Organoids In Personalized Breast Cancer Treatment

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Introduction

Definition of Cancer

Cancer is a disease in which abnormal tissue divides uncontrollably and affects healthy tissue nearby. Cancer can emerge in various body parts, such as the skin, tissue, blood, immune system, and brain (NCI). Breast cancer (BC) is the most commonly diagnosed cancer and the leading cause of cancer-related death among women worldwide (Yang & Liu, 2020). It affects 1 in 8 women in the US, affecting approximately 12% of the female population (Srivastava, 2020). Cancer is a dynamic disease that constantly changes at the genotypic level, such as its observable structure or arrangement of genetic information. These factors make it notably difficult to find treatment for BC (Campaner, 2020). There are also many kinds of breast cancers, so treatments that are effective against some cancers may be ineffective against others. Previous cancer research methods included patient-derived xenografts, tissue or organ transplants from a donor, and mouse models. Their main strengths were the ability to test the efficacy of drugs and gauge tumor cell responses. However, these models are ineffective and labor-intensive compared to organoids.

Definition of Organoids

Organoids are three-dimensional, self-organized structures that mimic organs and their function in vitro. They are derived from human tissue cells and artificially grown in the lab to be stored in biobanks. Organoids give scientists a more accurate view of how their in vivo counterparts grow, function, and react to different treatments. They better mimic their parent tissue's cellular and physical architecture than their in vitro counterparts.

Scientists studied BC in many ways before introducing the organoid model. Spheroids were developed in the 1980s and were the earliest three-dimensional cell culture form. They paved the way for organoids and served as its predecessor for a few decades, replicating natural cell responses and complexities. However, they cannot self-assemble or regenerate, thus making them unadvanced in comparison to organoids. Unlike spheroids, which are simple clusters of broad-ranging cells, organoids contain clusters of organ-specific cells that can accurately resemble organs in structures and function. This is critical in explaining why spheroids have arguably low tumor mirroring complexity, but organoids can accurately display tumor morphology (Neely, 2016)

Using patient-derived organoids, scientists have found a new ethical and revolutionary alternative to personalize BC treatment with the same benefits as previous models while overcoming the previous models' limitations. Scientists first implemented this method by developing BC organoids from extracted BC tissue from patients. These patient-derived organoids, abbreviated as PDOs, are then put through drug screenings and other tests to deduce the most effective therapy for an individual (Figure 1).

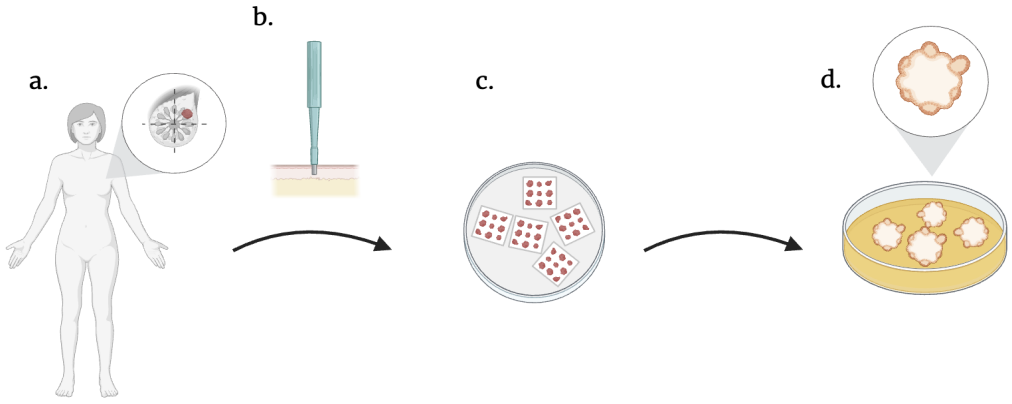

Figure 1. Overview example of breast cancer organoid generation. a) A breast cancer patient gives informed consent for a biopsy. b) The needle biopsy is performed. c) The collected tissue biopsy samples are stored in a biobank for research. d) Breast cancer tissue organoids are generated using the biobank samples and stored for future testing (Created with Biorender).

The main advantage of BC PDOs is that they are proven to maintain the tumor's original genotype and biological characteristics. PDOs have immense potential to provide BC patients with the appropriate treatment because of their ability to recapitulate tumor morphology, serving as the most reliable and intuitive model for individualized cancer research (Signati, 2021).

Definitions & Literature Review

Existing Experimental Models and Treatments For Breast Cancer

Traditionally, BC research uses patient-derived xenograft (PDX) mouse models and cell lines. However, this method has cross-contamination issues, and genetic information is difficult to retain in the passage process (Yu & Huang, 2020). PDX mouse models, in particular, are inefficient, labor-intensive, and take multiple months to develop, which makes it challenging to contribute to personalized therapy (Sachs, 2018). Many scientists have also raised concerns due to its unethical nature and how mice and their biological processes do not accurately reflect those of a human (Srivastava, 2020). Along with experimental models, scientists have also worked on several BC treatments, systemic chemotherapy, and endocrine therapy being the most widely used forms. Unfortunately, the main caveat of these methods is that they cause patients to develop recurring diseases and resist the initial treatment (Chen, 2021). Unlike previous methods and treatments, organoid models require less tissue, exhibit a high success rate, and avoid ethical disputes, making organoids a more optimal approach to individualized breast cancer therapy. (Yu and Huang, 2020).

Organoids in Research: Past & Present

History of Organoids

In 1907, scientists Henry Van and Peters Wilson established that manually isolated sponge cells could regroup and self-organize to produce whole organisms. This core discovery of cellular biology revealed the existence of stem cells, forming the foundation of the ideas that led to the development of the organoid. Since 1987, scientists have developed several 3D culture systems, utilizing stem cells to produce organoids that mimic organs and their biological processes. Around this time, organoid techniques for epithelial structures such as brain, lung, pancreatic, prostate, and breast organoids were developed (Yang & Liu, 2020). Breast organoids were heavily experimented on during this revolutionary cellular biological research period. For example, Li et al. demonstrated that breast epithelia could form 3D ducts and lumen when grown on a hydrogel to induce cell proliferation and promote tissue formation. These epithelia appeared to synthesize and secrete milk protein as opposed to two-dimensional (2D) cultures (Corrò, 2020, as cited in Li, 1987).

The Cultivation of Present Breast Cancer Organoid Models

Organoids can replicate genetic diseases, infectious diseases, parasitic infections, and cancer. Modeling cancer has been a primary focus of scientists over the last decade, creating organoid models derived from epithelial tumors (Pernik, 2021). Recently, breast cancer organoids have been studied and cultured using a method similar to other epithelial structures. BC organoids are created by extracting samples of human mammary tissue. The tissue removed is either healthy or cancerous, and the results of various tests can determine this. These tissues are then isolated through mechanical disruption and enzymatic digestion (Sachs, 2018). Developing BC PDOs from cultivation to testing takes 4-6 weeks (Yu & Huang, 2020). Once the organoid samples are created, they are stored in biobanks and put through drug screenings. In most cases, tissue samples are cut and treated with collagenase, an enzyme that cleaves the peptide bonds in collagen to release cells for research and help healthy tissue grow. They are then suspended and plated in Matrigel, a matrix gel used frequently in co-culture to mimic the tumor (Campaner, 2020). By creating and treating these organoids, scientists can conduct clinical trials to assess tumor sensitivity to specific drug treatments (Figure 2). PDOs serve as a concurrent platform to explore treatments for BC in a cost-efficient and ethical manner.

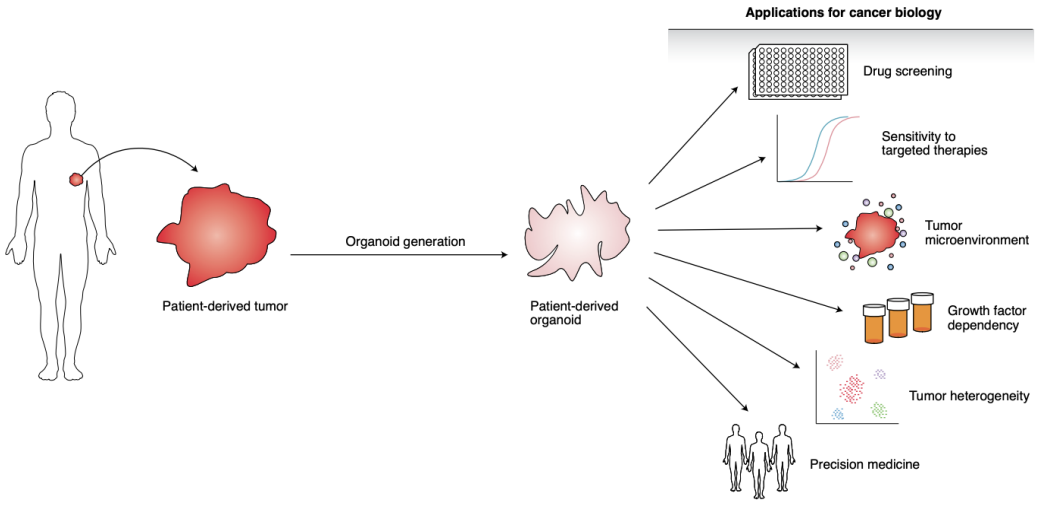

Figure 2. Cancer modeling in patient-derived BC organoids. Tumor-derived organoids from human patients have numerous applications for revolutionary cancer research. Potential uses include screening drugs and tumor sensitivity to targeted therapies, studying tumor microenvironment and heterogeneity, and its applications in precision medicine and response prediction (Lo, Kasper, & Kuo, 2020).
Note. Adapted from “Applications of organoids for cancer biology and precision medicine” by Lo, Karlsson, and Kuo, 2020.

Presentation of Evidence & Discussion of Data

Organoids Express Biomarkers For Breast Cancers

The majority of research findings to date affirm that organoids recapitulate BC tumor morphology. In a recent study, hematoxylin and eosin (H&E) staining was performed to compare the morphological features of organoids and original tumors. Staining results showed that the most common biomarkers for breast cancer - estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) - matched the parental tumors (Chen, 2021). Another study by Yu and Huang et al. reported that if a tumor sample contained over 5% of tumor stem cells, the organoid culture obtained 100% efficiency and expressed the exact biomarkers of the patient’s corresponding tissue (Yu & Huang, 2020). This statement is also supported by an analysis by Rosenbluth et al., which compared human and mouse cancer-prone BC tissue. Mammary epithelial cells are typically grouped into luminal and basal categories based on their location relative to breast tissue. These tissues are called stromal cells, which are components of the breast's connective tissue. Using standard markers to define stromal cells, they confirmed that the human major mammary lineages were preserved in culture, unlike the luminal and basal mouse mammary epithelial types, which presented differences in their organoid culture rather than matching the histopathology (Rosenbluth, 2020). Bhatia et al. developed a biobank of normal and cancerous BC organoids derived from patients

of various backgrounds. The biobank includes samples from patients of various ethnicities, races, ages, and breast cancer types. Scientists comprehensively viewed these organoids' genomic, transcriptomic, and cellular characterization. They found that all PDO models recapitulated the inherent characteristics of the originating patient tumors, enforcing the validity of PDOs as cancer models to study aspects of BC treatment and therapies (Bhatia, 2022).

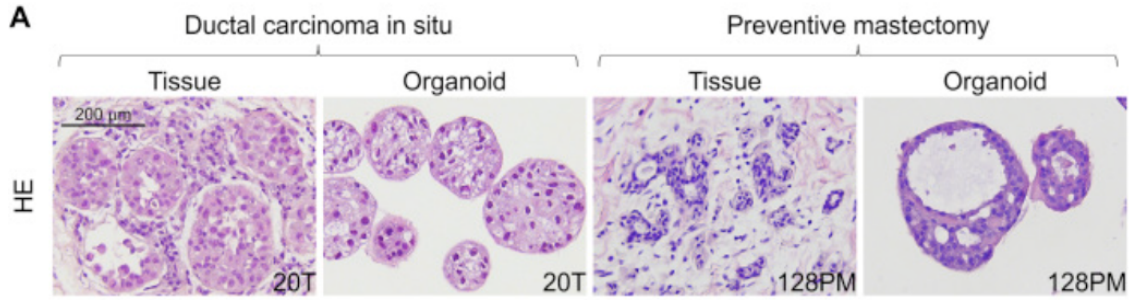

Figure 3. Comparative histological images of DCIS (left) and PM (right) tissues with their respective derived organoid lines. DCIS organoids show a structure architecturally reminiscent of the original tissue, while PM organoids display mildly inconspicuous characteristics of the tissue phenotype.
Note. Adapted from “A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity,” by Sachs, 2018.

Recent Discoveries Made Using Breast Cancer Organoids

Tsai et al. discovered that therapeutic resistance in tumors may be due to the selective outgrowth of cancer cells with genetic alterations enhancing their growth and survival. Treatment efficacy depends upon stimulating anti-tumor inflammation and efficient T-cell killing of tumor cells. Furthermore, tumor therapy resistance may arise because cancers elicit a pro-tumor inflammatory response, compromising T cell killing. Many solid human tumors are intrinsically resistant to anticancer therapy, limit the efficacy of treatments, and are the primary cause of patient mortality. An example of a discovery that was only possible through the use of BC organoids was implemented by Tsai et al., who found that human breast tumors can engage a chromatin repressor nuclear receptor compressor 2 (NCOR2) to enhance their resistance to chemotherapy, cell death receptors, radiation, and immune-mediated cell death. They identified this repressor by exploiting PDOs from patients with BC, which exhibited increasing intrinsic resistance to cytotoxic stress, a substance or process that can damage or cause cell death. By examining the transcript level of NCOR2 in multiple cohorts of neoadjuvant-treated patients with breast cancer, scientists noticed that NCOR2 expression is a strong predictor of therapeutic resistance. NCOR2 expression and nuclear activity are strongly linked to the therapeutic outcome of primary breast tumors and suggest that the multidrug resistance phenotype of tumors may be acquired at an early stage of tumor development before therapy (Tsai, 2022).

These discoveries have allowed scientists to better understand tumor behavior on an intrinsic level previously unattainable without BC organoid models.

Alternate Perspectives & Limitations

Patient tumors are vital in capturing the intricacy of cancer and studying therapy responses in a personalized, real-time manner. BC PDOs can recapitulate many of the core functions of the originating BC tissue, such as breast cancer cells, stroma, and cytokines (Yu & Huang, 2020). However, some caveats come with the production of PDOs. Procedures such as biopsy samples and surgical resections are well-established methods. However, isolating organoids from single cells to allow them to pass and multiply is time-consuming and demanding. While obtaining PDOs is simple, establishing cell lines from the extracted tissue is challenging. Fortunately, scientists have devised alternative protocols for biopsied and surgically resected tissue digestion, PDO culturing, and maintenance (Mazzucchelli, 2019).

PDOs are also often more time- and labor-intensive than their cancer model predecessors, such as patient-derived xenografts (PDXs), an invaluable culture vessel at the time. In a study by Guillen et al., they compared the therapeutic reaction duration of PDOs and patient-derived xenograft organoids (PDxOs), a fusion of the two three-dimensional structures PDOs and PDXs. As a result, outcomes from therapies tested with PDxO therapy had extended progression-free survival (PFS) and time-to-next treatment (TTNT), which took approximately 4-5 times longer than previous therapies. While this may seem like a consequential setback, PDxOs display feasible drug screenings and are more cost-effective than standard PDXs. PDXs (whether 2D or 3D) have restrictions because of their transient nature and ability to be contaminated by non-tumor cells. These human tumors become immediately unstable after being placed in culture, potentially affecting drug responses. Conversely, the PDxOs elicited concrete patient responses to drug screenings. Despite their slower establishment, PDOs react in the lab more efficiently when compared to their previous model counterparts (Guillen, 2022).

In addition to the laborious nature of maintaining 3D breast organoid cultures, it is essential to consider that they are also costly compared to most other in vitro breast cultures. Compared to the growth properties of human intestinal organoids, which can be passaged weekly with limited variation among samples, the proliferation rate of breast organoids is relatively slow. It can vary substantially between donors (Dekkers, 2021).

Another critical limitation of conventional organoid methods is a demonstrated lack of endogenous tumor-associated stromal components, particularly immune cells and fibroblasts. Thus, it is urgently needed to develop organoid systems to represent the tumor microenvironments more holistically. Ultimately, PDOs incorporating immune and other stromal components may open doors to new research, such as novel immunotherapies, and help accomplish the promise of precision cancer therapies (Lo, Kasper, & Kuo, 2020). Despite these hurdles, PDOs offer a more holistic, representative view of patient tumors and are thus an exemplary research model.

Conclusion

The primary challenge in translational research for developing therapy for BC lies in developing a model that can recreate patient-specific scenarios while preserving tumor heterogeneity and the complex tumor environment. With PDOs, researchers and clinicians gain insight into a drug's efficacy and toxicity before subjecting a patient to treatment or trial enrollment. There are technical hurdles to organoid development, such as culture duration, which can take up to 4-6 weeks. Additionally, many laboratories still face the challenge of cultivating successful organoids from patients since most PDOs are from surgical resection tissues, which requires the surgical removal of some or all of the tissue rather than a small sample. A diminished cell count in a biopsy specimen directly correlates with a decreased suitability of the resulting organoid model.

However, with continued innovation and sustained clinical trials using other methods, PDOs can serve as prospective tests for cancer patients. Sachs et al. have increased the success rate of BC organoid establishment to more than 80% with an optimized BC organoid culture medium using a multipoint biopsy method (Yu and Huang, 2020, as cited in Sachs, 2018). A multipoint biopsy is a multiple-core biopsy from at least four separate areas in the tumor. Furthermore, different teams of scientists have used other protocols and mixtures of inhibitors and growth factors to find the most suitable medium for BC organoid growth (Yu & Huang, 2020). Even though organoids are a relatively new model, they may present a breakthrough for precision cancer treatment. Overall, patient-derived organoids are the most optimal and revolutionary approach to treating breast cancer in individual patients.

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