1

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).

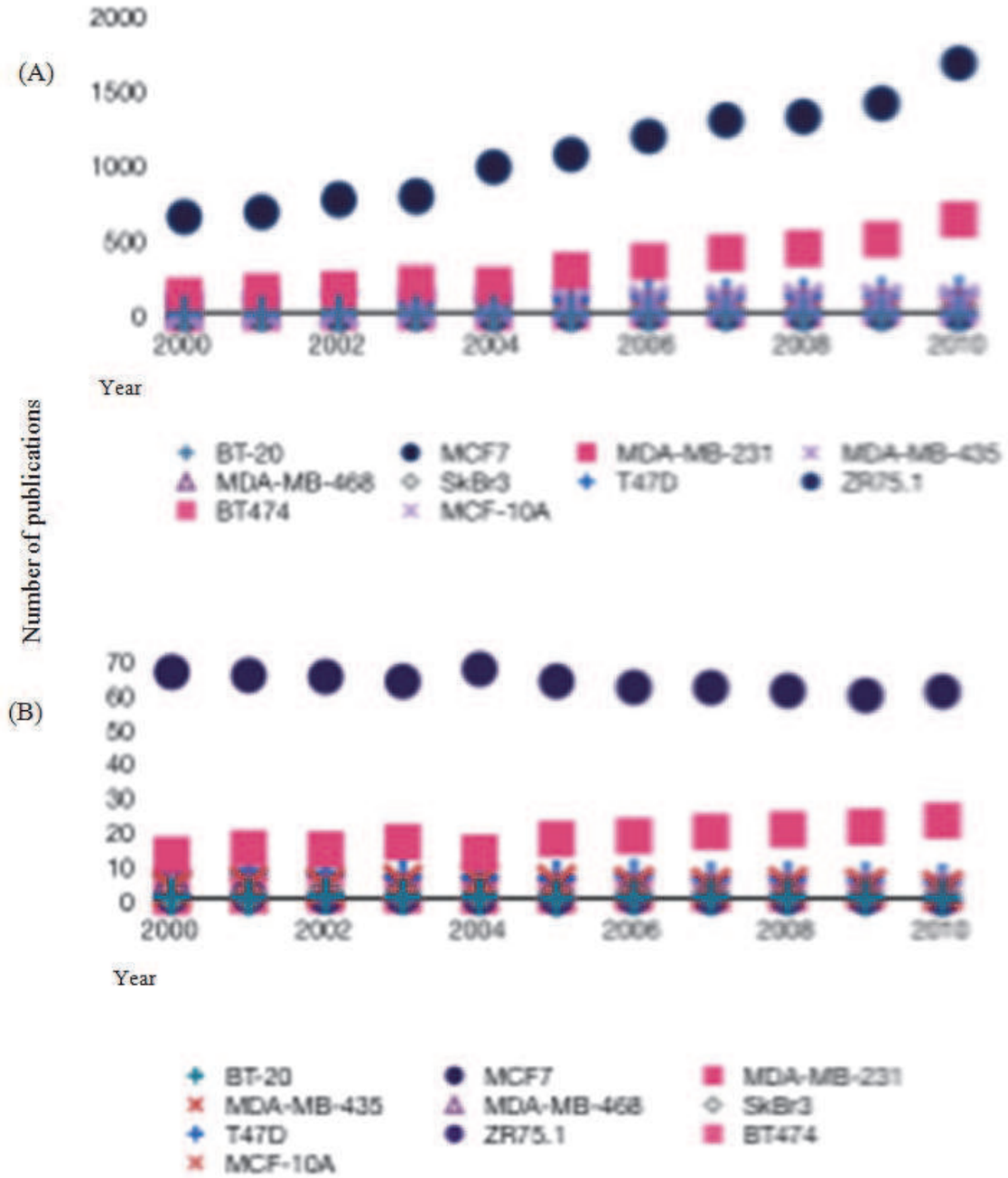

Fig. 1. The total number of publications per breast cancer cell line from 2000 through 2010. The most commonly used cell line is the ER+ MCF7 cell line, followed by ER - MDA-MB-231 cell lines. Many other cell lines are in use, however the number of publications using these models is quite small. A. Total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

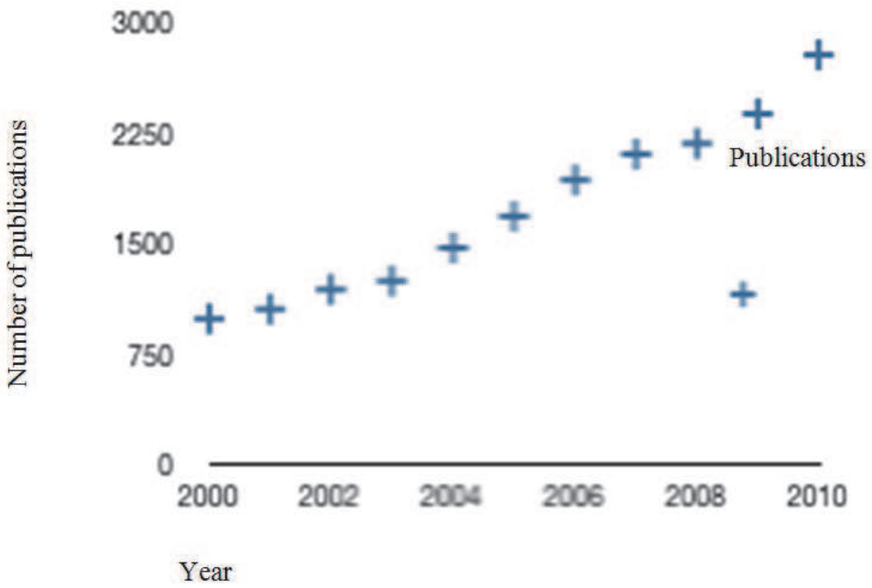

Fig. 2. The total number of publications using breast cancer cell lines from 2000 through 2010. Use of breast cancer cell lines has steadily been rising since 2000.

Fig. 3. Number and percent of papers published using MDA-MB-435 cells from 2000 - 2010. The tumor type that gave rise to MDA-MB-435 cells has been controversial since 2000. In 2004, STR profiling confirmed that MDA-MB-435 was not a breast cell line but rather has been contaminated with the M4 melanoma cell line. There has been a subsequent drop in the use and publication of these cells. Shown is the total number of papers published using MDA-MB-435 cells (green bars) and the percent of the total number of publications use MDA-MB-435 cells (blue circles). Arrow denotes when MDA-MB-435 were identified as M14 melanoma cells.

cell line	year established	origin	ER/PR status
BT-20	1958	primary tissue	-/?
SK-Br-3	1970	pleural effusion	+/+
SW13	1971	?	?
MDA-MB-134-VI	1973	pleural effusion	+/-
MDA-MB-157	1973	pleural effusion	?
MDA-MB-175-VII	1973	pleural effusion	?
MDA-MB-231	1973	pleural effusion	-/-
MDA-MB-361	1973	brain metastasis	?
MDA-MB-330	1973	pleural effusion	?
MDA-MB-415	1973	pleural effusion	?
MDA-MB-436	1973	pleural effusion	?
MDA-MB-453	1973	pleural effusion	-/-
MDA-MB-468	1973	pleural effusion	-/-
MDA-MB-157	1974	pleural effusion	?
MCF7	1974	primary tissue	+/+
CAMA-1	1975	pleural effusion	?
SW527	1977	?	?
Hs578Bst	1977	non-tumorigenic breast tissue	-/-
Hs578T	1977	primary tissue	-/-
ZR-75-1	1978	ascites	+/+
ZR-75-30	1978	ascites	?
BT483	1978	primary tissue	?
DU4475	1979	primary tissue	?
T47D	1979	pleural effusion	+/+
MCF10A	1984	non-tumorigenic breast tissue	-/-
MCF10F	1984	non-tumorigenic breast tissue	-/-
MCF10-2A	1984	non-tumorigenic breast tissue	-/-
184A1	1985	normal mammoplasty (transformed)	?
184B5	1985	normal mammoplasty (transformed)	?
UACC-812	1986	primary tissue	-/-
UACC-893	1987	primary tissue	-/-
HCC38	1992	primary tissue	-/-
HCC70	1992	primary tissue	-/-
HCC202	1992	primary tissue	-/-
HCC1008	1994	lymph node	-/-
HCC1143	1994	primary tissue	-/-
HCC1187	1994	primary tissue	?/-

cell line	year established	origin	ER/PR status
HCC1395	1994	primary tissue	-/-
HCC1419	1994	primary tissue	-/-
HCC1428	1995	pleural effusion	?
HCC1500	1995	primary tissue	+/+
HCC1569	1995	primary tissue	-/-
HCC1806	1995	primary tissue	-/-
HCC1937	1995	primary tissue	-/-
HCC1954	1995	primary tissue	+/+
HCC2157	1995	primary tissue	-/+
HCC2158	1996	primary tissue	-/?
HCC1599	1998	primary tissue	-/-
AU565	1998	pleural effusion	?

Table 2. Commercially available cell lines, their establishment date, and hormonal receptor status

Debate has also centered on whether cell lines grown in culture maintain the same genotypic/phenotypic changes that are detected in the primary tissues from which they are derived. Characterization of breast cancer cell lines has been ongoing since their establishment in 1958. In general, breast cancer cell lines are representative models of the primary breast tumors they are derived from (Kao et al., 2009). Initial characterization including karyotyping and comparative genomic hybridization (CGH) demonstrate that, when created and propagated in culture, cell lines maintain the same mutations and chromosomal abnormalities as their primary tumor samples (Lacroix and Laclercq, 2004). While new mutations and chromosomal instability develop in cultured cell lines, overall the genotype remains generally consistent between primary cells and cell lines (Lacroix and Laclercq, 2004). Due to differences in the *in vitro* environment, lack of surrounding naturally occurring microenvironment, and selection pressures, differentiation in culture can occur (Kao et al., 2009; Lacroix and Laclercq, 2004; Voskoglou-Nomikos et al., 2003). Because cancer cells are inherently unstable, differences between same cell line grown in different labs under different environments, even if the growth conditions are the same, are evident (Lacroix and Laclercq, 2004; Osborne et al., 1987). This impacts experimentation as data derived from one lab may not be reproducible in another lab, even is using the same cell line. Caution must be taken when relying on one or two cell lines to draw conclusions.

Use of more modern molecular techniques to characterize cell lines has revealed that while differences between primary cells and cell lines do exist. These techniques do confirm, however, that cell lines maintain the molecular distinction found the primary tumors. Gene expression changes detected in primary tumors are not dramatically different to those found in culture systems, even when cultures are grown directly on plastic in 2D cultures or in reconstituted 3D cultures (Vargo-Gogola and Rosen, 2007). Direct comparison of primary tissue to cultured cells revealed "close similarities" between molecular profiles (Dairkee et al., 2004). Indeed, even epigenetic changes found in primary cancers are similarly detected

in cell lines (Lacroix and Laclercq, 2004). This suggests that cell lines are an appropriate model of primary disease and, depending on the research focus, cell lines will faithfully reflect the processes of primary tissues.

Since cell lines generally remain faithful in terms of the molecular and genetic profiles of the primary tumor from which they are derived, it is critical to consider the correct model system. While ER/PR status of primary tumors leans predominantly toward ER+ expression (55-60%), most breast cancer cell lines have been derived from ER - tumors or pleural effusions (McGuire et al., 1978)(Table 2). Therefore it is of utmost importance to select the proper model to answer the experimental question. A detailed analysis of the applicability of cell lines to accurately model primary breast tumors revealed that overall breast cancer cell lines as a whole do model primary tumors, however on an individual basis, one specific cell line does not accurately mirror a primary breast tumor, even with the same gene expression profile. Since variability in cell lines exist, it is generally thought that to more accurately predict outcomes in primary tissue, a panel of breast cancer cell lines rather than just 1 or 2 individual lines should be tested. Using panels more accurately reflects primary breast tumors and will help translate findings from *in vitro* studies to *in vivo* therapeutic options (Dairkee et al., 2004).

Microarray analysis clearly defined primary breast tumors and breast cancer cell lines at the genetic level. Perou and others have conducted detailed studies using microarray platforms and determined a molecular signature of gene expression changes found in primary breast cancer tumors (Alizadeh et al., 2001; Perou et al., 1999b; Perou et al., 2000b; Ross et al., 2000; Sorlie et al., 2001). These signatures are used to understand the molecular basis of breast cancer and to define different subtypes of cancer that occur naturally in humans. It was also developed as a diagnostic tool to detect breast cancer tumors earlier and to facilitate proper treatment based on a gene signature. Based on these studies, 5 molecular signatures and types of primary breast tumors have been identified. These are luminal A, luminal B, basal-like, HER2+, and normal-like profiles (Perou et al., 1999a; Perou et al., 2000a; Ross et al., 2000; Sorlie et al., 2001). Prior to establishment of these molecular signatures, diagnosis was determined by receptor expression status, i.e. ER/PR/HER2, and treatment regimes assigned accordingly. Using this molecular approach, luminal A and luminal B tend to also be ER + expressing tumors, basal-like encompasses ER - tumors, HER2+ incorporate those HER2+ expressing tumors, and normal-like have similar expression patterns to non-cancerous cells (Perou et al., 1999a; Perou et al., 2000a; Ross et al., 2000; Sorlie et al., 2001). Such molecular characterization will lead to providing more personalized therapy to patients. Efficacy of drugs in different subtypes will be easily determined and accurately assigned to patients expressing a similar molecular profile. While such personalized medicine may be still in the future, some current breast cancer treatment options that exist today are based on the molecular profile of the tumor. For example, tumors expressing the estrogen receptor are treated with selective estrogen receptor modulator (SERM) or other similar anti-estrogen compound whereas tumors lacking ER do not receive the same therapy. Similarly, HER2+ tumors are susceptible to trastuzumab because of HER2 expression. In the future as molecular characterization improves and new chemotherapeutics are developed, more personalized options will be available.

Do cell lines reflect the molecular signature of primary tumors? In a direct comparison of the molecular profiles from cell lines and primary tumors, Kao et. al. found that instead of the 5 breast cancer subtypes identified in primary breast tumors, cell lines can be divided into three main groups, luminal, basal A, or basal B phenotypes (Kao et al., 2009). Luminal cells

contained all ER + cell lines, both Basal A and B consisted of all ER - cell lines. HER2+ cell lines were grouped into the luminal. Basal A contained the HCC cells and BRCA1 mutant cells, whereas basal B genotype contained non-tumorigenic lines including MCF10A cells (Kao et al., 2009). This highlights that breast cancer cell lines are a model of disease.

Cell lines are merely a model of breast disease that aim to provide clinical predictability of outcomes in humans. To directly test the applicability of breast cancer cell lines, xenograft cancer models, and mouse breast cancer models to clinical outcome, Voskoglou-Nomikos et. al. compared outcomes *in vitro* to those in xenograft models, to mouse models and phase II clinical trails (Voskoglou-Nomikos et al., 2003). In these comparisons, a general correlation between relative risk (predictive value of a drug in cell line) and the phase II human trial (tumor/control ratio) existed for *in vitro* cell lines. A general predictive value when using xenograft models to predict outcome to chemotherapy was detected, however this was dependent on the drug tested and the grade/type of tumor analyzed (Voskoglou-Nomikos et al., 2003). Overall, Vaskoglou-Nomikos et. al. concluded that cell lines and xenograft models were good predictors of clinical phase II trial outcomes, but are reliable predictors only when testing cytotoxic drugs and when using the correct model system. These models generally were not predictive of human outcomes when testing non-cytotoxic drugs (Voskoglou-Nomikos et al., 2003). Taken together, these studies emphasize the critical need to establish more breast cancer cell lines that model the heterogeneity of breast cancer and to employ many *in vitro* and xenograft model systems using multiple cell lines per experiment to reliably predict clinical outcome.

4. Contamination

Overt contamination of cell lines, such as bacterial, fungal or yeast infections, is readily detectable merely by altered appearance of the culture and can be rectified without impacting the quality or reproducibility of the data. Less overt contamination, such as mycoplasma and cell line cross-contamination, can occur undetected and can seriously jeopardize experimental findings. While it is well recognized that periodic testing for mycoplasma is a necessary requirement when using cell lines, cross-contamination with other cell lines is less recognized as a problem and therefore and cell authentication practices are not routine.

Cell line cross-contamination is most evident in the case of MDA-MB-435 cells. When Ross et. al. published the molecular profiles of breast cancer cell lines in 2000, the MDA-MB-435 cell line consistently fell outside the range of profiles of the other breast cancer cell lines and clustered with melanoma cell lines (Ross et al., 2000). This sparked great debate about the authenticity of the this line. Derived in 1976 from the pleural effusion of a 31 year old patient with metastatic adenocarcinoma of the breast, initial debate suggested that this was still a breast cancer cell line, but had been derived from a patient who may have also had undiagnosed melanoma (Cailleau et al., 1978). Data indicating that MDA-MB-435 cells expressed a mixture of both melanoma and epithelial markers fueled this debate, however the overwhelming belief was the these were indeed breast cancer cells (Chambers, 2009; Sellappan et al., 2004)(Figures 2 and 3). Indeed, early characterization of the cell line indicated that they were highly metastatic and secrete milk proteins, findings consistent with those of breast cancer cells (Howlett et al., 1994; Price, 1996; Price et al., 1990; Price and Zhang, 1990; Sellappan et al., 2004; Suzuki et al., 2006; Welch, 1997). Confusingly, MDA-MB-435 cells also expressed the melanocyte markers tyrosinase, melan A and S100 (Ellison et al.,

2002; Sellappan et al., 2004). Because of such conflicting results, these data just propagated the debate instead of satisfactorily squelching it as intended. MDA-MB-435 cells were still used and published as a breast cancer cell line (Figure 3).

Finally in 2007, DNA fingerprinting, or short tandem repeat (STR) analysis, in conjunction with SNP analysis, cytogenetic analysis, and comparative genomic hybridization using the earliest stocks of MDA-MB-435 cells revealed that these cells were identical to the M14 human melanoma cells and were melanoma rather than breast cancer cells (Garraway et al., 2005)(Rae et al., 2007). Rae et. al., who conducted the analysis, concluded that at some point early in passage, MDA-MB-435 cells were contaminated with M14 melanoma cells which took over the colony, leading to the establishment of a M14 melanoma cell line rather than a breast cancer line (Rae et al., 2007). This change was never detected. Stocks were unknowingly mislabeled, marked as MDA-MB-435 cells and distributed. Still, after the molecular characterization was published, debate as to whether MDA-MB-435 were really M14 melanoma cells or if M14 were really MDA-MB-435 breast cell still existed (Chambers, 2009). Ultimately, it was determined that MDA-MB-435 cells were really M14, based on the original 1974 publication that initially characterized the morphology, growth and tumorigenicity of MDA-MB-435 cells. In the original paper, MDA-MB-435 cells were reportedly non-tumorigenic in nude mice. After the initial creation in 1974, the MDA-MB-435 cells were not extensively used for testing until the 1990s when Price et. al. used these cells. At this time, MDA-MB-435 cells were characterized as a tumorigenic cell line (Cailleau et al., 1978; Price, 1996; Price et al., 1990; Price and Zhang, 1990).

While impossible to reconstruct that actually happened, this indirect evidence suggests that the MDA-MB-435 cells were contaminated with M14 melanoma cells and the original breast cancer cells died off. Subsequent frozen stocks were of the contaminating M14 cell lines, although they were labeled as MDA-MB-435 cells. No one was aware of this misidentification. Therefore, M14 cells were masquerading as MDA-MB-435 cells and used as a model of breast cancer until 2007. A total of 1803 PubMed indexed articles using MDA-MB-435 cells were published over that period (Figure 3). Since 2007, however, the number of publications using MDA-MB-435 cells has diminished, indicating that it is generally accepted that these cells are clearly not breast cancer cells and therefore should not be used as such.

4.1 Authentication

Cell line cross-contamination is hardly a new problem in tissue culture studies, although it still remains largely ignored. When HeLa were the only human cell line and few scientists studied them, cross-contamination was not a concern(Buehring et al., 2004; Skloot, 2010). Now, it is estimated that 20 - 30% of all cell lines are inadvertently contaminated (Alston-Roberts et al., 2010; Buehring et al., 2004; Gartler, 1968; Rojas and Steinsapir, 1983). Gartler et. al, was the first to highlight the problem in 1967 at the Second Decennial Review Conference on Cell, Tissue and Organ culture (Gartler, 1968). He was the first to demonstrate that many cultures from many labs were contaminated with other cell lines, primarily by HeLa cells. This meant that a significant amount of research was incorrectly interpreted because it was conducted in a different cell line and therefore the data were false. His findings were largely ignored. Over the years, others, including MacLeod, Freshney, Nardone, Alston-R, Buehring and Capes-Davis, have also documented contamination with HeLa and other cell lines, including cross-species

contamination, however this issue has rarely been adequately addressed (Alston-Roberts et al., 2010; Bartallon et al., 2010; Buehring et al., 2004; Capes-Davis et al., 2010; Freshney, 2008; MacLeod et al., 2008; MacLeod et al., 1999; SDO et al., 2010). Recent efforts have again been made to increase awareness of this problem and many calls for action have been published (Buehring et al., 2004; Capes-Davis et al., 2010; Freshney, 2008, 2010b; Lichter et al., 2010; MacLeod et al., 2008; MacLeod et al., 1999; SDO et al., 2010). A group of concerned scientists gathered and created the ATCC Standard Development Organization (ATCC SDO) to develop standards for cell authentication and with maintaining databases of STR profiles.

Eliminating contamination has an easy solution. Cell line authentication using a standardized technique, Short Tandem Repeat Analysis (STR), can provide an unique DNA fingerprint of the cell line (Azari et al., 2007; Bartallon et al., 2010; Masters et al., 2001; Nims et al., 2010; Parson et al., 2005). STR is inexpensive, standardized, and provides proven methodology to produce cell line identities that is reproducible between labs. An aliquot of DNA can be analyzed and compared with known STR profiles to authenticate the cell line. STR profiles for the most commonly used cell lines are freely available and STR services are available at many universities or companies. According to the standards developed by the ATCC-SDO, cells in active use should be authenticated by STR every 2 months (SDO et al., 2010). The ATCC-SDO also recommends that such documentation of authenticity be provided with grant applications and with manuscript submission. Many funding agencies and journals agree with this idea and suggest that scientists provide such documentation prior to acceptance of a manuscript, however at this time, this is merely a recommendation.

5. Future directions

Use of breast cancer cell lines as models of breast disease will not diminish in the near future. These cell lines are an excellent resource to test novel hypotheses and to gain greater understanding about how cells work and how breast cancer can be treated. On the whole, the established cell lines are a good model for disease, however additional cell lines should be created. The addition on new lines, especially those derived from various forms of breast cancer will only strengthen the data gleaned from them. Likewise, cell authentication should become a routine part of experimental procedures. By periodically ensuring the cell lines being tested are truly the correct lines will eliminate the generation and publication of false data. Authentication will save money and potentially careers if done of a routine basis.

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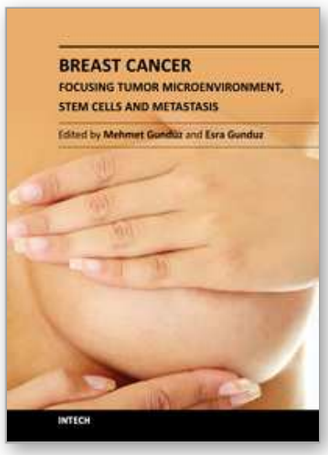

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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed characteristics of breast cancer cell, role of microenvironment, stem cells and metastasis for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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