Alternatives to Animal Use and Testing in Breast Cancer Research

A Senior Honors Thesis

Submitted in Partial Fulfillment of the Requirements

for Graduation in the Honors College

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December 17, 2021

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Abstract

While animals have played a key role in scientific research for many years, there are limits to the knowledge that can be gained through this model. Recently, alternatives to animal use were created as models for human cancer research. This thesis discusses the current uses of animals in breast cancer research and what has been learned from them, as well as the alternatives that are becoming increasingly available. The drawbacks of both animal use and alternatives were discussed and how these affect breast cancer research. Alternatives were found to be functional in breast cancer research and in many cases even more reliable than the animal models.

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Introduction

Animals have been used in laboratories as models for humans for years but recent studies have used alternatives instead, suggesting that they may be a viable option for use in a research setting, specifically breast cancer research. Animal research was first used by the ancient Greeks[1] and has since been used in almost every lab and in many different disciplines to solve the world's biological questions. Breast cancer affects about 1 in 8 women in their lifetime and has the highest death rate besides lung cancer[2]. While we have found treatments for many types of cancers, we have yet to find a cure through the use of animal research. This suggests that alternatives might shed new light on breast cancer research, offering new treatments and possibly even a cure.

In Russell and Burch's paper *The Principles of Humane Experimental Technique* [3], they discuss the use of animals in research and introduce the Three R's: Replacement, Reduction, and Refinement. Reduction and refinement are important considerations in animal use in labs, however this essay will focus on the ways in which replacement can better the study and treatment of breast cancer. Russell and Burch[3] define the replacement technique as "any scientific method employing non-sentient material which may in the history of experimentation replace methods which use conscious living vertebrates." This definition astutely describes what this essay will focus on in terms of the alternatives to animal use in breast cancer research.

To begin, the current uses of animals in breast cancer research will be discussed as well as what has previously been learned from these animal uses. Then the available alternatives and their uses in breast cancer research will be covered. Finally, the viability of these alternatives will be discussed along with a comparison of the pros and cons of the alternatives and the current uses of animals.

Animal Use in Breast Cancer Research

Animals have long been used as research subjects for several reasons, not the least of which being that it is easy to procure them. They are readily available to scientists that want to use them and many are bred specifically for use in labs. The cost of setting up a lab for animal testing can be expensive when buying all new equipment but many labs are already equipped for animal testing, avoiding these setup costs [4]. Animals can be purchased cheaply, but the cost of maintaining them can be high. Paying vet staff and the cost of food and bedding will also add to the total cost. On top of the direct cost, there are important regulations that must be followed when animals are used in laboratories. Most research institutions have lists of requirements for the care and housing of lab animals that include specifics about ventilation, temperature, humidity, light, and noise within their housing [5]. If the lab doesn't already meet these specifications, the indirect cost of building renovations may be excessive. There are also federal and state laws that need to be followed by any laboratory that uses animals for research purposes. The regulations for animal care in the laboratory aim to avoid unnecessary suffering of the animals and must be followed for their use in research to be legal [5].

Animals have been used specifically in cancer research as they allow the scientist to see how cancer affects not just the body part it has grown in but also the rest of the body, something that is hard to replicate outside of the body. Mice are the most heavily utilized species and have been used to look at specific parts of the progression of breast cancer. Using mice, scientists have isolated specific cellular processes that relate to the spread of breast cancer. Overall, the mouse model has taught scientists a lot about breast cancer, how it grows, and spreads through metastasis. The mouse model, while not a perfect replica of the human patient, can be used to gain much understanding of breast cancer.

Metastasis of breast cancer is the cause of a lot of complications and death in breast cancer patients. Animal analogs can be used to show how cancer metastasizes throughout the whole body, not just in the initial organ. Breast cancer doesn't just affect the breasts so all parts of the body must be taken into account in order to properly treat the disease. Su et al. [6] performed an experiment on mice that showed metastasis when they injected breast cell cultures into the tail vein of the mouse. Lung metastasis was found after 4 weeks showing the movement of the cancer cells from the tail to the lungs. Uses of the mouse model have yielded a greater understanding of the mechanisms of cancer metastasis and progression. Mice are frequently used given the similarities between mouse and human genetics. Mice can be given breast cancer through xenograft transplantation, which is the injection of human cancer cells into an immuno-compromised or genetically modified mouse [7]. The mouse will then grow a tumor in the area where the cancer cells were injected for study. This allows a scientist to have greater control over what type of cancer the mouse gets as well as the specific cell line the injected cancer comes from. If the scientist is trying to look at a specific type of breast cancer or cell line, then this is possible using the mouse model. The genetically modified mouse can also be used to study the role of specific genes in the creation and destruction of cancer cells [7]. Mouse tumor cells can also be labeled with fluorescent protein markers and then observed through intravital imaging technology, allowing scientists to observe in real time the movement of the cancer cells [8]. The mouse model can be used to better understand the communication process that goes on between the tumor and the part of the body where the tumor occurs [8].

Another animal used frequently in breast cancer research is dogs. Dogs are not thought of as similar to humans but canine mammary cancer has many similarities to human breast cancer. Breast cancer can form in dogs spontaneously, similar to humans, and therefore the tumor doesn't need to be genetically modified, as in most mouse models [9]. Canine mammary tumors also have many similar biomarkers to human breast cancer [10]. Some of these biomarkers have been found to link to the malignancy of the tumor. By examining the biomarkers present in both human and canine cancers, scientists can determine if the cancer is malignant or benign [11]. These similarities led to the use of human drugs in the treatment of dog cancers and vice versa. Drugs used to treat human breast cancers have already been used on dogs to treat both breast and lung cancers [12]. The dogs used in this study were pet dogs with naturally occurring breast cancer that were not bred in the lab. This means that the genetics of the pet dogs are unknown compared to the specifically bred mice from labs whose ancestors and genetics are known.

Despite the knowledge gained from animal use, there are several downsides to animal use. An animal's body differs from that of a human body in the crucial areas of drug metabolism, immune system, and genetics. As Leonard et al. [13] stated, the microenvironment of the tumor (breast or other) is dependent on the host. The environment within a mouse's body is necessarily quite different from that of a human patient. While many of the enzymes necessary for drug metabolism are conserved across species, there are minor differences between the amounts of these enzymes that can affect how well a drug works between species [14]. Drugs that treat breast cancer in mice may have no effect on dogs or humans [14]. This is a major risk that comes with using the animal model. The mice used in labs have been immunosuppressed, meaning that their immune systems don't react to the cancer. This is not realistic to the human progression of cancer as the human immune system is usually not suppressed in a cancer patient and even plays a part in the metastasis of cancer, giving the tumor more pathways for metastasis that are not found in the mouse model [9]. Even primates, which are most similar to humans, differ enough that any treatments or research done on primates cannot be guaranteed to work on humans. On top of these issues, many experiments involving animals are too small to draw accurate conclusions and are not easily replicable [15]. These problems highlight the need for alternatives to animal use which allow for experiments that better replicate the cancer being researched.

Alternatives Available

There are many different alternatives available to scientists and researchers today in lieu of using animals. This section will discuss six alternatives. Cell lines, organoid cultures, and computational methods were found by the director of the Johns Hopkins Bloomberg School of Public Health Center for Alternatives to Animal Testing to be the most promising alternatives [16]. Other alternatives currently in use include collagen based scaffolds, bioprinting, 'organ on a chip' or microfluidic devices, and genetics testing. All of these alternatives will be discussed along with how they can be used in breast cancer research and how they can help to advance this area of research.

Cell Culture

The first alternative to be discussed is grown cell lines or cell cultures which are cells that have been grown in a petri dish in conditions and solutions that mimic the environment of the human body's cells. This has been a common alternative for years and there are many cell lines available for use. Cell cultures have mainly been used to study specific parts of the cells. Cell lines are used in a similar way in cancer research. Cell cultures are used to study the pathways and parts of cancer metastasis as opposed to looking at the whole tumor or how to treat the whole cancer [17]. Finn et al. [18] used breast cancer cell lines to study specific cell cycle regulators and their effect on the progression of breast cancer. Breast cancer cell cultures are not grown in a body, like a tumor is and in this way they do not resemble how a real tumor will behave and grow [8]. While cancer cell lines have been useful for learning about cancer mechanisms overall, induced pluripotent cells are useful to understand specifics of an individual patient's unique cancer. Induced pluripotent cells are cells that were taken from a human patient, usually from the cheek or skin, and changed back into a form equivalent to embryonic stem cells; which can be grown indefinitely in culture [19]. This is important to research as these cells can be cancer cells isolated from a patient, allowing for greater specificity in the treatments that are created using those cells. Cell lines are not without fault and cannot fully encapsulate the effects of cancer on the body or how body systems, like the immune system, influence cancer. Additionally, cell lines are two-dimensional while tumors are three dimensional. Finally, treating tumors in a patient is much more complicated than treatment in a cell culture given the rest of the body systems involved. While they are not an all encompassing research alternative, cell lines give scientists a close up look at the cancer cells and their specific mechanisms. This information helps scientists to better understand breast cancer, which will lead to better treatments and maybe even a cure.

Organoid Cultures

Organoid cultures are another promising alternative to animal models that offers a look into how breast cancer works and how it can be treated. Organoids are in vitro tissue constructs that are used to mimic an organ in the human body in the treatment of diseases and in this case, cancers. Similar to cell cultures, organoids can be made using induced pluripotent cells or adult stem cells that are taken from the patient. Organoids are grown in a petri dish through a series of specific and timed changes to the environment involving the addition of growth factors, nutrients, and signaling molecules. Other molecules are added to differentiate the cell and cause it to grow into the intended organ [20]. This allows the cells to grow into an organ without the assistance of a scaffold as they self-organize into the structure [8]. The organoid is considered to be a small organ that can be used to study the mechanisms of diseases in that organ with the specificity of the patient whose cells were used. These realistic replica organs mimic those of the in vitro host cells allowing for the study of specific interactions with that organ. This has applications in the creation of treatments as well as research into the development of cancers [21]. The organoid can show the researcher how the treatment affects the whole organ and how the cancer spreads in the organ without the use of human or animal subjects [20]. This method accounts also for the tumor microenvironment within the organ and may lead to more specific breast cancer treatments, maybe even specialized for that patient's specific cancer type [22]. Organoids offer researchers a look into the working of an organ through a small replica that can be controlled and manipulated to learn more about how a disease affects an organ.

Three Dimensional Cultures

Collagen based scaffolds are similar to organoids but are grown on three dimensional culture platforms made using collagen [21]. Collagen is a structural protein in animals that is integral to the structure of the extracellular matrix (ECM) and can be used as a scaffold or frame for other cells to grow on. The ECM is integral to tumor growth and cell-ECM interactions can drive tumorigenesis and metastasis [23]. The collagen scaffold doesn't just act as a holder for the cells but actually plays a role in the creation of structures and regulation of cells in the ECM [24]. Collagen is an important factor in the development of cancers so the use of collagen in this alternative can offer a more realistic view of the growth of a tumor in an area of the body [21]. The interactions between the cancer cells and the ECM are important to cancer progression and different cancer types can change how the collagen acts in the ECM, so without the ability to see what role the ECM plays in cancer development, researchers are not getting the full picture of what's going on. Collagen can be purified from a variety of sources but is usually taken from animals, especially fish, after they have been used in the agricultural and food industries, as the leftover bones and skin contain collagen [24]. Human collagen has also been used but is harder to procure without human volunteers. The most common technique involves growing collagen on hydrogel, which is a growth platform that allows for some versatility in the porosity and stiffness of the collagen to help mimic the ECM in a human body [21]. There are a couple of other techniques to make collagen based scaffolds that mimic different organs and parts of the body which researchers can then use to study the mechanisms of disease in a specific organ.

Bioprinting

Bioprinting offers the advantage of three-dimensionality, similar to collagen based scaffolds, to show a more accurate portrayal of how cancer grows and spreads in the body. Leonard et al. [13] discuss the use of bioprinting and how the microenvironments around a tumor can have a major effect on the growth and response of the tumor to treatments and therapies. This is an area where alternatives are superior to traditional animal use because the use of animals cannot properly replicate the microenvironment around a human tumor. There are several different ways to bioprint but there are three main types currently in use. These are ink-jet based printing, microextrusion, and laser-assisted bioprinting [25].

Inkjet bioprinting uses extremely small "ink" droplets made of cells that are ejected onto a medium and then grown [26]. This form of printing was created from modified desktop inkjet printers [27]. It allows for precise tissue engineering in the bioprinted cells. The cells can also be ejected onto a scaffold to create the shape of an organ which can then be grown. There are three different types of printing, piezoelectric, thermal, and electromagnetic. Thermal and piezoelectric are most commonly used given their high cell viability [27]. In piezoelectric printing the cell ink droplets are ejected from the printer through a piezoelectric-based apparatus which uses acoustic waves to propel the cells out and onto the medium of choice [28]. In thermal printing, the nozzle is heated momentarily which creates an air bubble that pushes out the cells [27]. Inkjet bioprinting is low cost and widely available, while there can be low directionality for the droplets, the print speeds are high, so this form of printing has plenty of potential [28]. Since the cells are mechanically extruded from a nozzle there is the chance of increased cell death from this mechanical stress. In breast cancer research, this technique has potential to offer the ability to print out a breast tumor for testing. The "ink" can be made using primary cells, or cells taken from a patient [27]. This allows scientists to print tumor tissue from a patient's cells which can be used to test drug effectiveness.

Microextrusion bioprinting is similar except that the bioink is extruded through a nozzle using a mechanical or pneumatic force [25]. Instead of using droplets like in ink-jet printing, microextrusion uses a continuous stream of cells [25]. These streams can be layered to form a tissue which can then be used for research [27]. The resolution of this form of printing is lower and can only be used for larger, hard tissues. This reduces the usefulness of this form of printing. The other downside is the use of mechanical force, which causes a higher rate of cell death during the printing process [28]. This form of printing is thus less advantageous to breast cancer research but could still be used to print breast tissue.

The last type of bioprinting is laser-based bioprinting, a type of bioprinting that uses UV light to cure a layer of cells [25]. This process doesn't need a nozzle, so it negates issues with the last two forms in which the cells were killed during the ejection through the nozzle. A scaffold made of collagen is dipped into a container of bioink then brought back out and exposed to UV light. The light polymerizes the cell layer and can be repeated for as many layers as wanted [28]. There are several different modifications that can be added to the light source to allow for a more focused light that only polymerizes certain cells in a layer, leading to a specific pattern of cells [27]. This bioprinting type can be utilized to grow breast cancer tissues that can then be tested on. This system allows for the building of tissues without limitations on the type of bioink since it does not need to be extruded through a nozzle [28]. The main downside to this system is the use of UV light which can be damaging for some cells.

Organ-on-a-chip

Another alternative is 'organ-on-a-chip' (OOAC), an analog of an organ which is grown on a microfluidic chip. A microfluidic chip is a surface that allows the precise manipulation and control of fluids through the use of microscopic channels [29]. A scientist can use this chip to control the precise physical and chemical properties of the environment in order to simulate human physiology [29]. Similar to the previous alternatives, the organ is then grown on the microfluidic chip in conditions that closely resemble that of the human body. Through the use of these OOAC systems, a researcher can test breast cancer drugs on a grown breast cancer tumor which will accurately portray the results in a human body. This has uses in breast cancer drug testing, however, scientists realized that OOAC lacked the ability to simulate the interconnected responses of the whole body. To combat this, they created multi-organ-on-chip (MOC) systems. These contain several different OOACs that are connected via channels or fluidic networks [29]. These MOCs allow scientists to look at the effect of a tumor on the whole body, not just one organ [30]. MOCs help to visualize the pathways of metastasis and allow for the testing of drugs to combat it. OOACs and MOCs are more consistent in their similarity to human anatomy than mice and are more reproducible given the fact that human cells are used [31].

Early Diagnostic Genetics

Early diagnostic genetics is a large field of emerging sciences that aims to look at the base code of a person or their DNA to predict anything from hair color to their susceptibility to illness. Recently, genetics has been used as a way to preemptively find the threat of breast cancer before it has even grown. This alternative doesn't involve the use of a wet lab or cells while still offering an advantage to cancer research. Genetics is a noninvasive way to test specific patients. Samples of cells can be taken from the patient without invasive procedures and tested to determine their risk of breast cancer. There are two main gene mutations that are known risks for breast cancer. These are the BRCA1 gene and the BRCA2 gene. These two genes play a role in the creation of proteins that repair DNA so when they are mutated, there is a potential for more mutations in the DNA which can lead to cancer [32]. In one study, breast cancer was linked to the BRCA1 gene in 52% of cases and the BRCA2 gene in 32% of cases [33]. The American Cancer Society says that women with the BRCA1 or BRCA2 gene mutation are more likely to develop breast cancer at a younger age and have a 7 in 10 chance of developing breast cancer by 80 [32]. These increased risks can be assessed at a young age through genetic testing and allow for increased awareness in the patient and their doctor. Genetic screening for breast cancer can lead to early detection in at risk patients which saves lives. Genetics can give scientists an idea of what genes affect cancer which can be used for testing, early diagnosis and treatment.

Viable Options for Cancer Research

After examining the current use of animals in breast cancer research and the alternatives available, we return to the main question: are the alternatives better for use in breast cancer research than animals? This question is not an easy one to answer. One approach to an answer to this question is to look at the most common methods that are used by the research community. BioMed Central was searched using the key words “breast cancer”. This resulted in the 10 most recent studies published relating to breast cancer research. These included two studies using human cell cultures, two using genetics and biomarker research, three using patient studies, one using bioinformatics, and two using the mouse model. This gave the impression that the mouse model, while still used, is not as widely utilized compared to the alternatives in current breast cancer research. Genetics seems to have taken a front seat in this area of research, as a look into the next ten most recent studies shows four more studies focused on genetics research surrounding breast cancer.

There are several possible reasons why animals are still sometimes chosen over alternatives in research. First, alternatives may not be as widely available to researchers. Gruber et al. [15] said that many alternatives stay in the labs where they were created and added that there need to be incentives for scientists to use the alternatives instead of animals. Gruber et al. [15] also said that there needs to be a financial incentive to use alternatives to animals in research as well as a wider database of information on the available alternatives and how to use them. The research discussed throughout this paper suggests that the database for alternatives has grown since Gruber et al. wrote about this in 2004 but there are still alternatives that are hard to get as they have only been used in a couple labs. However, since 2004, the cost of procedures like gene sequencing has decreased to a much more reasonable and affordable price. While the cost of lab equipment can be expensive when first purchased, many labs already own the necessary equipment and thus can avoid these costs. With these financial barriers mostly surmountable, alternatives have become more popular in labs and are now used more than animals, as shown by the 10 most recent studies on breast cancer.

Conclusion

More and more alternatives to animal use are becoming available for research. This paper has only touched the surface of possible alternatives but when looking at the alternatives focused on in this paper, we can see that they are useful for research and have been used in breast cancer research as well as other forms of research. While animals will probably always be used in some capacity in the lab, these alternatives offer more accurate and reproducible options that don't cost any more to the scientist. This research shows that the alternatives that are currently available offer a more human centered approach to research that allows the use of a human patient's cells and may even lead to personalized medicine for each patient. We have gained a wealth of useful knowledge from the mouse model but alternatives offer new ways to learn more about specific diseases and how they affect the human body.

As discussed throughout this paper, there are downsides and problems to each type of research model and this holds true to alternatives as well. However, if these barriers can be overcome, there is evidence that these alternatives will offer more reliable and reproducible research models for many diseases, including breast cancer, as well as the creation of new treatments.

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