2025 Volume 10, Issue 1
Journal of Medicines Development Sciences
ISSN: 2382-6371(Online)
ISSN: 2382-6363(Print)

Research Progress of Breast Cancer Organoids and Application Prospect of Traditional Chinese Medicine

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Abstract:

Breast cancer is a highly heterogeneous malignancy that includes various subtypes differing in genetics, pathology, clinical treatment, and prognosis. The conventional breast cancer cell line research model does not fully align with the actual pathological process of clinical tumor pathogenesis and the characteristics of tumor heterogeneity. Breast cancer organoid, a model based on the 3D cell culture system, reproduces the heterogeneity of the primary tumor while preserving the molecular phenotype and genotype alterations of the patient's tumor. Consequently, it has been utilized in studies on tumor pathogenesis and anti-tumor drug screening. Currently, traditional Chinese medicine research on breast cancer primarily focuses on cell lines and patient-derived tumor xenografts, while organoid-related research remains relatively scarce. This review briefly introduces the advancement of the breast cancer organoid culture system and its application in life science research, aiming to provide insights for breast cancer-related studies involving traditional Chinese medicine.

Keywords:

Breast cancer
Organoids
Research progress
Traditional Chinese medicine
Outlook

Online publication: February 25, 2025

1. Introduction

Breast cancer is the most common malignancy among women, with its incidence rate ranking first among female malignancies [1]. It poses a serious threat to women's health. With the help of molecular biology techniques, the diagnosis and treatment of breast cancer have become increasingly detailed and in-depth. The development of models that align with clinical tumor pathogenesis and tumor heterogeneity is of great significance for the comprehensive treatment of breast cancer. Currently, traditional Chinese medicine research on breast cancer primarily relies on breast cancer cell

lines and patient-derived xenografts (PDXs). Breast cancer cell lines are simple to construct, economical, and allow for high-throughput drug screening, making them widely used in basic breast cancer research and drug sensitivity studies [2]. However, due to their lack of heterogeneity, cancer research often tends to be one-sided. Additionally, after thousands of generations of subculturing and circulation through numerous laboratories, the cellular genome [3] and behavior deviate from their original state [2–4]. Although PDXs can maintain a high degree of intra-tumor heterogeneity and molecular diversity, they have a long research cycle, low transplantation success rate, and are not suitable for high-throughput drug screening [4–6]. Breast cancer organoids refer to three-dimensional cell complexes formed by utilizing 3D culture techniques on fresh breast tumor tissue obtained during surgery. Compared to traditional tumor models, they share structural and functional characteristics similar to the source breast cancer tissue [7], effectively maintaining the heterogeneity of the original tumor. They hold vast potential for basic research and clinical diagnosis and treatment applications of traditional Chinese medicine in preventing and treating breast cancer. This article provides an overview of the development of the breast cancer organoid culture system and its applications in life sciences and traditional Chinese medicine.

2. Research progress of breast cancer organoids

In the 1980s, Roelofs *et al.* [8] took the lead in developing three-dimensional cultures. In 1990, they demonstrated the influence and importance of different extracellular matrices (ECM) on the morphology and function of mouse mammary cells. Lee *et al.* (2007) [9] described a 3D culture protocol for normal and malignant breast cells. Researchers have cultivated long-term intestinal organoids and applied them to organs such as the colon, lungs, prostate, stomach, liver, pancreas, and breasts, forming an organoid biobank [10–15]. Twigger *et al.* (2022) [16] reconstructed the three-dimensional tissue structure of the breast. In 2017, Rosenbluth *et al.* (2020) [17] established a human breast cancer organoid biobank, marking the maturity of the breast cancer organoid culture system.

3. Culture methods for normal breast tissue and breast cancer organoids

Linnemann *et al.* (2015) [18] from Germany adopted the floating collagen gel method to establish a breast organoid model. The breast tissue was shredded, digested, cryopreserved, and filtered to remove tissue fragments and cell aggregates. The cells were planted and cultured in 2D form, made into a gel, and cultured in a normal breast organoid medium (**Table 1**). Type I collagen was chosen as the substrate because it constitutes the main component of the extracellular matrix of human mammary gland (MG) cells and provides characteristics that can be used to simulate different microenvironments. Additionally, it was observed that breast cancer cell lines produced tubular structures in free-floating collagen gels. A study [9] obtained tumor tissue from post-breast cancer surgery patients, and combined mechanical and physical manipulations to isolate normal tissue and acquire breast cancer cells. The tissue fragments were then digested, the supernatant was removed, allowed to stand, and mixed into a homogeneous slurry. The cultured cells were mixed with matrix gel, and placed to form a gel, and the composition of the breast cancer organoid culture medium was prepared (**Table 2**). Simultaneously, it was discovered that neuregulin 1 is a ligand for human epidermal growth factor receptor (HER) tyrosine kinase-3 and -4, which is related to breast development and tumorigenesis. Adding it to the breast cancer organoid culture medium can efficiently generate breast cancer organoids and allow them to expand for up to 20 generations.

Table 1. Components of normal breast organoid culture medium

Name of organoid culture medium	
Advance DMEM/F12	FGF-10
Wnt3a	EGF
Neuregulin 1	B27 supplement
L-Glutagulin	N-acetylcysteine
ROCK inhibitor Y-27632	Primocin
Noggin	Hydrocortisone
Nicotinamide	β-estradiol
TGFβinhibitor A83-01	Forskolin

Table 2. Composition of breast cancer organoid culture medium

Name of organoid culture medium	
Advance DMEM/F12	TGFβinhibitor A83-01
R-Spondin 3	FGF-10
Penicillin/streptomycin	EGF
Neuregulin 1	SB202190
L-Glutagulin	B27 supplement
ROCK inhibitor Y-27632	N-acetylcysteine
Noggin	Primocin
HEPES	FGF 7
Nicotinamide	

4. Research and potential applications of organoids in breast cancer

4.1. Studying the mechanisms of tumor development

The deletion of BRCA1 leads to the development of breast tumors. Wang *et al.* (2019) [19] cultivated breast cancer organoids by constructing a mouse model of BRCA1 breast cancer and found that BRCA1-deficient organoids exhibited epithelial-mesenchymal transition (EMT) progression. Connexin 43 (Cx43) is generally downregulated in human breast cancer tissues [20]. When connexin is overexpressed in cancer cells, tumor growth is slowed, and cells regain some ability to differentiate structures. McLachlan *et al.* (2006) [21] studied the tumor-suppressing mode of connexin in MDA-MB-231 organoids, providing a model for the independent inhibitory effect of gap junction intercellular communication (GJIC) on tumors. Dekkers *et al.* (2020) [22] targeted and knocked out tumor suppressor genes in normal breast organoids, recapitulating the occurrence of breast cancer and increasing understanding of the key drivers in the process of breast tumor development. Bhatia *et al.* (2022) [23] established a biobank of patient-derived organoids (PDOs) for breast cancer, where the organoid models recreated large-scale breast cancer genomes [24–27]. These findings revealed cancer driver genes, providing insights into the mechanisms of breast tumor development.

4.2. Elucidating key mechanisms of tumor metastasis

Cheung *et al.* (2013) [28] determined through cultivating breast cancer organoids that the earliest invading cells were those with high expression of CK14. Breast cancer organoids with CK14 knocked out in mice lost their invasiveness, laying a theoretical foundation for studying the initial mechanisms of breast cancer metastasis. Nguyen-Ngoc *et al.* (2012) [29] used breast cancer organoids to simulate the microenvironment of invasive breast cancer and found that ECM-induced signaling changes can initiate invasion and local dissemination. Cheung *et al.* (2013) [28] discovered that specific cancer cells expressing basal epithelial genes (such as cytokeratin-14 and p63) promote collective invasion in the main subtypes of human breast cancer, indicating that heterologous interactions between epithelial subpopulations underlie collective invasion. Park *et al.* (2019) [30] analyzed the oncogenic functions of breast organoids and identified three splicing factors related to breast cell proliferation and invasion. Diermeier *et al.* (2016) [31] found that organoids grown from malignant and healthy mouse tissues non-coding RNAs lead to reduced branching in tumor-generating organoids, playing a specific role in cancer cell migration [8]. Thus, the above studies have elucidated the mechanisms of breast cancer invasion and metastasis through gene knockout in organoids and simulation of the breast cancer microenvironment *in vivo*.

4.3. Anti-tumor drug screening and development

One of the main uses of breast cancer organoids is for drug testing [8,32], where organoid lines generated from patient samples are exposed to standard treatments for different breast cancer subtypes [10,33]. Most organoids dependent on the human epidermal growth factor 2 receptor (Her-2) are sensitive to Her-2-targeted therapy, while most organoids that do not overexpress the receptor are resistant to it. It is worth noting that despite high receptor expression, some organoids were found to be resistant to Her-2-targeted therapy, indicating the presence of other resistance mechanisms in PDOs, which is consistent with clinical studies. This highlights the value of combining genomic research with *in vitro*

analysis of organoids [14]. Walsh *et al.* (2014) [33] exposed different subtypes of BC/PDOs to corresponding BC drug treatments to predict drug responses and found similar responses to those *in vivo*, confirming the heterogeneity of organoid responses. Chen *et al.* (2021) [34] divided 76 breast cancer organoid cell lines into two groups for drug screening. PDO drug phenotype analysis showed significant variability in PDO responses to drug treatment. Simultaneously, RNA sequencing was performed on 57 breast cancer organoid cell lines using six microtubule-targeted drugs. The results indicated that the response of breast cancer organoids to microtubule-targeted drugs is highly correlated with their transcriptional profiles, enabling the prediction of specific sensitive drugs.

4.4. Drug efficacy and toxicity evaluation

Organoids can predict the therapeutic response of xenografts and measure the antitumor drug response of organoids derived from human tumors [8], while simultaneously testing drug toxicity on patient-matched normal tissues [8,35,36]. Optical metabolic imaging (OMI) [33,37–44] can detect the downregulation of lactate dehydrogenase in breast cancer by trastuzumab, thereby evaluating the response of primary breast tumor organoids to clinical anticancer drugs [45,46]. In terms of drug toxicity evaluation, researchers have demonstrated through microchip cultivation of breast cancer organoids that this method's results in detecting the cytotoxicity of different anticancer drugs are consistent with the traditional MTT method [47].

4.5. Guiding individualized treatment for breast cancer

Organoids provide a valuable platform for studying the mechanisms of drug-genotype correlations, using CRISPR/Cas9 technology to investigate oncogenic transformation and model tumorigenesis [48]. Matano *et al.* (2015) [49] utilized CRISPR/Cas9 to study breast tumors, exploring the response mechanism of PARP inhibitors in brca1-deficient breast tumors. This will aid in investigating the impact of single or multiple genetic events in relevant patient materials.

5. Prospects for the application of breast cancer organoids in traditional Chinese medicine

The transcriptome profile of malignant cells is highly dependent on the microenvironment and the inherent characteristics of tumor cells. By comparing the genomic and transcriptome features of organoids with primary tumors, it can be determined whether organoids are true models of tumor heterogeneity. The morphology adopted by breast cancer cell lines in three-dimensional cultures reflects, at least partially, their gene expression profiles [50]. The emergence of the organoid model system has made it possible to simultaneously observe the morphology and function of organs under the microscope, which is significant for accurately evaluating the efficacy of traditional Chinese medicine, identifying drug targets, and revealing the mechanisms of traditional Chinese medicine formulations, especially compound prescriptions.

Traditional Chinese medicine guides clinical practice through holistic concepts and syndrome differentiation and treatment, acting on the body through multiple pathways, targets, and channels. Organoids, characterized by objectivity, visualization, and precision, utilize stem cells to construct tissue and organ models similar to those in the body *in vitro*. They have made breakthroughs in multiple areas such as simulating disease development, drug efficacy screening, new drug development, organ transplantation, and regenerative medicine [51]. Organoids can recreate the *in vivo* microenvironment, evaluate drug efficacy, detect changes in indicators in real-time, discover potential targets and pathways, and simultaneously explore and validate mechanisms of action. This aligns with the holistic approach and multi-target effects of traditional Chinese medicine, better reflecting its holistic diagnostic and therapeutic characteristics. Currently, the establishment of organoid biobanks provides a new approach to optimizing traditional Chinese medicine formulations, predicting adverse reactions of traditional Chinese medicine in humans [52], and guiding precise treatment with traditional Chinese medicine. It can be used not only for evaluating and optimizing formulations for specific diseases but also as an optimal model for precision medicine in individual patients [53].

Organoids play a crucial role in the research of traditional Chinese medicine for breast tumors. Koval *et*

al. (2018) [54] discovered that a plant from the Myrtaceae family in Cameroon, known as water peach, exhibits potent activity in breast cancer organoids by regulating cancer cells through the inhibition of the Wnt pathway. Phan *et al.* (2020) [55] found that an extract from the fragrant grass plant can enhance the therapeutic effect of chemotherapy drugs on breast cancer through hypoxia activation in a breast cancer organoid model. Ye *et al.* (2022) [56] revealed that resveratrol inhibits the proliferation of breast cancer cells by activating STAT3 in breast cancer organoids. Zhang *et al.* (2023) [57] discovered that YH677, a lead compound derived from the natural product harmine (HM), inhibits the expansion of CSCs by modulating the TGFβ/Smad signaling pathway. In a PDO model, it exerts inhibitory effects on the growth and metastasis of TNBC by inhibiting the TNBC/EMT process in a dose-dependent manner. Liu *et al.* (2023) [58] identified sulforaphane, the main active component of the traditional herbal medicine radish, as a natural small molecule antagonist of CRM1. It demonstrates significant activity against breast cancer stem cells by inhibiting the STAT3 signaling pathway in breast cancer organoids while protecting normal breast tissue cells. Lin *et al.* (2023) [59] discovered through a breast cancer organoid model that opamatin, a natural berberine-type alkaloid isolated from *Cortex phellodendri*, affects the progression of breast cancer by intervening in the PI3K/AKT, MAPK, and VEGFA-VEGFR2 pathways. Shan *et al.* (2023) [60] found that the ethanol extract of a medicinal fungus, a triterpenoid compound from the genus Inonotus, exerts anti-breast cancer activity by inhibiting the JAK2/STAT3 signaling axis in breast cancer organoids. Deng *et al.* (2022) [61] intervened in malignant tumor PDOs with cycloastragenol (CAG), an effective active molecule from Astragalus, and found that CAG combined with PD-1 antibodies enhances the tumor-killing ability of CD8+ T cells in organoids. Xu *et al.* (2021) [62] added a small molecule compound, atractylenolide 1 (ATT-1), from the traditional herbal medicine *Atractylodes macrocephala*, to genetic mouse models and PDO models of malignant tumors. They discovered that ATT-1 can enhance the activity of immune proteasomes, promote the presentation of tumor antigens to CD8+ T cells, and improve the efficacy of anti-PD-1-based immunotherapy. Celastrol (CEL) is a natural pentacyclic triterpenoid compound isolated from *Tripterygium wilfordii* [63]. By inhibiting STAT3, CEL suppresses the activity of tumor organoids, exhibiting potent anti-tumor effects. CEL has the potential to become a promising STAT3 inhibitor in cancer treatment. Fan *et al.* (2023) [64] used an organoid model of cardiac hypertrophy to evaluate the mechanistic and pharmacodynamic effects of the traditional Chinese medicine extract Guanxinning injection. Chen *et al.* (2019) [65] established intestinal organoids and showed that glycyrrhetinic acid increases the levels of human antigen R and the downstream proliferation-related nuclear antigen Ki67 to promote the development of intestinal organoids and maintain intestinal homeostasis. The establishment of colorectal cancer organoids has led to the identification of celastrol as a potent inhibitor of organoid growth, surpassing the positive drug L-OHP [66]. The concepts of traditional Chinese medicine treatment and organoids align in terms of individualized and precise treatment, and the vast diversity of traditional Chinese medicines provides a rich selection for organoid research.

6. Conclusion

Effective simulation of cellular functions and diseases is crucial for clinical medical research. Organoids are a promising tool, particularly for the study of malignant tumor diseases, where they offer significant advantages over two-dimensional cell lines and PDX models. Currently, organoids have made considerable progress in exploring the development, metastasis mechanisms, and key genes and cells involved in breast cancer. However, basic research on breast cancer tumor microenvironments using organoids is still relatively limited and could be further explored to more realistically reflect the pathophysiological changes that occur in the tumor microenvironment during the onset and progression of breast cancer [67]. The current standard treatment for cancer targets cancer cells, and research on the tumor microenvironment can contribute to further improvements in cancer therapy by directly targeting regulated cell populations in the tumor microenvironment [68]. This provides new insights for clinical drug treatment and new drug development [69]. It also has broad applications in the screening of anti-breast cancer drugs, new drug

development, evaluation of efficacy, and identification of toxicity, which can benefit clinical patients by promoting tumor dissipation, reducing surgical risks, decreasing the likelihood of postoperative recurrence and metastasis, and effectively extending patients' survival [70].

In the field of Chinese herbal medicine, research on breast cancer organoids is relatively scarce. Studies on single herbs and their active components have focused on the tumor-killing effects of breast cancer tumor organoids. Future research could explore pathway blockade, mechanism regulation, and protein synthesis intervention. Additionally, Chinese medicine-containing sera, freeze-dried powders of Chinese medicines, and Chinese medicine pairs and compounds, as external intervention methods for tumor treatment in traditional Chinese medicine, have not been systematically studied in breast cancer-related organoids [71]. These approaches could provide new insights for basic and clinical research on the intervention of Chinese medicine in breast cancer organoids and offer objective evidence for selecting Chinese medicine prescriptions. It is important to note that the dosages and administration methods of Chinese medicines in organoid experiments need further validation to guide future research.

Research on breast cancer organoids in traditional Chinese medicine is still in its infancy, and related studies are relatively limited. Organoids can be used to construct pathophysiological disease models that more closely resemble the real-world onset of breast cancer (BC) in patients. This allows for the identification of potential therapeutic targets, mechanisms of action, and signaling pathways, providing more realistic experimental data to support the prevention and treatment of breast cancer with traditional Chinese medicine and accelerating related research. Traditional Chinese medicine emphasizes the connection between internal and external organs, as well as the relationship between the superficial and internal aspects of the body, in the diagnosis and treatment of breast diseases. It focuses on overall regulation while addressing breast health, which aligns with the hormonal regulation axis of thyroid-breast-uterus in Western medicine. This provides a scientific basis for the "syndrome differentiation and treatment" approach in traditional Chinese medicine and could play a significant role in studying the characteristics and mechanisms of breast cancer syndromes in traditional Chinese medicine. However, it's important to note that organoids are still *in vitro* models that lack blood vessels, nerves, immune systems, and their interconnectedness as seen in the human body [72]. The syndromes of breast cancer in traditional Chinese medicine represent a series of pathological changes, including disease etiology, location, progression, and the relationship between pathogenic and healthy factors, at a certain stage of breast tumor development. They are a generalization guided by the "holistic concept" of traditional Chinese medicine, and there are inherent differences between the two. Additionally, for studies on the improvement of immune function by traditional Chinese medicine, individual organoid construction cannot simulate the immune status of the body. Therefore, combining organoid chip technology to mimic hormonal regulation axes and simulate physiological and pathological functions as well as immune responses in breast cancer states, along with the guidance of the holistic concept of traditional Chinese medicine in clinical diagnosis and treatment, provides possibilities for holistically evaluating the scientific basis of traditional Chinese medicine in the treatment of breast cancer [73].

Funding

National Natural Science Foundation of China Youth Fund, "Mechanism Study of Xianling Lianxia Recipe Upregulating GPX1 to Induce Immunogenic Cell Death and Synergistically Enhance PD-L1 Therapy for Triple-negative Breast Cancer" (Project No.: 82205114)

Disclosure statement

The authors declare no conflict of interest.

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