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Review

Mammary Cancer Models: An Overview from the Past to the Future

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Abstract. *Breast cancer research heavily relies on diverse model systems to comprehend disease progression, develop novel diagnostics, and evaluate new therapeutic strategies. This review offers a comprehensive overview of mammary cancer models, covering both ex vivo and in vivo approaches. We delve into established techniques, such as cell culture and explore cutting-edge advancements, like tumor-on-a-chip and bioprinting. The in vivo section encompasses spontaneous, induced, and transplanted models, genetically engineered models, chick chorioallantoic membrane assays, and the burgeoning field of in silico models. Additionally, this article briefly highlights the key discoveries made using these models, significantly enhancing our understanding of breast cancer. In essence, this article serves as a comprehensive compass, charting the trajectory of mammary cancer modeling from its early beginnings to the promising vistas of tomorrow.*

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Carcinogenesis is the multistep process through which normal cells are transformed into cancer cells. This process can take from a few months to many years, depending on the aggressiveness of the tumor. It consists of four phases: initiation, promotion, progression, and metastasis (1, 2). Initiation is the first phase and involves spontaneous or induced irreversible genetic damages in a normal cell, leading to its conversion into an initiated cell. Genetic damages can be induced by chemical compounds, physical agents (radiation) or biological agents (bacteria or viruses). Genetic damages can lead to the activation of oncogenes or the inactivation of tumor suppressor genes (3). The second phase is promotion, which involves the expansion of initiated cells into a larger mass of abnormal cells. The uncontrolled division of initiated cells can be viewed as a relatively long and reversible process induced by the administration of drugs that affect the division rate of neoplastic cells (2). The third step, designated as progression, involves the transformation of the preneoplastic cells into neoplastic cells. It is characterized by the acquisition of additional genetic mutations that enhance the ability of neoplastic cells to become malignant, invade other tissues, and spread to other

parts of the body, more or less distant from the original site, in a process called metastasis (3). Only the malignant neoplastic cells can spread from the primary tumor, through the blood or lymphatic system, to other organs, such as the bone, liver, lung, and brain. However, it is worth noting that metastasis is a complex process and not all malignant cells have the ability to metastasize (2, 4).

According to the World Health Organization (WHO), cancer is a leading cause of death worldwide. In the year 2022, over 18.7 million new cases of cancer were diagnosed globally and accounted for nearly 9.7 million deaths (5). Breast cancer, with 2.3 million new cases in 2022, is one of the most frequently diagnosed cancers and a leading cause of cancer-related deaths among women worldwide (666,103 deaths in 2022) (1, 5). Several factors can influence the development of breast cancer, commonly known as risk factors. These include family history, exposure to physical, chemical or biological agents, and lifestyle (6). Breast cancer is typically first diagnosed through ultrasound, mammography, or magnetic resonance imaging (MRI). In some cases, a biopsy is performed to confirm the diagnosis or clarify any uncertainties. The evolution of screening methodologies contributes to a better prognosis and a higher survival rate (4). Breast cancer can also occur in men, but it is over 100 times less common than in women, and it typically has a poor prognosis due to late diagnosis. Breast cancer can arise from different types of cells, as explained in a previously published article by our research team (6). Increasing awareness of the diverse cellular origins of breast cancer underscores the importance of tailored screening approaches for early detection and improved outcomes across all populations.

The use of animals for research purposes dates back to 384-258 B.C., when Aristotle and Erasistratus described the first and second evidences of animal use for research purposes, respectively (7) (Figure 1). Over the years, researchers have developed various models to study breast cancer and gain a better understanding of the mechanisms involved in its development, progression, and treatment. These models are also essential tools to understand the impact of genetic and environmental factors in breast cancer evolution. Animal models can also aid in the evaluation of new and more effective therapeutic approaches, which can improve the lifespan and the quality of life of oncologic patients (8). In 1854, Crisp described the first mouse mammary tumor as a "hard, scirrhous-like tumor, with a size of a large nut", located in the right pectoral muscle (9). Later, in 1906, Hugo Apolant described a spontaneous mammary tumor in a mouse (9, 10). The development of the first transplantable mouse mammary tumor line dates to 1903 by Jensen in collaboration with the Borrel laboratory. He pioneered a strain of mice known for their susceptibility to developing mammary tumors at an accelerated rate. His experiments conclusively showed that the proliferation observed was attributed to the transplanted tumor tissue itself rather than the host animal's own tissues (11). Afterwards, in 1911, Rous and Murphy produced the first transplanted primary tumor tissue on the highly vascularized chicken embryo chorioallantoic membrane model (CAM) (12). In 1918, Yamagiwa and Ichikawa developed the first cancer model (7). They experimented with multiple approaches to induce irritation in the epithelial and subcutaneous layers of the ears of domestic rabbits, resulting in persistent abnormal growth of the epithelium. Additionally, they successfully showed the occurrence of metastases in the lymph nodes located at the base of the ear and in the submaxillary region (13). It was only in 1958 that Lasfargues and Ozzello established the first breast cancer cell line (BT-20) (14). This cell line was originated from an invasive ductal carcinoma in a 74-year-old woman, the tumor has since demonstrated a pattern of growth characterized by epithelial cells (15). Three years later, in 1961, Charles Brenton Huggins induced mammary cancer development in Sprague-Dawley rats for the first time using 7,12-dimethylbenz[a]anthracene (DMBA), thus describing the first rat model of DMBA-induced mammary cancer (16). Since then, the use of animal models in breast cancer research has become increasingly accepted. The first cell-line derived xenograft (CDX) of breast cancer, achieved by transplanting human breast cancer cells into an immune-deficient mouse, was documented in 1962 (17). Genetically engineered models (GEMs) were introduced in 1980 (7, 10). In 1984, Philip Leder generated the first transgenic mice model using mouse mammary tumor virus (MMTV). In this genetically modified strain, the c-Myc oncogene is activated by the mouse mammary tumor virus long terminal repeat, leading to the spontaneous formation of adenocarcinomas (18). Four years later, in 1988, the Leder laboratory generated the first transgenic mouse model expressing an activated form of rat NEU (NEU-NT) under the transcriptional control of the MMTV promoter (MMTV-BEU-NT mice) (19). In 1993, the first orthotopic metastatic breast cancer PDX fragment was transplanted into the nude mouse fat pad (10). In 1998, scientists discovered the therapeutic benefits of Herceptin, which is now widely used to treat adult patients with HER2-positive breast cancer. This discovery was based on studies using a CDX model of human epithelial growth factor receptor 2 (HER2)-positive breast cancer cells (10). Herceptin is a monoclonal antibody that targets HER2 receptors found on the surface of HER2-positive tumor cells. By binding to these receptors, it inhibits their ability to receive growth signals and marks them for destruction by the immune system (20). One year later, the first GEM model of BRCA1 breast cancer was generated (14). This model offers insights into the development and progression of BRCA1-associated breast cancer, aiding in the exploration of novel therapeutic strategies (21). In 2004, Viravaidya and Shuler conducted the first study on microfluidic cell culture systems (22).

As stated above, researchers have developed various models over the last hundred years to study breast cancer. Thus, this

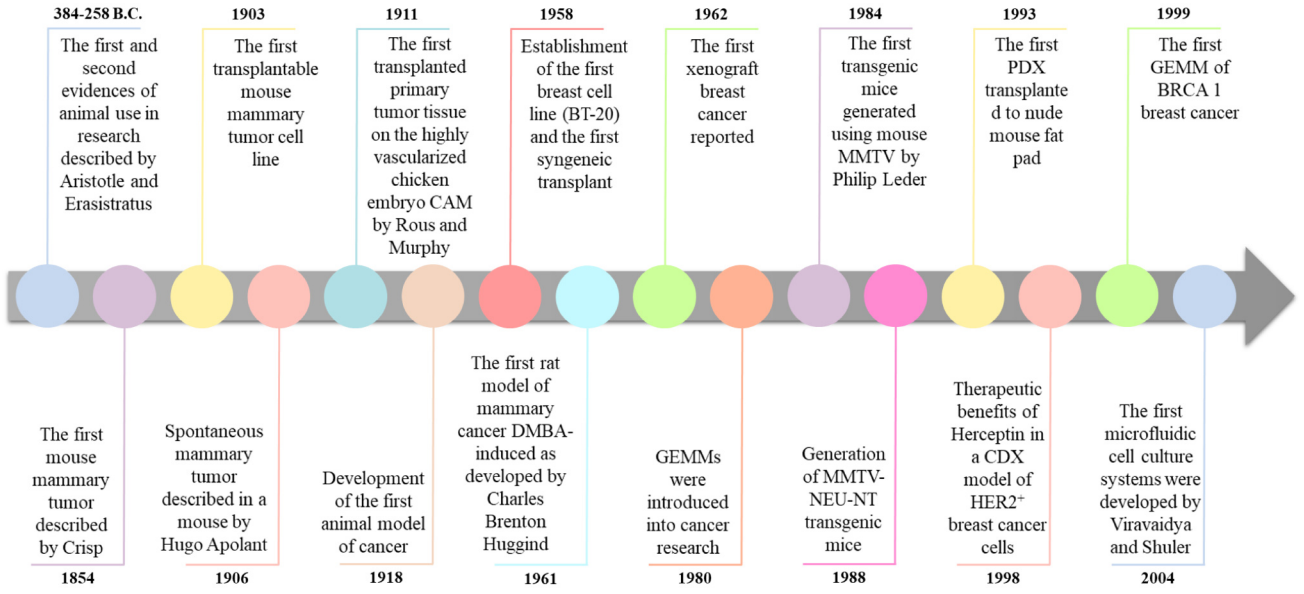


Figure 1. *Timeline highlighting important milestones in the use of animal models for breast cancer research (7, 9-11, 13-18). CAM: Chorioallantoic 3 membrane model; DMBA: 7,12-dimethilbenz[a]anthracene; PDX: patient-derived xenograft; GEMMs: genetically engineered mammary models; MMTV: mammary tumor in vitro; CDX: cell-derived xenograft; HER2+: human epithelial growth factor receptor 2 positive.*

work provides an overview of the *ex vivo* and *in vivo* models used in breast cancer research, as well as their applications, advantages, and disadvantages, serving as a guide for researchers who wish to implement research in breast cancer and need to select the best model according with their aims.

Ex Vivo Models of Breast Cancer

Ex vivo models of breast cancer, including cell culture, spheroids, organoids, tumor-on-chip, bioprinting, and microfluidic chip, are laboratory-based models that involve the growth and study of breast cancer cells outside of the body (23) (Figure 2). These models are valuable tools for investigating breast cancer biology, including the behavior of cancer cells, their response to treatments, and the underlying molecular mechanisms of carcinogenesis (24). While these models cannot fully replicate the complexity of tumors growing in all body, they provide valuable insights because they are significant tools in drug discovery and to understand cellular processes during carcinogenesis. Table I summarizes the advantages, limitations, and applications of *in vitro* models.

Breast cancer cell lines. The culture of breast cancer cell lines is a crucial method in breast cancer research as it provides information about the biology, behavior, and therapy in breast cancer cell lines (25). Breast cancer cell lines can be cultured in different formats, including monolayers (cells adhered to the surface of a culture dish), suspension cultures (cells growing in a liquid medium without surface attachment), and three-dimensional (3D) cultures that more accurately mimic the tissue microenvironment (26). The growth and maintenance of breast cancer cell lines is based on the appropriate choice of growth medium, supplements, and culture vessel (27).

The first human breast cancer cell line established in 1958 was the BT-20 that was derived from an infiltrating ductal carcinoma obtained from a 74 year-old woman (15). Currently, there are over 50 human breast cancer cell lines, most of which are derived from tumor metastasis in patients who have undergone multiple cycles of chemotherapy, radiotherapy, and/or even hormone therapy without success (28). The most common breast cancer cell lines include MCF-7, T47D, MDA-MB-231, and ZR-75-1 (14). These cell lines offer a renewable source of cancer cells that can be cultured and propagated for an extended period. Molecular classification of breast cancer cell lines is crucial in determining which cell line should be use according to each study objectives and characteristics (29).

Like other models, cell culture also has some limitations. Cell lines and primary cultures are simplified models of the complex tumor microenvironment in the human body (14); they do not have interactions with other cell types, extracellular matrix components, and the physiological conditions present in living tissues. Additionally, mechanical and/or enzymatic disaggregation disrupts the normal tissue architecture and cell-cell connections (30). When cell lines

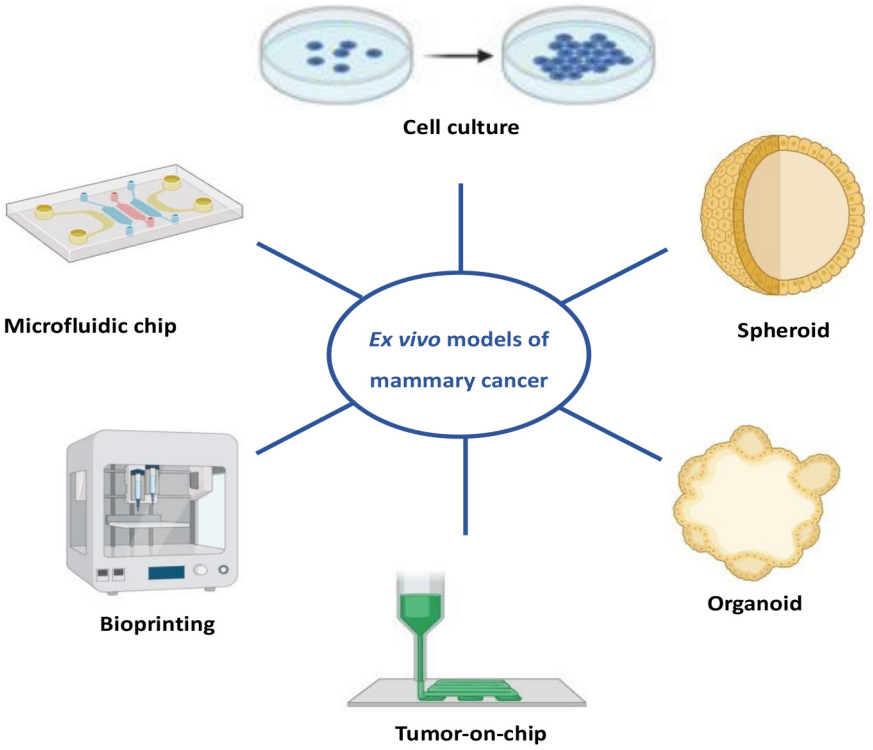


Figure 2. *Ex vivo models available for mammary cancer research.*

are established from primary cultures of tissues collected during surgical procedures, mechanical fragmentation is necessary, allowing the separation of tissue cells into a homogeneous suspension, facilitating their subsequent culture and study in the laboratory. Therefore, findings from cell culture experiments should be validated using more accurate models (27).

Spheroids. In the 1970s, Sutherland used a methodology with spinner flasks to induce rotation of cells and form spheroids (31). These spheroids were later used as model systems for conducting pharmacological studies. In 1978, Yuhas *et al*., systematically investigated the capacity of nine [2 human (Hs578Bst, Hs578T), six rat (13762, 13762-A, 3M2N, R323OAC, DMBA-1, DMBA14), and one mouse (MCa-1)] breast cancer cells to generate multicellular tumor spheroids using the agar-based method (32).

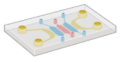
The spheroids are 3D cell culture models that bridge the gap between simplistic two-dimensional (2D) cell culture and the *in vivo* complexity of tumors, contributing to advances in our understanding of breast cancer and the development of novel therapeutic approaches (18, 28, 32).

The spheroids are aggregated cells developed in suspension, either with or without an extracellular matrix that replicates the architecture and metabolism of their original tissue (33). These models aim to better mimic the complexity and microenvironment of tumors considering all body. Spheroids can provide valuable insights into tumor growth, drug response, and metastasis (34). Spheroids models are frequently employed for the evaluation and analysis of immunotherapeutic approaches, primarily because they offer a cost-effective alternative to other *ex vivo* models and are well-suited for studying tumor penetration dynamics (33).

Organoids. In 1906, Ross Harrison placed a fragment of the embryonic nerve cord in a drop of lymph on a coverslip. He then inverted and sealed it over a hollowed slide (35). This pioneering work laid the foundation for subsequent advances in tissue culture techniques. Later, in 1981, Yang *et al*. discovered a novel method for the continuous growth of human mammary epithelial cells in monolayer cultures, distinct from traditional 3D organoid cultures (36).

Organoids are self-organizing 3D structures that develop from stem cells and have organ-specific cell types as well as structural, functional, and molecular similarities to the tissue of origin. Patient-derived organoids can be created using induced pluripotent stem cells (23). Although well-established cancer cell lines have been commonly used as a single cell cancer model, their limitations should be considered. This model shares similarities with the cell-line

Table I. *Ex vivo models: advantages, limitations, and applications [adapted from (23, 38, 116)].*

Models	Advantages	Limitations	Applications
2D 	Low cost	Cell-line derived Cannot represent tumor microenvironment	Initial nanomedicine Biological characterization
3D	Different tumor microenvironment representation	Optimized growth of different cell types	Cell type-targeted therapies
Spheroid 	Cell-cell and cell-matrix interactions Do not require highly specialized techniques	Expensive than monolayer culture Time consuming	Tumor penetration studies Immunotherapies discovery
Organoid 	Small size samples Maintenance of inter and intra tumor heterogeneity Can be preserved as biobanks	Very expensive Complex establishment and maintenance	Drug discovery Study of invasion and metastasis
Tumor-on-chip 	Supports microfluidic model	Very complex Highly specialized techniques and material	High-throughput screening, study of metastasis Personalized medicine
Bioprinting 	High spatial control High throughput	Highly specialized techniques and material Lower cell viability High cell number needed	Tissue and organ fabrication Study of migration, and angiogenesis Drug discovery
Microfluidic chip 	High accuracy and resolution Inter-cellular interactions Small size samples	Very expensive Complex technique Difficult reproducibility	Study of invasion, vasculature, and metastasis Modeling tumor microenvironment

spheroid model due to the presence of cell-cell and cell-matrix interactions (37). It does not require highly specialized techniques, but it is more expensive than monolayer culture and time-consuming (23).

In 2020, Rosenbluth *et al*. cultured organoids from normal and cancer-prone human breast tissue and concluded that they provide an excellent model for investigating mammary transformation, differentiation, and breast cancer risk (38). In recent years, several studies have utilized organoids as model for breast cancer research. Researchers, including Dekers *et al*., Li *et al*., Pan *et al*., and Luo *et al*., utilized surgical specimens from various breast cancer tissues. All of these studies reported the effectiveness of organoid models in exploring the clinicopathological and genomic characteristics of tissue to identify potential treatments (39-42).

Tumor-on-chip. Tumor-on-chip technology, also known as organ-on-chip, has gained prominence in cancer research, including in breast cancer. In 2016, Gioiella *et al*. proposed a tumor-on-chip system that closely mimics the characteristics of its native counterpart, including multicellularity with both tumor epithelial cells and stromal cells, as a model for breast cancer (43).

Tumor-on-chip model mimics the microenvironment and physiological conditions of tumors and surrounding tissues more closely than traditional spheroids (44). Microfluidic platforms are commonly used to replicate the complex interactions between different cell types, the extracellular matrix, and the flow of fluids, such as blood or lymph (23, 24, 44). Researchers can use tumor-on-a-chip models to monitor how drugs interact with the tumor, evaluate their effects on cancer cells, and assess potential side effects on healthy tissues (24).

These models are suitable for studying different stages breast cancer metastasis, allowing researchers to investigate how cancer cells invade surrounding tissue, enter the bloodstream or lymphatic system, and establish secondary tumors in distant organs. These models can also be generated using patient-derived cells, offering the potential for personalized medicine (24, 44).

Bioprinting. The development of the first bioprinter in the early 2000s can be directly attributed to the work of Thomas Boland's group at Clemson University, Clemson, SC USA. Boland recognized the similarity in size between ink droplets and cells in the human body and initiated the process using a conventional inkjet printer (45, 46). In 2018, Wang *et al*. demonstrated that 3D bioprinting of stromal cells from the breast cancer microenvironment replicates *in vivo* conditions and provides better models for studying breast cancer biology and drug discovery (47). The development of biomaterials and tissue engineering methods has been increasingly successful due to advances in *ex vivo* printing technology (37).

In this model, cells are printed together with extracellular matrix components, biomaterials and bioactive factors to reconstruct 3D tissue (33). Bioprinting allows control of the spatial organization of cells, the formation of biomolecular gradients, and the formation of vasculature with micron-scale resolution (23). It is therefore very useful not only for the fabrication of tissues and organs, but also for studying migration and angiogenesis, discovering drugs, developing tumor microenvironment models and screening studies (23, 37). However, it requires highly specialized techniques and materials and a large number of cells while having low cell viability (23). Most studies using bioprinting in breast cancer research have focused on breast cancer metastasis and drug resistance (37).

Microfluidic chip. Microfluidic models are systems that integrate micron-sized fluidic channels, along with tubing for pumping peripherals, fluids, and cells, into a single platform (48). In 2000, microfluidic systems were first employed to pattern proteins and mammalian cells on a flat substrate (49). These models differ from *in vitro* models in that they use microfluidic technology (29). The combination of cell

culture and microfluidic devices offers numerous advantages for achieving a closer approximation to *in vivo* systems. Cost reduction is possible by using microchannels to reduce the amount of chemicals required (50).

In the context of breast cancer research, microfluidic models offer several advantages. They allow greater fidelity to the tumor microenvironment by incorporating multiple cell types, including cancer cells, stromal cells, immune cells, and blood vessel-like structures, in a controlled spatial arrangement (50). They also allow researchers to study cell-cell interactions, cell migration, invasion, and the influence of the microenvironment on tumor behavior by mimicking the cellular and extracellular components of breast tumors (48). Microfluidics shows potential for personalized medicine in breast cancer. Researchers can use it to assess individual patient responses to different treatments, identify specific drug sensitivities, or evaluate the efficacy of targeted therapies by incorporating patient-derived cells or genetic material into microfluidic models (29).

The first microfluidic cell culture systems were developed by Viravaidya and Shuler in 2004. They established a micro cell culture analog to study the bioaccumulation of a new drug through absorption, distribution, metabolism, excretion, and toxicity pathways (22). Microfluidic models can have several applications, including disease diagnosis, drug discovery and delivery, disease modeling, tissue engineering, organ-on-a-chip, point of care testing, and biosensing (51).

Although microfluidic models have many advantages, they also present certain challenges. These include device fabrication, optimization of culture conditions, and the need for expertise in microfluidics and related techniques (52). In addition, as with any *in vitro* model, it is impossible to fully replicate the complexity of the human body, and validation through complementary *in vivo* and clinical studies remains essential (50).

Microfluidics has the potential to enhance our understanding of breast cancer biology, accelerate drug discovery, and improve patient care by bridging the gap between traditional cell culture and *in vivo* models.

In Vivo Models of Mammary Cancer

Rodent models can closely mimic human breast cancer in terms of gene expression and histopathological patterns, making them a valuable tool for studying carcinogenesis and testing potential treatments (53). Despite this, it is important to note that rodent models may not fully replicate the complexity and heterogeneity of human breast cancer, and their findings may not always be translated to human clinical trials (8).

In animal models, mammary cancer can be induced through the use of chemical carcinogens, and/or hormone administration, GEMs, and tumor cell transplantation (10, 54, 55) (Figure 3).

Rats have six pairs of mammary glands, whereas mice typically have five pairs (6). Furthermore, rats have a more robust immune system than mice, which may affect their suitability for certain tumor or drug response studies (56, 57).

The mouse is the most commonly used model organism for preclinical research among rodents (53). Mice offer numerous advantages over other model species, including a genome that is 99% identical to that of humans, a robust genetic and molecular toolkit, and the small size of the animal which allows for large-scale, high-throughput and cost-effective research (53). Therefore, by addressing a number of identified bottlenecks, their potential to improve the efficiency of medical research, particularly drug development, could be boosted (54). Rats are also commonly used in research, but there are some differences between rats and mice. Additionally, rats and mice have different metabolism and pharmacokinetics. Although mice are less expensive and more widely available than rats, it is important to consider the impact of these differences on the study results (58, 59). The choice between them depends on the specific goals of the study, the resources available, and the need to accurately mimic the characteristics of human breast cancer. Researchers should consider the advantages and limitations of each species when designing experiments to ensure that the chosen model aligns with their research objectives.

Spontaneous models. Spontaneous models are valuable research tools for studying breast cancer. These models involve the development of mammary tumors in animals, typically mice, without the intentional administration of chemical substances, specific genetic alterations or inoculation of specific cell lines (1). Instead, these tumors arise naturally in the animals' mammary glands. They closely mimic the development and progression of human breast cancer, relying on the natural biological process of tumor initiation and growth (18). Although these models are considered "spontaneous", as tumors arise naturally, they may still have underlying genetic factors that contribute to their development (60). Certain strains of mice are more prone to developing mammary tumors due to their genetic background (61). The BALB/c and C3H mouse strains are commonly used mouse strains as spontaneous mammary tumor models due to their higher predisposition to develop mammary tumors (61, 62). The Fischer 344 (F344), Sprague-Dawley, and Wistar strains are commonly used to induce mammary tumors in rats (63, 64). Despite this, spontaneous models typically have a long latency period, meaning that it can take several months for tumors to develop (1, 65). They are therefore suitable for studying the gradual progression of breast cancer over time. Working with these models can be challenging due to the unpredictability of tumor onset and the variability in tumor types. Researchers may need to carefully manage and monitor the animals over a long period (61).

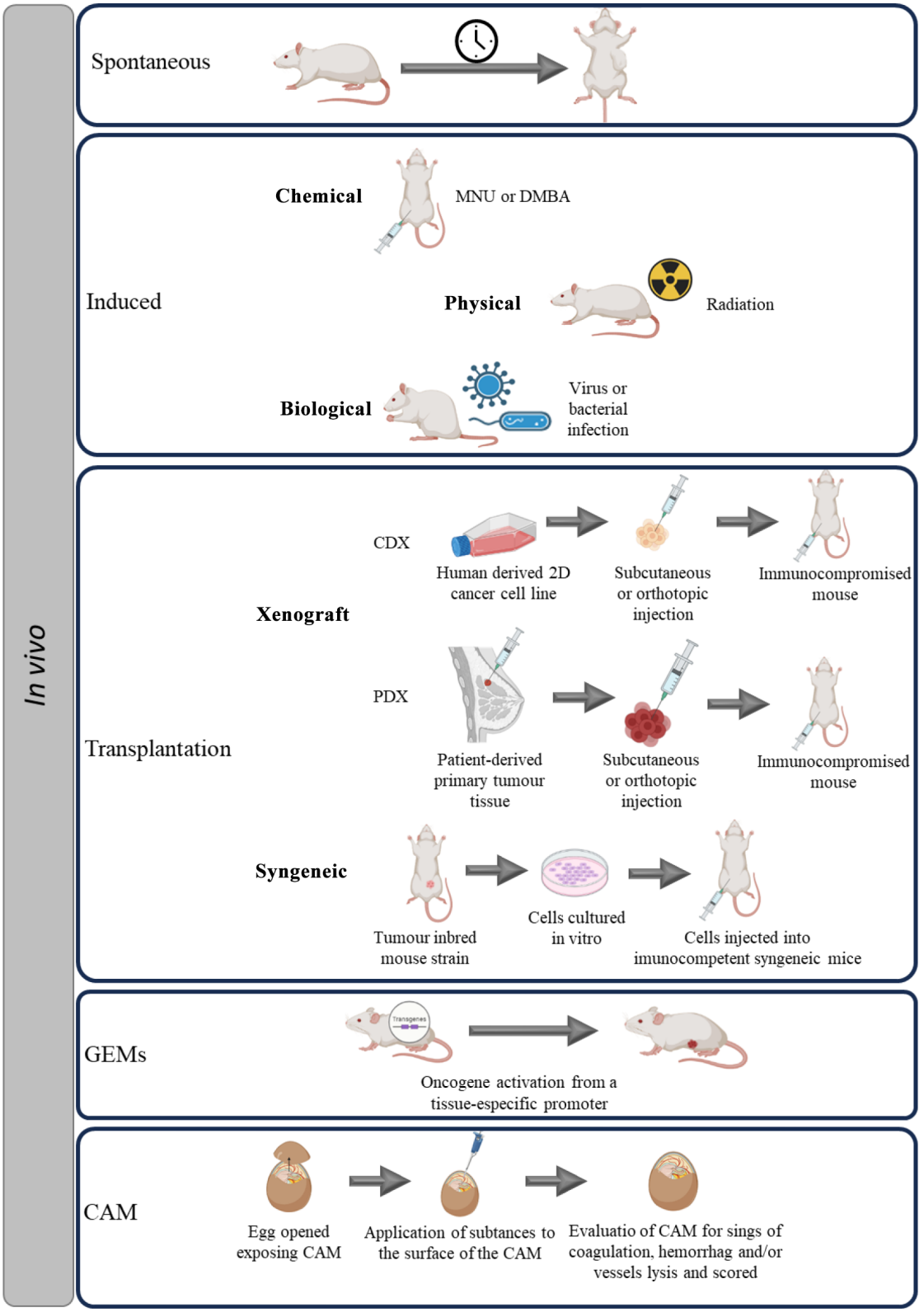


Figure 3. *Schematic showing an overview of in vivo pre-clinical breast cancer models, including induced, transplanted, genetically engineered models (GEMs), and chorioallantoic membrane (CAM) models. MNU: N-methyl-N-nitrosourea; DMBA: 7,12-dimethylbenz[a]anthracene; CDX: cell-derived xenograft; 2D: two-dimensional; PDX: patient-derived xenograft.*

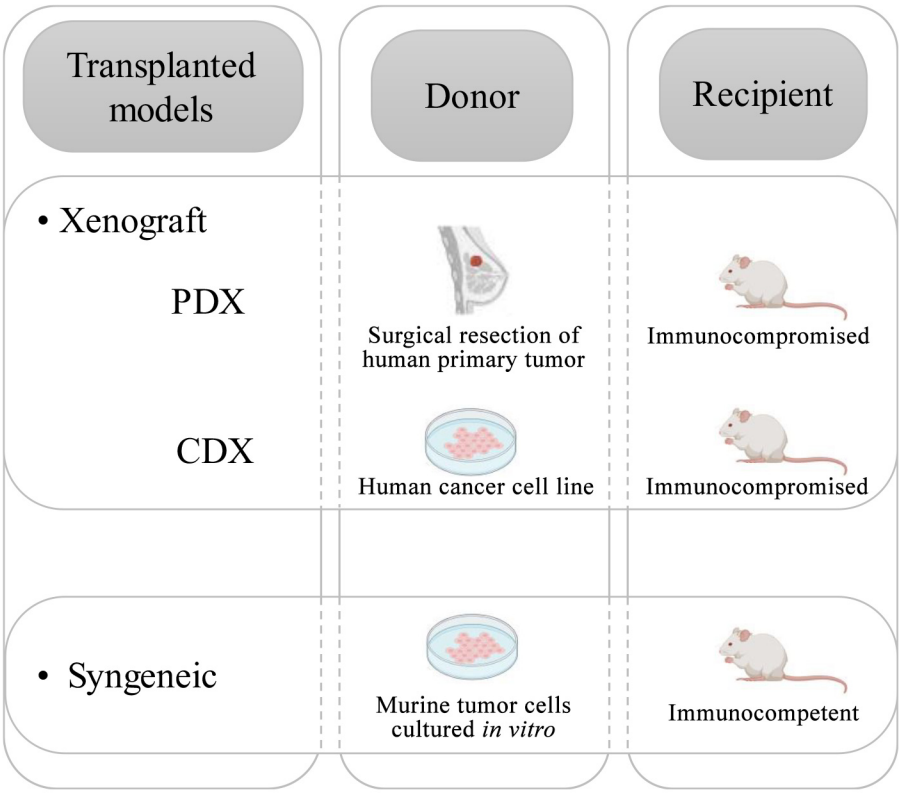


Figure 4. *Transplanted models (2, 55, 75).*

Induced models. Chemically and/or chemically and hormonally induced models. Chemically and hormonally-induced models are useful for studying the different stages of carcinogenesis (initiation, promotion, and progression), and the relationship between environmental factors and the development of breast cancer.

Chemical carcinogens, such as DMBA or *N*-methyl-*N*-nitrosourea (MNU) can induce tumors in several tissues depending on their route of administration, age and sex of animals (66). Chemically induced tumors have some advantages over other animal models of mammary cancer, such as their short latency period and high reproducibility (67).

The administration of DMBA or MNU by intravenous, intraperitoneal, or intragastric routes, is the most commonly method to induce mammary cancer in Sprague-Dawley, Fischer 344 or Wistar rats (1, 66, 67). In 1975, Gullino *et al*. reported a 73% incidence of mammary tumors in Sprague-Dawley rats, 86 days after an intraperitoneal injection of MNU, at a dose of 50 mg/kg (1). Since then, many scientists have used this model to induce mammary cancer and have demonstrated its advantages, such as high specificity and reproducibility, low cost, and the development of mammary tumors with histopathological patterns similar to those described in women, and ER-positive tumors (68).

Both MNU and DMBA can be used to induce mammary tumors and to study the molecular and cellular changes that occur during tumor development and the efficacy of potential therapies (67). Unlike MNU, DMBA requires prior metabolic activation by liver cytochrome P450 enzymes (66). This carcinogen also has a slower carcinogenic activity and a longer latency period (2). DMBA is used less commonly than MNU to induce breast cancer in rats, because it is more difficult to prepare (it is oil soluble), whereas MNU is water soluble (69). Histopathologically, as described by Alvarado *et al*., 2017, tumors induced by both DMBA and MNU are classified as hormone-dependent with immunoreactivity to estrogens and progesterone. However, MNU-induced mammary carcinomas showed higher levels of Ki-67 proliferative index and mitotic activity index compared to those induced by DMBA, suggesting increased aggressiveness and poorer prognosis in the former (66).

Hormonally induced models involve the use of hormones or hormone-like drugs to induce tumor development. These models are based on the fact that hormones, such as progesterone and estrogen, have a significant impact on the development and progression of human breast cancer (29). Estrogens can promote the growth of mammary tumors in mice (2). Progesterone can interact with estrogens to modulate

Table II. *Advantages and disadvantages of genetically engineered models (29, 54, 117-119).*

Advantages	Disadvantages
Creation and analysis of safe, useful, and recombinant products to be used in Man	Requires sophisticated genetic engineering techniques
A way to study disease mechanisms in a complex organism	Different inflammatory and desmoplastic responses
Understand the mechanisms underlying human diseases enabling the creation of effective and focused treatments	Different metastatic pattern when compared with humans
Specific to study molecular and pathophysiological pathways of breast cancer	Harder to mimic the time of tumor development
Specific reproduction of tumor formation, progression ad induced specific mutations	Limitation of the translatability of findings to humans

the hormonal response in breast tissue, which may lead to potentiating the proliferative effects of estrogens (70, 71).

Although chemically and hormonally induced models are well-described and tested, they have some disadvantages, such as uncontrolled latency periods and tumor size, as well as a lack of control over location and lack of clear evidence of metastasizing (29).

Radiation induced models. Radiation can be used alone or in combination with chemical carcinogens or hormones to induce mammary carcinogenesis (68). Mammary cancer can be induced by exposure to ionizing radiation, such as X-rays, gamma rays or neutron radiation, either in the whole body or in a specific irradiated segment (1). As with chemically-induced models, susceptibility to radiation-induced tumorigenesis varies between rat strains and is dependent on the type of radiation and magnetic fields (2). This model is useful for studying the effects of radiation and of fractionated doses, but further studies are needed to clarify the different models (29). Considering that inducing cancer through radiation requires specific equipment and general care by handlers to avoid radiation exposure, these models are rarely utilized.

Biologically-induced models. Biologically-induced models are obtained by administrating lentiviruses which mediate over-expression of oncogenes, or silencing of tumor suppressor genes, in laboratory animals. MMTV is a retrovirus that has been extensively studied in the context of mammary cancer research (1). It can integrate its genetic material into the host genome, and the tumors that develop closely resemble some aspects of human breast cancer, making it a valuable model system for studying this disease These aspects may include similarities in tumor morphology, gene expression patterns, signaling pathways, and responses to treatment (18). This model of mammary cancer has a higher incidence rate, shorter latency, and more reliable predicted outcomes compared to spontaneous models (8). However, they have low efficiency, variable incidence and long latency, and different pathological characteristics (1).

Transplanted models. These models are established by transplanting either spontaneous or induced breast cancer cells or a piece of a solid tumor from a donor into a laboratory animal (10). This can be done from a human donor to an animal recipient (xenograft) or between genetically identical animals of the same species (syngeneic). Transplantation models are also classified according to the transplantation site as orthotopic or ectopic (72). Ectopic transplantations can be classified as subcutaneous, tail vein injection or left ventricular injection. Xenograft models can also be further subclassified as CDX or PDX (1).

Although there are different immune-compromised murine models that can produce transplanted models under different transplant conditions and sample types, there is still no agreement on the optimal host to utilize (17). While mice are frequently employed in cancer research, laboratory rats offer a viable alternative with unique advantages, such as the capability for non-invasive imaging, the ability to develop larger tumors, and simpler surgical manipulation (73).

Nowadays, the human xenograft model is the most widely used animal model for testing new treatments (54). This model offers advantages, such as short cycles, low cost, minimal variation, and high rates of tumor growth (1). As a disadvantage, these models lack an immune system, compromising the final evaluation of treatments. Syngeneic models are suitable for studies that focus on evaluating the interaction between the immune system and tumors, as well as tumor development (2, 54, 74) (Figure 4).

Xenograft. In xenograft models, human cancer cell lines are transplanted into immunocompromised animals that lack a functional immune system (72). These cells can be derived from a patient's tumor or from a cell line established from a patient's tumor (2).

PDX models are derived directly from human tumors, making them more similar to patients in terms of genetic abnormalities, gene expression, metastatic potential, drug response, and pathological parameters. This model benefits from the heterogeneity and genetic diversity within tumors and the representation of different human tumor types. It also incorporates features of the tumor microenvironment, including stromal and vascular cells, and allows for easy and accurate measurement of tumors (10, 54).

PDX models can be used to identify biomarkers for personalized drug selection in clinical treatments (1). However, there are some limitations to their use. For example, they require an immunodeficient host, and their subcutaneous location may not allow for the presence of key tissue-specific stromal infiltrates. Additionally, surgical implantation is required, and there is a cross-species divide, as the stromal components are derived from mice, whereas the tumor cells are derived from humans. Finally, there is genetic and phenotypic drift with passage (10, 54).

In the CDX model, tumor cells originate from cell lines established in the laboratory that might have originated from human tumors but have been cultivated for extended periods (74, 75). These cell lines can undergo genetic modifications or mutations over time (1). While this model is valuable for various applications, such as high-throughput drug screenings and basic tumor biology studies, it may lose some of the original tumor heterogeneity due to prolonged culture, potentially impacting its ability to accurately reflect the biological complexity of the original tumor, including the tumor microenvironment (23). It can develop tumors with characteristics that mimic those found in humans, and these tumors grow quickly and are easy to study (10, 29, 54, 76).

CDX model has some limitations, such as the immunodeficiency of the host, which prevents the immune system from responding therapeutically and contributes significantly to the development and progression of cancer (10, 23). Additionally, there is excessive homogeneity within the tumor, which does not adequately mimic the intratumoral heterogeneity observed in the clinical setting and the CDX model generally does not metastasize (10, 29, 54, 76).

Syngeneic. Syngeneic models of breast cancer involve the implantation of murine breast cancer cells into immuno-competent mice of the same genetic background as the cancer cell. These models are valuable for studying interactions between the immune system and cancer, as well as for evaluating immunotherapies and other treatment modalities (77, 78). Tumors tend to grow rapidly, may metastasize, and can be resected; however, an immuno-competent host is required (10). They can also be used for screening potential anticancer drugs and combination therapies, providing insights into treatment responses and mechanisms of action. However, it is important to note that the genetic and histological composition of the tumors may not reflect the human condition (29).

Overall, syngeneic models of breast cancer offer a valuable tool for studying the complex interactions between tumors and the immune system, as well as for evaluating novel therapeutic strategies in a preclinical setting.

Genetically engineered models (GEMs). In 1984, Philip Leder generated the first transgenic mice using the mouse mammary tumor virus (18, 79). Genetically modified rats and mice are useful models for studying the role of genes, such as Ras, BRCA1, and BRCA2 in the progression of malignant tumors. These models can also be used to investigate the effects of growth factors and receptors, viral and nuclear oncogenes, Ras genes, INT genes, genes affecting the cell cycle and growth suppressor genes (29). GEMs were generated because xenografts do not accurately mimic the genetics and histology of human breast cancer. These organisms have altered DNA sequences through transgenic, knock-in, or knock-out techniques, resulting in genetic modifications not typically found in nature (2, 53, 80). These models must be validated, reproducible, robust, and cost-effective to be considered a good model by the pharmaceutical industry (53).

The ability to produce genetically modified animals has set new standards for the scientific community, allowing researchers to investigate new ways of treating disease, understand molecular causes, and develop specific drugs (2). However, there are concerns regarding the welfare and health of these animals due to the potential unfavorable side effects of the integration and expression of recombinant genes (81). Table II summarizes the advantages and disadvantages of genetically engineered models.

The mammary mouse model virus promoter and the polyomavirus middle T antigen (MMTV-PyMT) are among the most widely used transgenic animal models for studying breast cancer (82). Female MMTV-PyMT mice typically develop mammary tumors starting around 4-6 weeks of age, with rapid tumor progression leading to advanced metastatic disease by 10-12 weeks of age. This mouse model closely resembles human liminal B-type breast cancer histologically (82). There are several other GEMs utilized in breast cancer research, including: BRCA1/BRCA2 Knockout Mice (mice genetically modified with deletion in the BRCA1 or BRCA2 genes) (83, 84), HER2/neu Transgenic Mice (transgenic mice expressing the HER2/neu oncogene resembling the HER2 amplification seen in certain breast cancer subtypes) (85, 86), p53 Knockout Mice (mice lacking the p53 tumor suppressor gene) (87), PIK3CA Mutant Mice (mice harboring mutations in the PIK3CA gene)

(88, 89), and PTEN Knockout Mice (mice with deletion of the PTEN tumor suppressor gene) (90, 91). These represent just a subset of the diverse genetically modified models available for studying breast cancer. Each model possesses unique attributes and can be employed to investigate various facets of breast cancer biology, progression, and treatment.

Chorioallantoic membrane model (CAM). Chorioallantoic membrane models are used in breast cancer research. This model leverages the highly vascularized extraembryonic membrane found in developing avian embryos, serving as a vital platform for studying tumor angiogenesis, metastasis, and drug delivery in the context of breast cancer (92, 93). The model originated in 1911 with the pioneering work of Rous and Murphy, who transplanted chicken sarcoma onto the vascularized chicken embryo (12). This model has since been recognized for its cost-effectiveness and time efficiency compared to mammalian *in vivo* models for pre-clinical oncological studies.

In breast cancer research, CAM models offer a unique opportunity to graft breast cancer cells or tumor fragments onto the CAM, allowing researchers to observe their interaction with the host vasculature (93, 94). This facilitates the study of angiogenic factors released by tumor cells, vascular recruitment to the tumor site, and the assessment of angiogenesis inhibitors or anti-angiogenic therapies (95).

Furthermore, CAM models enable the evaluation of breast cancer cell proliferation and response to treatment interventions. By administering anti-cancer drugs, targeted therapies, or experimental interventions, researchers can monitor tumor size and proliferation rates, and evaluate treatment effects (96). Various drug formulations, nanoparticles, or targeted delivery strategies can be applied directly to the CAM or can be embedded in the tumor graft to assess drug penetration, distribution, and therapeutic efficacy in a highly vascularized environment (97). The assessment of CAM models for imaging and analysis is straightforward, employing microscopy techniques to observe tumor growth blood vessels development, and the expression of molecular markers or protein patterns (93). However, it's crucial to note that while CAM models offer valuable insights, they do have limitations. They do not fully replicate the intricate tumor microenvironment and immune system interactions found in humans. Additionally, CAM models lack key immune responses and stromal components, which can influence tumor behavior, progression, and response to therapies (98).

Computational (*in silico*) Models of Mammary Cancer

Faced with all the disadvantages associated with *in vitro* and *in vivo* models, *in silico* models have been developed to perform biological investigations without employing biological entities. This approach offers greater control over experiments, allowing to test more parameters and variables, and eliminates the ethical concerns associated with *in vitro* and *in vivo* models (29). It is not necessary to obtain legal authorizations to perform *in silico* studies.

In silico models combine complex mathematical equations with powerful computational tools to simulate and analyze biological mechanisms. It is based on data collected from *in vitro* and *in vivo* studies, as well as modelling and prediction approaches (99). In recent years, *in silico* models have become increasingly important due to their ability to incorporate and assess large amounts of biological and clinical data. These models support the development of personalized treatment plans and provide valuable insights into the biology of breast cancer (100). Information from databases and previously published research allows the creation of simulations for key aspects of breast cancer, including tumor growth, metabolism, and solute transport in tumor tissues (29).

In silico models provide a computational framework to understand the intricacies of the disease, generate predictions, guide experimental design, and promote personalized medicine approaches. These models play a crucial role in breast cancer research (99), advancing our understanding of breast cancer biology and improving patient care. In 2018, Cava *et al*. optimized a computational method for uncovering novel drug target pathways in different cancer subtypes by inhibiting pathway crosstalk. Their findings suggested that pathway crosstalk inhibition could provide valuable strategies for identifying more personalized and effective treatments, particularly in heterogenous cancer diseases (101). Later in 2022, Uchida and Sugino employed a variety of bioinformatics tools to pinpoint genes associated with tumor progression, aiming to validate their potential as prognostic indicators or new therapeutic targets in breast cancer (102).

Top Research Discoveries in Breast Cancer

Recent advancements in breast cancer research have significantly enhanced our comprehension of this complicated disease, with many groundbreaking discoveries originating from animal studies involving animal models. These models are indispensable for unraveling the mechanisms underlying breast cancer initiation, progression, metastasis, and response to treatment. Animal models have played a pivotal role in identifying novel biomarkers associated with the growth and progression of breast cancer. Through analysis of tumor samples from these models, researchers have revealed critical molecular signatures and genetic alterations that drive tumor growth and dissemination (103). Over the past four decades, various animal models of breast cancer including PDX, CDX, and GEMs, have been employed to assess drug efficacy, identify biomarkers, and investigate resistance mechanisms (104-109).

Animal models have provided valuable insights into the tumor microenvironment and its role in breast cancer progression (110). Research in this area explores the intricate interactions between cancer cells and their surrounding microenvironment, offering potential targets for targeted therapies (110). In a study conducted by Tan *et al*. in 2023, the composition, structure, and functional significance of the extracellular matrix derived from breast cancer tumors of two molecular subtypes, luminal-A and triple negative breast cancer, were compared. This study, involving the surgical implantation of tumors into BALB/C-NU mice, illuminated the maintenance of phenotype-specific tumor extracellular matrix, its impact on treatment sensitivity, and its role in cancer progression (111).

Furthermore, animal models have facilitated the development of personalized medicine approaches for breast cancer treatment (1). By tailoring treatment based on individual tumor characteristics, genetic makeup, and patient response, personalized medicine allows for more precise and targeted interventions, optimizing therapeutic outcomes while minimizing side effects (112, 113).

While spontaneous and induced breast cancer models are rarely used for routine screening of anti-tumor drugs, transplantation and transgenic models are currently predominant. Xenograft models and GEMs are extensively employed to elucidate the underlying mechanisms of drug resistance, breast cancer pathogenesis and metastasis, as well as drug efficacy and toxicity (114).

In conclusion, animal models have played a critical role in advancing our understanding of breast cancer, leading to numerous significant discoveries. These findings have not only deepened our comprehension of the disease but have also paved the way for the development of novel treatments and customized treatment plans for breast cancer patients.

Conclusion

The choice of an appropriate animal model for breast cancer research depends on the specific aims and research questions. It is important to define the objectives of the study before selecting the suitable model. The choice of model depends on whether the objective is to investigate initiation, progression, metastasis, drug testing, genetic factors, or specific aspects of breast cancer biology. Additionally, the complexity of the chosen model should also be considered. In vitro models offer controlled conditions but lack the *in vivo* context. *In vivo* models, such as spontaneous models, entail long latency periods, while induced models are unpredictable and carry risks. GEMs provide a highly complex and *in vivo*-like environment. Conversely, *in silico* models enable simulations and predictions.

Furthermore, researchers should evaluate the resources and budget available for the research, as some models may be more cost-effective than others. Validation against experimental data is crucial to ensure the accuracy and relevance of the chosen model to the specific research question.

Different models possess unique advantages and limitations, and they can often complement each other. Integrative research utilizing multiple models may offer a more comprehensive understanding of breast cancer and its underlying mechanisms.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

All Authors contributed to the concept and design. Data collection and analysis were performed by Jessica Silva. The first draft of the manuscript was written by Jessica Silva and all Authors commented on previous versions of the manuscript. All Authors read and approved the final manuscript.

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