy-resistant breast cancer. The present review discusses published evidence for growth inhibitory efficacy of natural products on cellular models for molecular subtypes of clinical breast cancer and development of drug-resistant stem cell models. Collectively, this evidence validates mechanism-based experimental approaches to screen and prioritize efficacious bioactive agents from natural products as novel drug candidates that may function as therapeutic alternatives for breast cancer.

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Key words: breast cancer, natural products, herbal extracts, drug candidates

1. Introduction

Development of metastatic breast cancer remains a major cause of death in women. The American Cancer Society estimates predict 287,850 newly diagnosed female breast cancer cases and 43,250 breast cancer related deaths in 2023 (1).

Global gene expression profiling of differentially expressed genes in molecular subtypes of breast cancer has provided valuable endpoint markers to select effective therapeutic options. Based on the gene expression status of hormone and growth factor receptors the major molecular subtypes are classified as Luminal A, Luminal B, HER-2-entiched and triple-negative, respectively (2).

Conventional chemotherapy with multi-drug combination using cytotoxic DNA damaging agents represents a widely accepted option (3). More recently, small molecule-based pathway selective targeted therapy has been used together with conventional chemotherapy. These selective small molecule inhibitors include estrogen receptor modulators, estrogen receptor degraders, cyclin-dependent kinase inhibitors, aromatase inhibitors and poly (adenosine ribose) polymerase inhibitors that are used depending on the specific molecular subtype of clinical breast cancer (4). Collectively, long-term use of these therapeutic options is associated with systemic toxicity, intrinsic/acquired tumor resistant, and emergence of therapy resistant stem cell population that is endowed with cancer initiation, cellular plasticity and metastatic properties.

Natural products such as dietary phytochemicals, nutritional herbs and constitutive bioactive agents have documented human consumption, lack of systemic toxicity, and off-target unfavorable side effects. These advantages may provide an unmet need to investigate natural products as testable therapeutic alternatives against therapy-resistant metastatic breast cancer. It is also notable that several mechanistically distinct natural products have documented efficacy against cancer stem cell population (5-8).

Present review provides a systematic discussion of published evidence relevant to i) Growth inhibitory efficacy of natural products such as dietary phytochemicals and nutritional herbs on cellular models for molecular subtypes of clinical breast cancer, ii) Potential mechanistic leads responsible for

the efficacy of these agents, and iii) Development and characterization of drug-resistant stem cell models for molecular subtypes of clinical breast cancer. Collectively, this review validates multiple mechanism-based preclinical experimental approaches to identify and prioritize efficacious natural products that may represent novel drug candidates for therapy resistant breast cancer. Additionally, published data on the models from human carcinoma-derived cell lines provide a scientifically robust rationale for future investigations on patient-derived cancer explants and organoids. These investigations may minimize extrapolation of preclinical data for their clinical relevance and translatability.

2. Experimental models

Parental cell lines. Human tissue derived tumorigenic cell lines represent valuable cellular models for clinical cancer subtypes. Human breast carcinoma-derived MCF-7 cells represent a model for the Luminal A breast cancer subtype. HER-2 expressing tumorigenic human mammary epithelial cells 184-B5/HER cells represent a model for the HER-2-enriched subtype. Human carcinoma-derived MDA-MB-231 cells represent a model for the triple-negative cancer subtype. Table I details the characteristics of the three parental cell lines.

These cell lines are notable for accelerated cell cycle progression, downregulated cellular apoptosis and anchorage independent colony formation (AICF) *in vitro*, and tumor development *in vivo*. At the mechanistic levels, these growth properties are associated with altered expression status of proteins responsible for signal transduction, cell cycle progression and cellular apoptosis.

Drug-resistant breast cancer stem cells. Cancer stem cells are characterized by resistance to chemo-endocrine or targeted therapy. Table II illustrates the developed breast cancer subtype specific stem cell models. These models are established by positive selection of resistant phenotypes. The resistant phenotypes are isolated by long-term treatment with maximum cytostatic doses of prototypic therapeutic agents. This treatment eliminates drug-sensitive phenotypes and provides selective growth advantage to the drug-resistant phenotypes. The drug-resistant phenotypes are expanded in the presence of positive selective pressure by relevant therapeutics.

The MCF-7/TAM-R model represents the Luminal A subtype that exhibits resistance to the selective estrogen receptor modulator tamoxifen (TAM). The 184-B5/HER-LAP-R model represents the HER-2-enriched model that exhibits resistance to the EGFR/HER-2 inhibitor lapatinib (LAP). The MDA-MB-231/DOX-R model represents the triple-negative breast cancer (TNBC) model that exhibits resistance to the DNA damaging chemo-therapeutic agent doxorubicin (DOX).

Stem cell markers. Tumor spheroid formation represents a specific biological property of cancer stem cells, and is used as a biological marker. In addition, the expression status of cell surface protein cluster of differentiation44 (CD44), and select transcription factors such as DNA binding homeobox transcription factor NANOG and octamer binding transcription factor-4 (OCT-4) together represent specific molecular markers for the cancer stem cell population. The quantitative end points and the assays optimized for the status of stem cell marker expression are detailed in Table III.

The quantitative immuno-fluorescence assay involves flow cytometry-based monitoring of cells stained with relevant fluorescein isothiocyanate (FITC)-conjugated fluorescent antibodies. The data are corrected using FITC-conjugated IgG antibody as isotype control, and expressed as relative fluorescent units (RFU) per 10^4 fluorescent events.

3. Conventional/targeted therapy

The molecular subtypes of clinical breast cancer are classified based on the gene expression status of hormone and growth factor receptors. These molecular characteristics dictate the selection of treatment options. Table IV provides examples of the use of specific conventional/targeted therapy for individual breast cancer subtype.

The major limitations of long-term conventional and targeted chemotherapy include chemo-resistance and emergence of cancer initiating stem cells. These limitations emphasize identification of efficacious testable alternatives.

The term testable alternatives defines natural products such as dietary phytochemicals and nutritional herbs that exhibit growth inhibitory efficacy against breast cancer subtypes. Unlike pharmacological chemotherapeutics used as treatment options, natural products have documented human consumption and low systemic toxicity. Several mechanistically distinct natural products exhibit preclinical efficacy.

4. Natural products

Several mechanistically distinct dietary phytochemicals and nutritional herbs used in traditional Chinese medicine have documented growth inhibitory efficacy in the cellular models for breast cancer subtypes (9,10). Non-fractionated aqueous extracts used as herbal formulations in traditional Chinese medicine are likely to contain multiple potential bioactive agents functioning via targeting specific signaling pathways. These aspects may be responsible for the growth inhibitory efficacy. The dietary phytochemicals are selected based on their documented chemo-preventive efficacy in preclinical models for organ site cancer. The nutritional herbs are selected based on their use in traditional Chinese for general health issues and as palliative treatment of cancer (11). Table V presents efficacious dietary phytochemicals and nutritional herbs, their source and constitutive bioactive agents. The bioactive agents present in herbal formulations may also represent potential testable alternatives as stem cell targeting drug candidates against therapy resistant breast cancer subtypes.

Mechanistic leads for efficacy. Distinct mechanistic leads for efficacy may be operative.in individual breast cancer subtype. The sequence of rank order is specific for individual quantitative end point. Rank ordering of efficacious test agents provides evidence for relative efficacy of individual test agent. For example, in the MCF-7 model cellular metabolism of estradiol represents a potential mechanism predominantly due to generation of anti-proliferative metabolites (12). In the HER-2-enriched model inhibition of HER-2 signal

Table I. Parental cell lines for breast cancer.

Model	Cellular marker	Clinical subtype
MCF-7	ER/PR positive, HER-2 negative	Luminal A
184-B5/HER	ER/PR negative, HER-2 positive	HER-2-enriched
MDA-MB-231	ER/PR negative, HER-2 negative	Triple-negative

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

Table II. Drug-resistant breast cancer stem cells.

Model	Resistance	Clinical subtype
MCF-7/TAM-R	TAM	Luminal A
184-B5/HER/LAP-R	LAP	HER-2-enriched
MDA-MB-231/DOX-R	DOX	Triple-negative

DOX, doxorubicin; HER-2, human epidermal growth factor receptor-2; LAP, lapatinib; R, resistant; TAM, tamoxifen.

Table III. Stem cell markers.

Marker	End point	Assay
Biological		
TS	TS number	TS formation
Molecular		
CD44	RFU	Antibody uptake
NANOG	RFU	Antibody uptake
OCT-4	RFU	Antibody uptake

CD44, cluster of differentiation 44; NANOG, DNA-binding homeobox transcription factor; OCT-4, octamer-binding transcription factor-4; RFU, relative fluorescent unit; TS, tumor spheroid.

Table IV. Conventional and targeted therapy for breast cancer.

	Therapy	
Clinical subtype	Conventional	Targeted
Luminal A	DOX, PCT, CPT	SERM, SERD, CDKI, AI
Luminal B	DOX, PCT, CPT	SERM, SERD, CDKI, AI, HER-2I
HER-2-enriched	DOX, PCT, CPT	HER-2I
Triple-negative	DOX, PCT, CPT	PARPI

AI, aromatase inhibitor; CDKI, cyclin dependent kinase inhibitor; CPT, carboplatin; DOX, doxorubicin; HER-2, human epidermal growth factor receptor-2; HER-2I, human epidermal growth factor receptor-2 inhibitor; SERD, selective estrogen receptor degrader; SERM, selective estrogen receptor modulator; PARPI, poly adenosine (ribose) polymerase inhibitor; PCT, paclitaxel.

transduction may represent one of the potential mechanism. In addition, most of the natural products induce cellular apoptosis. The process of apoptosis is associated with modulated expression of anti-apoptotic BCL-2, and/or of pro-apoptotic BAX, and induction of pro-apoptotic caspases (13). In the TNBC model the growth inhibitory efficacy of nutritional herbs is associated inhibition of RB, RAS, PI3K and AKT mediated signaling pathways (10).

5. Effects of natural products

Compared to the non-tumorigenic cells, hyper-proliferative tumorigenic cells exhibit accelerated cell cycle progression and anchorage independent colony formation (AICF). Anti-proliferative effects of natural products are commonly seen as inhibition of cell cycle progression and lack of cell-substrate adhesion leading to AICF. The latter property represents an *in vitro* marker for tumorigenic cells and exhibits a positive correlation with tumor development *in vivo*. Thus, AICF represents an *in vitro* surrogate end point for tumorigenic cells. The rank order of efficacy is distinct for individual end point biomarker. Thus, rank order provides mechanistic evidence for relative potency of test agents.

The results summarized from published primary data (9) illustrate that treatment with phytochemicals reduces AI colony number, exhibiting a rank order sequence of carnosic acid (CA)=ursolic acid (UA) > carnosol (CSOL) > genistein (GEN)=epigallocatechin gallate (EGCG)=indole-3-carbinol (I3C). Reduction in the number of AI colonies in response to treatment with nutritional herbs exhibits a rank order sequence of *Dipsacus asperoides* (DA) > Cornus officinalis (CO)=*Lyceum barbarum* (LB)=*Epimedium grandiflorum* (EG) > *Tabebia avellanedae* (TA)=*Psoralia corylifolia* (PC) (10). Thus, data on inhibitory efficacy of natural products on AICF provide a rationale for subsequent experiments to identify potential mechanistic leads for natural products.

The anti-proliferative effects of natural products are associated with modulation of distinct mechanistic pathways in models for molecular subtypes of breast cancer. These pathways include estrogen metabolism in Luminal A, HER-2 signaling in HER-2-enriched, and RB signaling in TNBC subtypes.

Cellular metabolism of estradiol. 17β-estradiol (E_2) functions as a physiological ligand for estrogen receptor mediated multi-step signal transduction cascade that culminates in

Table V. Growth inhibitory natural products.

Natural product	Origin/source	Bioactive agent
Dietary phytochemical		
CA	Rosemary, leaf, stem	Terpene
CSOL	Rosemary, leaf, stem	Terpene
EGCG	Green tea, leaf	Polyphenol
GEN	Soy	Isoflavone
I3C	Broccoli, cabbage	Diindolyl methane
UA	Rosemary, leaf, stem	Terpene
Nutritional herb		
CO	Fruit	Anthocyanin
DA	Root	Saponin
EG	Leaf	Prenylflavone
LB	Fruit, bark	Flavone, lignin, tanin
PC	Seed	Terpene
TA	Bark	Naphthofuran dione

CA, carnosic acid; CSOL, carnosol; EGCG, epigallocatechin gallate; GEN, genistein; I3C, indole-3-carbinol; UA, ursolic acid; CO, *Cornus officinalis*; DA, *Dipsacus asperoides*; EG, *Epimedium grnadiflorum*; LB, *Lyceum barbarum*; PC, *Psoralia corylifolia*; TA, *Tabebuia avellanedae*.

the activation of E_2 target genes. This signal transduction is essential for the cellular and molecular effects of E_2 (14,15). In addition to this genomic mechanism, cellular metabolism of E_2 plays a significant role in breast carcinogenesis. E_2 metabolites have divergent growth modulatory effects on breast cancer cells. E_2 is converted to estrone (E_1) via the C17-oxidation pathway and E_1 functions as a common precursor for the formation of 2-hydroxyestrone (2-OHE_1) via C2-hydroxylation pathway, and the formation of 16α-hydroxyestrone (16α-OHE_1) via the C16-hydroxylation pathway (16,17).

The methodology for determining the formation of E_2 metabolites includes the tritium exchange assay wherein the cells are treated with stereo-specifically labelled [^{3}H] E_2, and the formation of [^{3}H] H_2O is measured. The data are expressed as radioactive counts of [^{3}H] per 10^6 cells. Additionally, the identity of individual metabolites is confirmed using the GC-MS assay. The data for the formation of individual metabolite are expressed as ng per 10^6 cells.

The 2-OHE_1 metabolite of E_2 has documented anti-proliferative effects, while 16α-OHE_1 metabolite exhibits growth promoting effects in the MCF-7 model (18,19). Distinct growth modulatory effects of these metabolites are commonly expressed as 2-OHE_1: 16α-OHE_1 ratio. Published evidence has suggested that several nutritional herbs alter the metabolite ratio favoring formation of anti-proliferative 2-OHE_1. The data summarized from published results (12) illustrate that in the ER positive MCF-7 model select nutritional herbs increase the 2-OHE_1: 16α-OHE_1 ratio, exhibiting a rank order sequence of EG>LB>CO.

At the molecular levels, mechanistic leads identified for the growth inhibitory efficacy of non-fractionated aqueous extract prepared from the inner bark of Tabebuia avellanedae (TA) on the MCF-7 model include inhibited expression of several genes responsible for cell proliferation, modulated expression of apoptosis related genes and upregulated expression of genes related to CYP1A1 and CYP1B1 mediated cellular metabolism (20).

The peripheral estrogen biosynthesis during menopause via aromatase results in formation of E_2 from testosterone and E_1 from androstene dione. In post-menopausal aromatase expressing breast cancer small molecule inhibitors of aromatase including letrozole (LET) and exemestane (EXM) are used as pharmacological inhibitors of aromatase activity. Preclinical studies on aromatase expressing MCF-7^{AROM} cells have provided evidence that resistance to individual inhibitor is associated with cross-resistance to other inhibitors (21), and LET-resistant MCF-7^{AROM} phenotype exhibits upregulated expression of HER-2 (22). In the MCF-7^{AROM} model treatment with TA results in potent aromatase inhibitory activity and reduced expression of select estrogen target genes including ESR-1, PS2, GRB2 and cyclin D1 (23). Additionally, based on the content of its bioactive agent Naphtho-furan dione (NFD), TA exhibits greater potency for aromatase inhibition than either LAT or EXM.

HER-2 signaling. In Luminal B and HER-2-enriched breast cancer subtypes HER-2 expression is positively correlated with tumor growth and downregulated response to conventional chemotherapy. In these subtypes HER-2 targeted therapy is commonly used. Post-translational modification resulting in phosphorylation of growth factor receptor is essential for the activation of the signal transduction pathway. The expression of phosphorylated HER-2 (pHER-2) or phosphorylated epidermal growth factor receptor (pEGFR) is variable, while that of HER-2 or EGFR remains essentially unaltered (24-26). The status of activation of EGFR and HER-2 is commonly expressed as the ratio of phosphorylated protein: total protein. The data summarized from published results (9) suggests that select dietary phytochemicals inhibit the pHER-2: HER-2 ratio with a rank order sequence of UA=CA>CSOL>EGCG in the 184-B5/HER cells, a model for HER-2-enriched breast cancer.

RB signaling. The tumor suppressor RB gene is essential for regulation of cell cycle progression via the G_1 to S phase transition and functions via the cyclin D1-CDK4/6-pRB-E2F axis. The post-translational modification of RB via phosphorylation is essential for the signal transduction process (27,28). The TNBC molecular subtype of breast cancer is notable for defective tumor suppressor function of the RB gene, and pRB status represents a marker for RB signaling. During active signal transduction the expression of pRB is altered, while that of total RB remains essentially unchanged. Thus, pRB: RB ratio represents an important marker for activation of RB signaling pathway (29,30). The results summarized from published primary data (10) illustrate that select nutritional herbs inhibit pRB: RB ratio exhibiting a rank order sequence of DA=PC>CO, and thereby, may reduce the defective RB function in the MDA-MB-231 model for TNBC.

Cellular apoptosis. It is well-established that in the intrinsic mitochondrial pathway of cellular apoptosis, altered membrane permeability, cytochrome-c release, apoptosome-mediated activation of caspase-9 and subsequently of caspase-3/7, and reciprocal expression of anti-apoptotic BCL-2 and of pro-apoptotic BAX are critical for the apoptotic process (31,32). The pro-apoptotic effects of natural products are commonly seen as increase in the sub G_0 (apoptotic) phase of the cell cycle and modulation of several regulatory pathways. Phytochemicals in the HER-2-enriched model increase the number of cells in Sub G_0 phase, decrease BCL-2 expression and increase BAX expression, thereby reducing BCL-2: BAX ratio (9). Nutritional herbs in the TNBC model increase apoptotic cells, reduce BCL-2: BAX ratio and induce caspase 3/7 activity (10,33).

6. Drug-resistant cancer stem cells

The stem cell population plays important roles in target organ sites, as well as in cancers developing in the target sites. In epithelial organ sites the stem cells regulate normal cell proliferation, differentiation and apoptosis required for cellular homeostasis during organ regeneration. These processes are regulated by Wnt/β-catenin, Hedgehog and Notch signaling pathways (34). In the cancer stem cells these regulatory pathways are disrupted, and cell survival pathways are activated via RAS, PI3K, AKT and mTOR signaling (35), thereby, providing growth advantage to the chemo-resistant cancer cell phenotype. The common and unique characteristics of normal and cancer stem cells represent the basis for development of reliable cancer stem cell models. Intrinsic or acquired resistance to long-term conventional and targeted therapy results in emergence of drug resistant cancer stem cell population. Development of reliable cancer stem cell models provides valuable experimental approaches to identify efficacious new drugs that target chemo-resistant stem cells. The methodology for isolation of drug resistant stem cells essentially involves long-term treatment with select therapeutic agents, and selection and expansion of surviving cell population in the presence of the therapeutic agent. The resistant phenotypes are examined for the status of select stem cell markers.

Tumor spheroid formation. Tumor spheroid (TS) formation represents a specific biological marker for stem cells. The results summarized from published primary data illustrate that TS number is substantially increased in the TAM-resistant, LAP-resistant and DOX-resistant phenotypes.

Molecular markers. Nuclear transcription factors such as OCT-4, Klf-4, SOX-2, c-Myc and NANOG represent essential factors for the maintenance of stem cell population (36,37). In addition to TS select cell surface proteins such as cell differentiation proteins CD44, and CD133 and transcription factors including NANOG and OCT-4 also represent sensitive and specific molecular markers for drug-resistant cancer stem cells (38). Commonly used methodology for quantification of these markers involves monitoring of the cellular uptake of relevant fluorescently labeled antibodies.

The specificity and sensitivity of molecular markers of stem cells represent important aspects for characterization of drug-resistant stem cells. The results summarized from published primary data (38) illustrate that TAM-R, LAP-R and DOX-R phenotypes exhibit upregulated expression of CD44, NANOG and Oct-4, relative to the respective drug sensitive phenotypes.

An overview of substantial body of published evidence suggests that natural products exhibit growth inhibitory efficacy via multiple mechanisms. Mechanisms of action relevant to dietary phytochemicals and nutritional herbs, susceptible models for breast cancer subtypes and preclinical evidence for growth inhibitory effects are summarized in Table VI.

Dietary phytochemicals are tested for their efficacy in Phase I and Phase II randomized clinical trials on patients at risk for breast cancer and those with diagnosed breast cancer. Selection of these agents is based on their documented mechanisms of action from preclinical studies. The clinical evidence is summarized in Table VII.

In preclinical and clinical efficacy of dietary phytochemicals susceptibility of signaling pathways relevant to estrogen receptor, HER-2, MAPK, PI3K, AKT, ERK, RB, Wnt/β-catenin, and NFkB signaling represent mechanistic pathways to identify potential molecular targets (10,39,40). Additionally, glutamine metabolism and one-carbon metabolism has been reported as susceptible pathways in the TNBC subtype (41). The pro-apoptotic effects of natural products have been associated with modulated expression of BCL-2, BAX, c-jun and PARP (39,42).

It is noteworthy that efficacy of individual bioactive agents present in nutritional herbs commonly used in traditional Chinese medicines is essentially unknown. However, the stem cell targeting efficacy of dietary phytochemicals and Chinese nutritional herbs has been reported (5-8,43).

Preclinical and clinical efficacy of dietary phytochemicals via multiple signaling pathways (39), cancer stem cell targeting efficacy of Chinese medicines and dietary phytochemicals (5,43) and combinatorial efficacy of dietary phytochemicals with drug candidates (44) provide valuable leads for clinical relevance and translatability of natural products for breast cancer therapy.

7. Conclusions

This review provides a systematic analysis of published evidence development of cellular models for select molecular subtypes

Table VI. Growth inhibitory efficacy of natural products in preclinical studies.

First author/s, year	Natural product	Model	Mechanism of action	(Refs.)
Telang, 2022	CO, LB, EG	Luminal A; MCF-7	Cell cycle arrest, estrogen metabolism	(12)
Telang, *et al*, 2019	TA	MCF-7^{AROM}	Cell cycle arrest, apoptosis, aromatase, ESR-1, PR, AROM, cyclin D1, PS2, GRB2, BCL-2, BAX	(23)
Telang, 2020	CA, CSOL, GEN, I3C, UA	HER-2-enriched; 184-B5-HER	G_1 arrest, apoptosis, BCL-2, BAX	(9)
Telang *et al*, 2021	CO, DA, PC	TNBC; MDA-MB-231	CO, PC: Cell cycle arrest, apoptosis, cyclin D1, RB, BCL-2, BAX; DA: Cell cycle arrest, apoptosis, RB, RAS, PI3K, AKT	(10)
Muniraj *et al*, 2019	CUR, EGCG, GEN, RES	Breast cancer cell lines	CUR: Apoptosis, AKT, mTOR, PARP, caspase; EGCG: Apoptosis, BAX, PARP, caspase; GEN: Apoptosis, BCL-2, ATM; RES: Apoptosis, BCL-2, BAX, caspase	(39)

CA, carnosic acid; CSOL, carnosol; CO, *Cornus officinalis*; CUR, curcumin; DA, *Dipsacus asperoides*; EGCG, epigallocatechin gallate; EG, *Epimedium grandiflorum*; GEN, genistein; HER-2, human epidermal growth factor-2; I3C, indole-3-carbinol; LB, *Lyceum barbarum*; PC, *Psoralia corylifolia*; RES, resveratrol; TA, *Tabebuia avellanedae*; TNBC, triple negative breast cancer; UA, ursolic acid; ESR-1, gene for estrogen receptor-α; PR, progesterone receptor; AROM, aromatase; PS2, estrogen responsive gene; GRB2, growth factor receptor binding protein; BCL-2, B cell lymphoma-2; BAX, BCL-2 associated X protein; RB, retinoblastoma; RAS, Rous sarcoma; PI3K, phospho-inositol-3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; PARP, poly adenosine (ribose) polymerase; ATM, Ataxia telangiectasia mutant.

Table VII. Growth inhibitory effects of natural products in clinical studies.

First author/s, year	Natural product	Breast cancer type/study cohort	Phase I and phase II randomized trial NCT identifier	(Refs.)
Muniraj *et al*, 2019	CUR	HER-2-negative metastatic breast cancer	NCT00852332	(39)
Muniraj *et al*, 2019	EGCG	Hormone receptor-negative breast cancer	NCT00516243	(39)
Muniraj *et al*, 2019	GEN	Patients with documented high risk and patients with diagnosed breast cancer	NCT00290758	(39)
Muniraj *et al*, 2019	RES	Patients with diagnosed breast cancer	NCT03482401	(39)

CUR, curcumin; EGCG, epigallocatechin gallate; GEN, genistein; RES, resveratrol; HER-2, human epidermal growth factor-2; NCT, National Clinical Trial.

of clinical breast cancer and of chemotherapy-resistant cancer stem cell models. Effective pathway selective targeted therapy depends on specific molecular subtype of breast cancer. For example, the Luminal A sub type responds to estrogen receptor modulators, receptor degraders and aromatase inhibitors. Luminal B subtype responds to endocrine inhibitors and HER-2 targeted therapy. TNBC subtype responds to only PARP inhibitors. The limited therapeutic response of TNBC emphasizes discovery of therapeutic alternatives (45). Nutritional herbs widely used in traditional Chinese medicine may target multiple signaling pathways functional in hyper-proliferative breast cancer (46,47). Published evidence has documented susceptibility of the parental cells to growth inhibition by mechanistically distinct natural products such as dietary phytochemicals and Chinese nutritional herbs. Collectively, these aspects suggest that bioactive agents from the natural products may represent testable new drug candidates for stem cell targeted therapy against clinical breast cancer. In the drug discovery program prioritization of new drug candidates includes high-throughput screening assays to identify effective candidates, genomic, transcriptomic and metabolomic analysis to identify molecular targets, and tumor inhibitory efficacy for anti-cancer properties. These directions are essential for identification of new drug candidates.

8. Future research

Human tissue-derived cellular models for breast cancer provide valuable experimental approaches for the understanding of cellular and molecular aspects of breast carcinogenesis and for chemotherapeutic efficacy of drug candidates from natural products.

Resistance to conventional and targeted therapy and cross-resistance between individual therapeutic agents (21,22) represent a formidable challenge for treatment options, and thereby, emphasizes identification of testable alternatives functioning independent of therapy resistance. In this context, the following published evidence may represent scientifically robust rationale for future studies.

The human telomerase reverse transcriptase (hTERT) represents a universal marker for immortalized cancer cells and for cancer initiating stem cells that is independent of therapy resistance (48,49). This enzyme adds a hexameric repeat of 5' TTAGGG 3' sequence to telomeres on the chromosomal ends, Thus, hTERT represents an attractive cancer therapeutic target for natural products and pharmacological compounds (50-52).

Epigenetic modifications impact transcriptional activity via nuclear histones, DNA methyltransferases and promoter methylation. Small molecule pharmacological inhibitors or natural products functioning as epigenetic modifiers (53) may be effective in therapy-resistant cancer stem cells.

The epithelial-mesenchymal transition (EMT) characterizes cellular plasticity in cancer stem cells. This process is associated with reciprocal modulation of epithelial specific cytokeratins, cadherins and mesenchymal specific vimentin proteins. The expression status of transcription factors SNAIL, SLUG and ZEB also plays an important role in metastatic progression of therapy-resistant cancer stem cells (54,55). Additionally, inhibitors of NFkB signaling pathway and modifiers of the JAK/STAT signaling pathway (56) may represent testable drug candidates.

However, evidence from carcinoma derived cell lines is strongly dependent on extrapolation for its clinical relevance and translatability. This limitation can be reduced by investigations using patient derived tumor explants (57), and organoids (58-60) obtained from therapy resistant cancer patients. These future research directions may provide clinical relevance and translatability.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

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

Authors' contributions

NTT reviewed the published data and prepared the manuscript. Data authentication is not applicable. The author read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The author declares that they have no competing interests.

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