Breast Cancer

Tumor Microenvironment and Biology

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Edited by **Sandra Lekin**

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Preface

Every book is initially just a concept; it takes months of research and hard work to give it the final shape in which the readers receive it. In its early stages, this book also went through rigorous reviewing. The notable contributions made by experts from across the globe were first molded into patterned chapters and then arranged in a sensibly sequential manner to bring out the best results.

The causes of cancer are diversified. Cancer is the principal reason of death in the majority of countries and it results in enormous financial, societal and mental burden. Breast cancer is the most diagnosed kind of cancer and the foremost reason of death due to cancer amongst women. This book deals with various aspects of breast cancer. It discusses topics related to breast cancer cell lines, classification of tumors and stem cells. It also elucidates the microenvironment of breast cancer. It intends to help students and experts in gaining more knowledge regarding the topic.

It has been my immense pleasure to be a part of this project and to contribute my years of learning in such a meaningful form. I would like to take this opportunity to thank all the people who have been associated with the completion of this book at any step.

Editor

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Part 1

Breast Cancer Cell Lines, Tumor Classification, *In Vitro* Cancer Models

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Breast Cancer Cell Line Development and Authentication

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1. Introduction

Inarguably, the development of cell culture and the ability to grow human cells *in vitro* has revolutionized medicine and scientific research. In the nearly sixty years since the first successful culture of immortalized human tumor cells in the lab in 1952, new fields of research have emerged and new scientific industries have been launched. Without cell lines, medicine would not be as advanced as it is today. Modern techniques that allow for manipulation of cell have allowed for a more complete understanding of the of fundamental basics of cellular and molecular biology and the biological system as a whole.

Different types of cell lines exist. Lines are maintained as continuous cultures, are established as primary cultures for transient studies, are created as explants of tumor or tissue samples, or cultivated from a single individual cell. Cell lines, especially cancer cell lines, are ubiquitous and are used for everything. By using cell lines, our understanding of cells and genes, how they function or malfunction, and how they interact with other cells has increased the pace of discovery and fundamentally changed how science is conducted. Cell lines have been established as a model of specific disease types. Individual cell lines have been derived from specific disease states and therefore possess specific characteristics of that disease state. Therefore, they are exceptionally useful to gain insight into normal physiology and how that physiology changes with onset of disease. Novel treatments and therapeutic strategies are investigated in cell lines in order to gain a fundamental detailed understanding of how a cell will react. Initial protocols are developed and tested in cell lines prior to use in animal models or testing in humans. This has enormous implications in discovery and reducing unintended side effects.

The first breast cancer cell line was established in 1958. Today, lines modeling the varied types of breast cancer help to develop targeted therapy and to provide a molecular signature of gene expression. Cell lines of estrogen/progesterone receptor (ER/PR) positive, ER/PR negative, triple negative (ER/PR/Her2), normal mammary epithelium, metastatic disease, and more are so widely used that it is nearly impossible to identify a recent discovery that hasn't used cell line models at some point during development.

Unfortunately, significant shortcomings of the use of cell lines exist. Cell lines are a model system. They do not always predict the outcome in humans and therefore, do not replace use of whole organisms. They are grown and tested in isolation, therefore the influence of neighboring cells or organs is non-existent in cell culture systems. Over time, cells can differentiate resulting in a change in phenotype from the original culture. Cell lines can

become contaminated by infectious agents such as mycoplasma or even by other cell lines. Such contamination may not be readily detectable and can result in dramatically different results leading to false or irreproducible data. Some of these issues can be addressed to thwart the waste of reagents, money, and time. This includes testing and authenticating cell lines while they are actively grown and in use in the lab. Companies exist that can test for mycoplasma infection or DNA fingerprinting of cell lines to authenticate a particular cell line. Other shortcomings are merely inherent to this model system and must simply be identified and addressed.

2. A brief history of cell culture

Since the first successful establishment of a human cancer cell line in 1952, cell lines have been the backbone of cancer research. They have provided the understanding of systems at the molecular and cellular levels. Cell lines are used in the vast majority of research labs to understand the fundamentals of basic mechanisms as well as the translation to clinical settings.

Modern tissue culture techniques were made possible through the contributions of many scientists across the world whose attempts to understand physiology and to establish a source of tissue to study lead to fundamental changes in our understanding of biology and medicine. Among the contributions include those of Sydney Ringer at the University College London, who determined the ion concentrations necessary to maintain cellular life and cell contractility, and ultimately created Ringers Solution. Through his seminal work in the 1880s, Ringer described the concentrations of calcium, potassium and sodium required to maintain contraction of a frog heart and began the steps towards modern day cell culture (Miller, 2004; Ringer, 1882, 1883). In 1885, Wilhelm Roux at the Institute of Embryology in Germany cultured chicken embryonic tissue in saline for several days. This was followed by the work of Ross Harrison at the Johns Hopkins University in 1907, who was the first to successfully grow nerve fibers in vitro from frog embryonic tissues. While this was the outgrowth of embryonic tissue, these tissue cultures were successfully maintained *ex vivo* for 1 - 3 weeks (Skloot, 2010)(Ryan, 2007b). In 1912, Alex Carrel at the Rockefeller Institute for Medical Research successfully cultured the first mammalian tissue, chicken heart fragments. He claimed to maintain beating chicken heart fragments in culture for over 34 years and outliving him by one year (Ryan, 2007a). Although controversy as to whether these cultures were authentic or supplemented with fresh chicken hearts still remains (Skloot, 2010). This controversy may have slowed progress towards the establishment of cell lines in culture to some degree, it did not prevent work to create a source of material and model systems to allow for testing *in vitro*.

It would be another 40 years before the establishment of the first continuously growing human cell line, however steady advances towards that goal were ongoing. Carrel, working with Charles Lindbergh, worked to create novel culturing techniques that included use of pyrex glass. This glass could be heated and sterilized to reduce, or preferably eliminate, bacterial contamination. This led to the creation of the D flasks in the 1930s which improved cell culturing conditions by reducing contamination (Ryan, 2007c).

Tissue culture took another leap forward in 1948 when Katherine Sanford at Johns Hopkins was the first to culture single mammalian cells on glass plates in solution to produce the first continuous cell line (Earle et al., 1943; Sanford et al., 1948). Prior to this, tissues were attached to coverslips, inverted and grown in droplets of blood or plasma.

Her work set the stage for modern practices of growing cells in media on plates or flasks (Sanford et al., 1948).

2.1 Establishment of the HeLa cell line and cell line production

Indoubtedly, the most important factor to change biomedical research and our understanding of disease at the cellular and molecular levels was the establishment of the first continuously growing human cell line, the HeLa cell (Gey et al., 1952). In 1952, Henrietta Lacks was a patient with adenocarcinoma of the cervix treated at the Johns Hopkins Hospital. A portion of her tumor was used in the laboratory of George Gey at Johns Hopkins University and the revolution of modern biomedical research began. These cells were grown in roller flasks in specialized medium containing serum developed by Evans and Earle et al. and continued to proliferate (Evans et al., 1951). Almost 60 years later, these cells are still proliferating in laboratories across the globe and used to increase our understanding of cellular mechanisms from cell signaling, to the implications of weighlessness/zero gravity on cellular aging, and everything in between. The implications of establishing this cell line have been tremendous and is still ongoing. HeLa cells have not stopped growing and neither has the vast amount of knowledge gleened from them.

In 1953, Gey demonstrated that HeLa cells could be infected with the polio virus and therefore were a useful tool for testing the efficacy of the polio vaccine that was under development. This set the stage for the mass production of cell lines for distribution and use worldwide. The National Science Foundation established the first production lab at the Tuskegee Institute in 1953 that would provide HeLa cells to scientists involved in the development of the polio vaccine (Brown and Henderson, 1983). The goal was to ship at least 10,000 cultures per week. At the peak of production, 20,000 cultures were shipped per week and a total of 600,000 cultures were shipped in the two years the lab was in existence (Brown and Henderson, 1983). This, along with the Lewis Coriell's development of the laminar flow hood to reduce contamination of cell cultures and methods to freeze and recover cell lines (Coriell et al., 1958; McGarrity and Coriell, 1973, 1974)(Coriell and McGarrity, 1968; Greene et al., 1964; McAllister and Coriell, 1956; Silver et al., 1964), led to the establishment of cell repositories to house and distribute cells. It also led to the development of tumor specific cancer cell lines that created models of different types of human cancer and to an explosion of understanding of how cells work without the influence or perturbation of other cells. These models were also an ideal system to test novel therapeutics and treatment strategies without use of whole animals or humans.

2.2 Culturing cells

The terms tissue culture and cell culture are used interchangeably, but in reality they are two distinct entities. While both methods are derived from specific cells isolated from the whole organism, the cultures established are quite different and used for different endpoints (Freshney, 2010a).

Tissue, or primary, cultures are established from isolated tissue or organ fragment, most commonly from tumor slices (McAteer and Davis, 2002). These primary cultures can be used either for immediate experimentation to determine how primary cells operate or to establish a continuous cell line. Generally, primary cultures are established through placing an organ explant into culture media and allowing for outgrowth of cells or by digesting the tissue fragment using enzymatic or mechanical digestion. By definition, these cultures are

transient. Primary culture refers to the period of time the primary tissue/organ fragment is kept in culture *in vitro* prior to the first passage or subculturing of cells, at which time they are referred to as a cell culture. This could range from days to a few weeks at most (MacDonald, 2002).

Cell lines are primary cultures that have been subcultured or passaged and can be clonal, terminal or immortalized cells (McAteer and Davis, 2002). Clonal cell cultures are created by selecting a single cell that will proliferate to establish a single population. Terminal cell lines are able to grow in culture for a few generations before senescence occurs and the cell line can no longer survive in culture media. Immortalized cell lines are able to grow in culture forever. These immortalized cell lines can occur naturally, such as HeLa cells, or through transformation events, such as Epstein-Barr Virus transformation. All types of *in vitro* cell cultures are used in breast cancer research.

3. The establishment of human breast cancer cell lines

The first human breast cancer cell line, BT-20, was established by Lasfargues and Ozzello in 1958 from an explant culture of a tumor slice from a 74 year old caucasian woman (Lasfargues and Ozzello, 1958). These cells are estrogen receptor alpha (ER) negative, progesterone receptor (PR) negative, Tumor Necrosis Factor alpha (TNF-α) positive, and epidermal growth factor receptor (EGFR) positive (Borras et al., 1997). While BT-20 is the oldest established breast cancer cell line, it is not the most commonly used line. By far, the most widely used breast cancer cell line worldwide is the MCF-7 cell line (Table 1 and Figure 1)(Burdall et al., 2003). Established in 1973 by Soule and colleagues at the Michigan Cancer Foundation, from where it derives its name, MCF-7 cells were isolated from the plural effusion of a 69 year old woman with metastatic disease (Soule et al., 1973). Since its establishment, MCF7 has become the model of ER positive breast cancer (Lacroix and Laclercq, 2004). Establishment of other cell lines has followed, including ones from other breast cancer types such as BRCA mutant, triple negative, HER2 overexpressing, and those derived from normal mammary epithelial cells such as MCF-10A cells (Soule et al., 1990) (Table 2).

Cell line use in labs is ubiquitous and continues to increase. From 2000 - 2010, the publication of manuscripts using the 10 most commonly used cell lines has almost tripled (2.8% increase) (Figure 2). Clearly demonstrating that the importance of, need for, and use of breast cancer cell lines will not diminish in the near future. Evaluation of the existing lines indicates that most breast cancer cell lines in use are derived from metastatic cancer and not other breast cancer phenotypes (Borras et al., 1997). Indeed, the overall success rate of establishing a cell line is only 10%. Most of the cell lines that exist today have been derived from pleural effusion instead of from primary tumors and are primarily ER - lines (Table 2 and reviewed in (Lacroix and Laclercq, 2004). This is surprising since ER - breast cancer is detected in only 20 - 30% of all primary tumors, whereas ER + tumors are detected 55-60% of the time (Ali and Coombes, 2000; McGuire et al., 1978). The reason for this discrepancy remains unknown, however it has been postulated that this could be because ER - cells are easier to establish in culture than ER + or that as cells are grown in culture, the epithelial like phenotype is lost while more mesenchymal traits are retained, therefore cells in culture appear to undergo a endothelial to mesenchymal transition (EMT) *in vitro* which is associated with the ER - phenotype (Lacroix and Laclercq, 2004). This suggests that culture systems are a model of metastatic disease that can grow in isolation and not a model the

wide heterogeneity of disease that is detected clinically. Although current cell lines are derived form only a subset of primary cancers, overall these lines are a reliable model to study the fundamental questions concerning cell growth, death, and the basic biology of breast cancer. Indeed, many advances in breast cancer biology have been made using cell culture systems and should not be dismissed because of these concerns.

Cell line	No of publications 1/1/2000 to 12/31/2010	origin
BT-20	79	breast
MCF7	11813	pleural effusion
MDA-MB-231	3489	pleural effusion
MDA-MB-435 *	719	pleural effusion
MDA-MB-468	486	pleural effusion
SkBr3	372	pleural effusion
T47D	1168	pleural effusion
ZR75.1	96	ascites
BT474	251	pleural effusion
MCF-10A	451	subcutaneous mastectomy
* not a breast cancer cell line		

Table 1. List of commonly used cell lines, the number of citations and their origin

3.1 Breast cancer cell lines as models of primary tumors

Using breast cancer cell lines clearly hold advantages over use of animal or human models. Beyond the ethical implications of animal or human use, the advantages to using cell lines include the ease of obtaining cell lines (can be purchased from commercial sources), the ease of harvesting large numbers of cells (can be grown in culture for long periods of time to accumulate the necessary concentration), and the ability to test an individual cell type without confounding parameters such as other cell types or local microenvironment (to date, no two cell lines can grown simultaneously in culture for extended periods). Conversely, much debate has circulated concerning the applicability of the data derived from isolated cell lines to the predicted outcomes in humans. One area that this debate has been most contentious has been regarding the importance of the immune system in cancer development. Clearly, the microenvironment and infiltrating immune cells contribute to development and progression of disease, therefore individual cells grown in isolation will lack the influence of other neighboring cells (Voskoglou-Nomikos et al., 2003). Genetic, epigenetic and cytotoxicity studies that focus on outcomes in breast cells clearly benefit from use of cell culture systems. The fundamental understanding of the underlying genetic or molecular pathways involved in breast cell growth and its response to cytotoxic agents are best understood in isolated cell culture systems (Voskoglou-Nomikos et al., 2003).

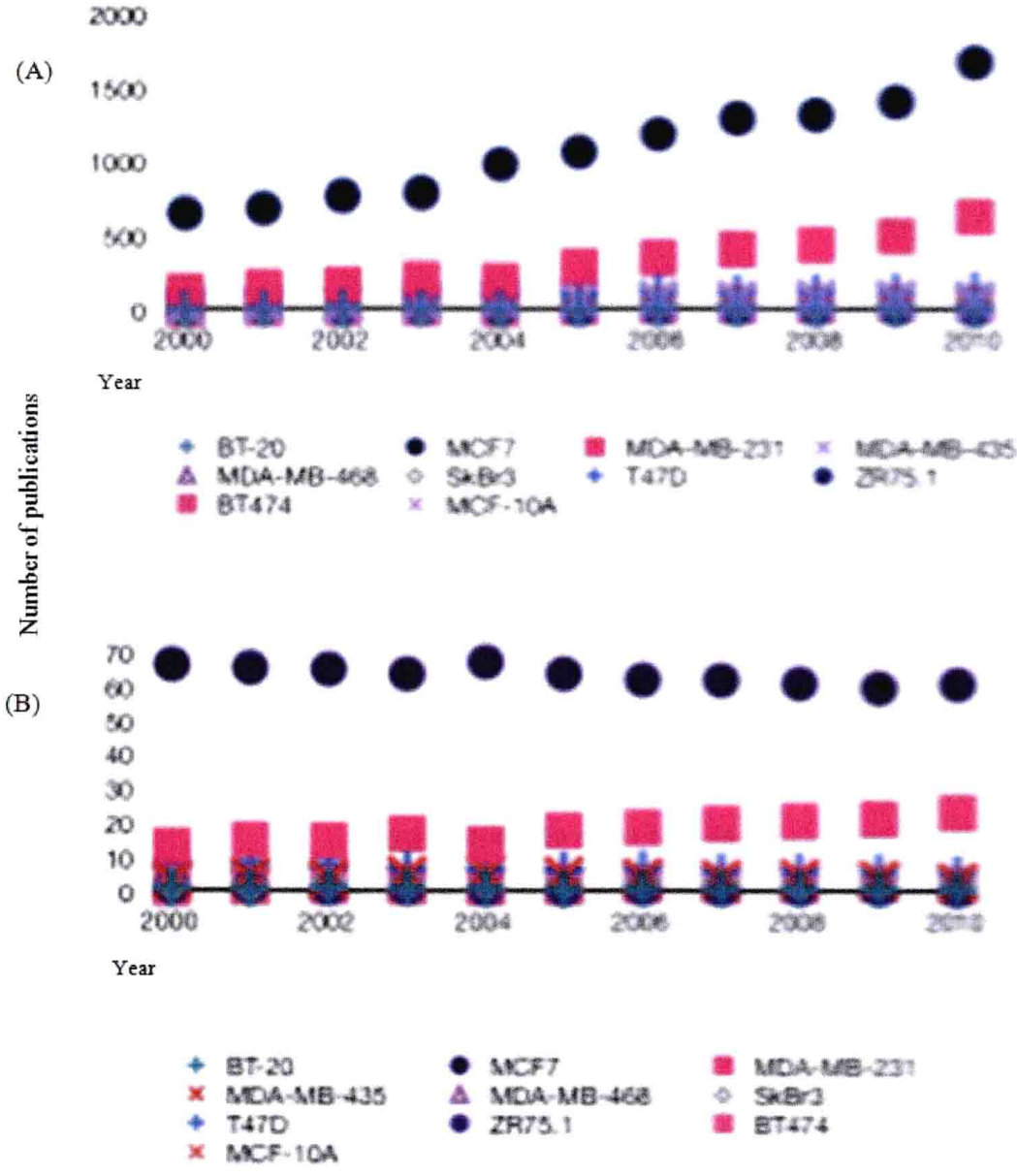

Fig. 1. The total number of publications per breast cancer cell line from 2000 through 2010. The most commonly used cell line is the ER+ MCF7 cell line, followed by ER - MDA-MB-231 cell lines. Many other cell lines are in use, however the number of publications using these models is quite small. A. Total number of publications using breast cancer cell lines. B. Each breast cancer cell line as a percentage of the total breast cancer cell lines used per year.