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Review

Cell cultures as model systems in breast cancer research

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Abstract

Despite undoubted success in early diagnosis and treatment of breast cancer, it remains one of the most common and lethal neoplastic diseases in the world among women. Adequate model systems, including cell cultures, can help us better understand breast cancer biology as well as for the discovery of new diagnostic, prognostic and therapeutic strategies. This review summarizes some of the main characteristics of cell cultures (primary cell cultures and immortal cell lines; 2D and 3D cell cultures) used in current biomedical research and their strengths and weaknesses.

Keywords: Primary cell cultures and immortal cell lines; 2D and 3D cell cultures, breast cancer

INTRODUCTION

The challenges of breast cancer research

Cancer is a group of related complex diseases characterized by uncontrolled cell division – the cells are insensitive to growth control signals, replicate limitlessly and avoid apoptosis. In addition, tumor cells have the potential to invade the surrounding tissue and/or spread to other parts of the body, which is often fatal (Hanahan, Weinberg, 2011). Cancer treatment becomes complicated because malignant cells are not pathogens that have specific treatments, but are the body's own cells that must be killed or physically removed (Levi et al., 2001). Different obstacles prevent successful therapy of neoplastic diseases such as tumor heterogeneity, (multi)drug resistance, side / toxic effects, and limited knowledge of the molecular and cellular biology of tumor cells.

Malignant neoplasms are the second cause of death, after heart diseases, and it is estimated to rank first beginning with 2010 (American Cancer Society, 2011). Worldwide 14.1 million (in Europe 3.4 million) new cases were diagnosed as cancer in 2012, and it caused death for 8.2 million (1.75 million in Europe) people (Ferlay et al., 2012; Torre et al., 2012).

Globally, breast cancer (BC) is the most frequently diagnosed cancer in women, with an estimated 25% (1.7 million) new cases and 15% (more than 0.5 million) of deaths from all cancers every year (Torre et al., 2012).

Awareness of breast cancer, public attentiveness, and advancement in early diagnosis and treatment (including targeted anticancer therapy with monoclonal antibodies and/or tyrosine kinase inhibitors) has led to significant progress in screening and diagnosing BC as well as increasing five-year survival rate (Akram et al., 2017). Although enormous progress has been made over the last decades, the mobility of breast cancer is still increasing and associated with relative high mortality and high economic and social costs (Ferlay et al., 2010; Eccles et al., 2013; Asif et al., 2016). It is worthwhile to mention here that overall mortality has decreased by only 30% with > 90% sensitivity of current breast cancer screening approaches (Longacre et al., 2016).

Table 1. Breast cancer subtypes and permanent cell lines established from them

Subtype	Characterization	Permanent cell lines (examples)
Luminal A	ER positive PR high Ki-67 low (<14%) HER2 negative	MCF-7 HCC1428 EFM19 T47D
Luminal B (HER2 negative)	ER positive Either Ki-67 high or PR low HER2 negative	
Luminal B (HER2 positive)	ER positive Any PR Any Ki-67 HER2 over-expressed or amplified	BT474 UACC812 ZR7530 MDA-MB-361
HER2 positive	ER and PR absent HER2 over-expressed or amplified	SKBR3 SUM190PT SUM225CWN HCC1954 HCC1569 HCC202 AU565
Basal like / Triple negative	ER and PR absent HER2 negative	BT20 HCC38 HCC1143 HCC1395 MDA-MB-231 MDA-MB-157 MDA-MB-435 SUM52PE SUM149PE SUM229PE

According to Neve et al., 2006; Chavez et al., 2010; Subik et al., 2010; Holliday, Speirs, 2011; Goldhirsch et al., 2011; Inic et al., 2014; Dai et al., 2017.
ER - estrogene receptor, PR - progesterone receptor; HER2 - human epidermal growth factor receptor 2.

Breast cancer subtypes

Breast cancer displays intra- and inter-tumor heterogeneity due to genetic and non-genetic alterations. As a result it is presented by a spectrum of different subtypes with distinct biological features that lead to variations in response to various treatment modalities, tendency to metastasis, prognosis and clinical outcomes [Alexandrova et al., 2001; Holliday, Speirs, 2011; Koren, Bentires-Alj, 2015; Dai et al., 2017]. As such, breast cancer heterogeneity is one of the most important and clinically relevant areas of research.

Classification of breast cancer was based on the following main criteria: histological type, tumour grade, lymph node status, the presence / absence of hormonal (estrogene and / or progesterone) receptors and human epidermal growth factor receptor 2 (HER2), expression of Ki67, a marker of proliferation. The development of immunohistochemical techniques and molecular biological methods enable a deeper study of the variety of forms in breast cancer and facilitate their identification. The St. Gallen International Expert Consensus 2011 proposed a classification system for breast cancer to divide into five subgroups (Luminal A; Luminal B – HER2 negative; Luminal B – HER2 positive; HER2 positive and Basal like / Triple negative), that was further refined [Goldhirch et al., 2011; Inic et al., 2014; Dai et al., 2017]. A brief description of these breast cancer subtypes is summarised in Table 1. Each subtype may in turn be subdivided into different forms. For instance, triple negative breast cancer (TNBC) is presented by at least four subtypes, i.e., core basal, claudin-low, metaplastic and interferon-rich breast cancer, each with distinct molecular characteristics and clinical behavior (Chavez et al., 2010; Dai et al., 2017).

Cell cultures as experimental models in cancer research

One of the greatest challenges standing in front of modern science is the necessity adequate experimental models to be created, which allow us to perform studies on the biology of tumor cells, the mechanisms of cancerogenesis, recurrence / metastasis, drug resistance, as well as testing innovative treatment approaches and identification of new markers for early diagnosis.

Table 2. Advantages and disadvantages of permanent cancer cell lines and primary cell cultures

Permanent cell lines (PCL)	
Advantages	**Disadvantages**
• Cost effective • Easy to handle • Can provide large number of cells • Avoid ethical concerns associated with the use of animal and human tissue • More homogenous as compared to PCC • Suitable for studies in different biomedical scientific fields (including cancer research and oncopharmacology) as well as for the needs of biotechnology (production of vaccines, monoclonal antibodies, biologically active substances) and tissue engineering (for example cyto- / biocompatibility assessment of new materials for bone implants and wound dressings)	• Establishment of PCL is not easy, with low success rate • Often differ genetically and phenotypically from their tissue of origin due to continuous propagation in laboratory conditions • Possible contamination with cells from other cell lines • Risk of mycoplasma contamination that can persist undetected in cell cultures for a long period of time and can affect gene expression and cell behavior • The establishment of PCL may eliminate some types of cancer initially present in the original tissue samples • One and the same PCL cultured in different laboratories and/or under different conditions, may evolve into distinct modifications
Primary cell cultures (PCC)	
• Still express many of the important markers and functions seen in vivo and represent better the cells of origin • Can be useful to test whether proposed therapies for the particular cancer patient will be effective – the hallmark of personalized cancer treatment • Avoid problems of misidentification, contamination or other artefacts that accompany long-term cell cultures • Application in tissue engineering and regenerative medicine	• The isolation process makes PCC vulnerable to contamination by bacteria or stromal cells • More heterogenous than PCL • More sensitive • Slow growth rates that make large-scale studies with PCC difficult • Short life • Often require additional nutrients not included in classical commercial media or even special media customized for each cell type

According to Alge et al., 2006; Pan et al., 2009; Alston-Roberts et al., 2010; Lorsch et al., 2014.

Animal models closely recapitulate *in vivo* human physiology and are important in cancer research, however their application is associated with ethical concerns; it is not clear whether they can fully predict human disease outcome; they are also consuming more money, time and resources. Furthermore, patient derived human cancer xenografts experience mouse-specific tumor evolution when implanted in immuno-suppressed mice (Carranza-Rosales et al., 2018; Byrne et al., 2017).

Primary cell cultures (PCC, cells, isolated directly from animal or human tissue, without cell proliferation *in vitro*) and especially immortalcell lines (PCL, cells that have been continually propagated in laboratory conditions over a long period of time - over 80-100 passages, immortalized through spontaneous mutations or genetic manipulation) have been successfully applied in various fields of biomedical research and biotechnologies. Some of the main advantages and weaknesses of PCL and PCC as well as of two-dimensional (2D, monolayer) and 3D cell cultures are summarized in Tables 2 and 3.

The first human cell line (HeLa) was established from cervical carcinoma over 60 years ago by George Gey and named after Henrietta Lacks, the women from whom the tumor tissue was obtained [Gey et al., 1952; Scherer et al., 1953]. Since then it is widely accepted that PCL play important role in our understanding of cancer and have been used extensively in the discovery and characterization of new antineoplastic drugs. For example, due to its highly sensitive to hormone through expression of estrogen and progesterone receptors MCF-7 cell line (Luminal A type breast cancer) has been useful for the study of the estrogen signaling pathway and the development of efficacious anti-hormonal therapies such as tamoxifen [Levenson, Jordan, 1997; Osborne, 1998]. The antitumor effect of the anti-HER-2 mouse monoclonal antibody (which was later humanized to create trastuzumab / herceptin) as a single agent and in a combination with chemotherapeutic agents, demonstrated in breast cancer cell lines that could amplify HER-2.Thus, it opened the door for clinical trials of trastuzmab (Hudziak et al., 1989; Pietras et al., 1994; Pegram et al., 1999; Sliwkowski et al., 1999). Furthermore, HER2-amplified cell lines (such as SKBR3 and HCC1954) are helpful to study mechanisms of resistance to anti-HER2 therapies and to search for new agents/strategies that can restore cancer cell sensitivity to the treatment (D'Alesio et al., 2017; Baldassarre et al., 2017).

The molecular profiles of a large number of human cancer cell lines are available in the Cancer Cell Line

Table 3. Advantages and disadvantages of 2D- and 3D-cell cultures

2D cell cultures	
Advantages	Disadvantages
• Less expensive and easier to maintain • A lot of literature available • Well established • Easier environmental control • Easier observation and measurement -can be conveniently analyzed by almost any kind of imaging	• Decreased compatibility with living organisms • Not representative of real cell environment • Lack of predictivity
3D cell cultures	
• More physiologically relevant and predictive cell models • Allow interactions between different types of cells • Reduce the application of animal models • Provide more realistic way to grow and treat tumor cells	• More expensive and difficult to maintain • Many 3D culture techniques are cumbersome and time-consuming and are not suitable for drug development screening and research • Challenges in microscopy and measurement due to the larger size as compared to 2D cell cultures • Diffusional transport limitations • Culture dependent alterations in gene expression

According to Edmondson et al., 2014; Katt et al., 2016.

Encyclopedia (Barretina et al., 2012), and these profiles can be compared to the profiles of a large number of human tumors, compiled as part of the Cancer Genome Atlas Research Network (Holliday and Speirs, 2011; Cancer Genome Atlas Research Network et al., 2013; Domcke et al., 2013; Katt et al., 2016).

The principles of good laboratory practice should be kept while working with cancer cell cultures. Following these regulations will ensure the safety of staff and the achievement of reliable results.

Among the problems associated with cell culturing are:

i) Mycoplasma infection (resistant to commonly used antibiotics and invisible to the naked eye) is among the main problems in cell culturing. It has been estimated that about 5 to 30% of the world's cell lines are contaminated with mycoplasmas (Nikfarjam, Farzaneh, 2012).

ii) The cell cultures can be contaminated with other cells. For instance evidences suggest that HeLa cells contaminated and overgrew other cell lines. Cultures, supposed to be of breast cancer or mouse origin, were proved to be HeLa cells (Lucey et al., 2009).

We must keep in mind that *in vitro* cultured cancer cells may undergo different genetic and epigenetic alterations. For example, Schmidt et al. reported the loss of estrogen and progesterone receptors in cultures breast cancer cells, derived from pleural effusion (Schmidt et al., (2007).

Breast cancer cell lines – past, present and future

The first breast cancer cell line (BT-20) was established in 1958 from a 74-year old Caucasian female [Lasfargues, Ozzello, 1958]. Various breast cancer cell lines were obtained in the next years including the “MD Anderson series” presented by nineteen human breast carcinoma cell lines derived from pleural effusions (16 cell lines), brain metastases (two cell lines) and pericardial fluid (one cell line) [Cailleau et al., 1978]. “HCC series” cell lines were isolated at Hamon Cancer Centre [Gazdar et al., 1998]. Another popular and widely used cell line – MCF-7 (acronym of Michigan Cancer Foundation-**7**), was established in 1970 from breast adenocarcinoma of a 69-year-old Caucasian woman (Soule et al., 1973). The list includes also the immortal cell lines derived from breast primary tumours, pleural effusions or various metastatic sites in individual patients [Vandewalle et al., 1987; Minafra et al., 1989; Bover et al., 1991; Ethier et al., 1993; Zoli et al., 1997]. Many of these cell lines are now widely available through commercial cell banks – some of them are presented in Table 1.

The establishment of new breast cancer immortal cell lines continues to be an important challenge for biomedical professionals because of the following reasons:

i) Relatively low number of BC cell lines are available at the moment – there are < 100 cell line available until today (Holliday, Speirs, 2011). The establishment of a new cancer cell line is not an easy task mainly because of technical difficulties in extracting viable tumor cells from the surrounding stroma, the problems accompanying long-term propagation during cultivation in laboratory conditions and at least in some countries (for example in the United Kingdom partly due to ethical restrictions concerning the use of human tissues for research (Holliday, Speirs, 2011; Dai et al., 2017).

ii) A wide range of BC cell lines are needed to cover the full spectrum of breast cancer development and progress as well as inter-tumoral heterogeneity including cell lines from normal mammary tissue, premalignant breast formations, different (including rare) subtypes of breast cancer and ideally metastases from all major sites

as well as relapsed BC cell lines (Eccles et al., 2013). Most of the known BC cell lines (including those established from “good prognosis” subtypes such as luminal A) are obtained from metastases or pleural effusions due to the higher adaptability and durability of more aggressive (metastatic) cells. As a result, currently, there is no cell line to resemble adequately the good prognosis luminal A subtype - the most frequently diagnosed breast cancer subtype in women (Neve et al., 2006; Holliday, Speirs, 2011; Prat et al., 2013, Dai et al., 2017). We do not have cell lines representing the currently known TNBC subtypes (Chavez et al., 2010; Dai et al., 2017).

iii) Not all of the available BC cell lines are suitable (due to different reasons including technical easiness) for routine laboratory cultivation which narrows the range of the available *in vitro* cell model systems. The most frequently used BC cell lines are MCF7, T-47D, and MDAMB231 (Dai et al., 2017).

vi) Three dimensional (3D) cell culture systems that resemble better than monolayer (2D) cell cultures the in vivo biology and behavior of neoplastic formations and adequately represent the functions of 3D tissues with their extensive cell-cell and cell-matrix interactions, as well as markedly different diffusion /transport conditions have attracted the attention of the scientific community in recent years. Clinically relevant (multi)drug-resistant cell lines established in vitro in conditions representing the experience which oncology patients undergo during chemotherapy are also needed. They can contribute to better elucidate the molecular signaling pathways involved in drug resistance, as well as to find strategies to overcome and to identify reliable new biomarkers for response or relapse. Model systems that allow us to study the interactions between tumor cells and the immune system are also necessary (Jenkins, 2017).

Three dimensional (3D) cell cultures

Different methods and protocols for 3D cell cultures of various cancer cell lines are available. They can be also subdivided into liquid-based and scaffold-based 3D-models (Thoma et al., 2014). The most commonly used scaffold-free 3D cells cultures techniques are the forced-floating method, the hanging drop method and the agitation based method. Scaffold platforms for 3D cultures are made of synthetic or naturally-derived polymers that provide a support for cell growth and mimic extracellular matrix conditions. Various types of 3D cell culture systems have been described including:

Tumor-derived spheroids - one of the most common scaffold-free 3D cell cultures; either self-assembling or stimulated to grow as cell clusters starting from single cell suspensions; purposed for the enrichment of cancer stem cells. However, for many cancer cell lines, the efficiency of spheroid formation is low. In addition, the production of spheroids with different sizes and shapes may influence drug efficacy and toxicity, leading to artificial results (Yamada et al., 2007; Ishiguro et al., 2017; Verjans et al., 2018).

Tumor-derived organoids – 3D constructs obtained from primary tumor tissue or developed from stem cells; epithelial cell cultures that lack a tumor stroma, vasculature, and immune cells, although involved interactions with a basal membrane (typically Matrigel) (Ishiguro et al., 2017; Xu et al., 2018). For example, 3D cancer organoids obtained from genetically engineered mouse models for BRCA1- and BRCA2-deficient cancers have been recently reported. Orthotopically implanted these organoids produce mammary tumors that recapitulate the epithelial morphology and preserve the drug response of the original tumor, can be easily genetically modified and can serve as suitable tool for investigations in the fields of tumor biology and drug resistance (Duarte et al., 2018).

Organotypic multicellular cultures attempt to maintain the native stroma and tumor heterogeneity that is lacking in organoid cell culture system – an increasing amount of data support the importance of intra-patient tumor heterogeneity and tumor-stromal interactions for cancer behaviour and response to therapy.

Multicellular tumor shperoids are 3D growing aggregates of cancer cells that more closely represent in vivo characteristics of the tumor microenvironment (TME). TME consists of extracellular matrix (ECM), cells (myofibroblasts, fibroblasts, neuroendocrine, adipose and immune-inflammatory cells), and the blood and lymphatic vascular networks (Chen et al., 2015) and has been suggested to play a crucial role in cancer initiation, progression, and invasion (Wang et al., 2017). Cell model systems that preserve the native TME are suitable for the evaluation of ex vivo drug responses as well as for investigating the role of stromal cells in cancerogenesis and especially interactions between tumor cell and immune cells.

CONCLUSION

Cell cultures have gone a long and successful way in recent decades and have become a valuable and widely used research and biotechnological tool that is also important for regenerative medicine. 2D and especially 3D breast cancer cell cultures can help us to better understand biological processes involved in this disease as well as the identification of potential therapeutic targets and diagnostic / predictive markers. In addition 3D cell cultures are suitable as a preclinical model facilitating the translation from experimental oncology and oncopharmacology to clinical practice and supporting personalized medicine.

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