Advances in the development of improved animal-free models for use in breast cancer biomedical research

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Received: 6 June 2017 / Accepted: 3 July 2017 / Published online: 26 July 2017 © The Author(s) 2017. This article is an open access publication

Abstract Through translational research, the outcomes for women (and men) diagnosed with breast cancer have improved significantly, with now over 80% of women surviving for at least 5 years post-diagnosis. Much of this success has been translated from the bench to the bedside using laboratory models. Here, we outline the types of laboratory models that have helped achieve this and discuss new approaches as we move towards animal-free disease modelling.

Keywords Breast cancer · Ex vivo models · Tissue banks

Introduction

Laboratory models to study breast cancer behaviour and response to therapy have been instrumental in contributing to improving patient outcome. Starting from simple cell culture models using immortalised human cell lines derived from patient tumours grown in two dimensions (2D), these have gradually evolved into more complex three-dimensional (3D) multi-cellular models and, lately, towards patient-derived organoid models (Soule et al. 1973; Wang et al. 2002; Debnath et al. 2003; Nash et al. 2015; Bruna et al. 2016). Animal models have also been employed first using cells lines growing as xenografts (Deome et al. 1959) and, more recently, using so-called patient-derived xenograft (PDX) models (Whittle et al. 2015). The guiding principles for the improved welfare of animals used in research were introduced in 1959 and termed the 3Rs: replacement, reduction and refinement (Russell and Burch 1959). These have been implemented in many countries to support the humane use of animals in laboratory research. There are now specific funding bodies which exclusively support research which either completely replaces (e.g. Animal Free Research UK; https://www.animalfreeresearchuk.org), reduces or refines the use of animals in research (e.g. the National Centre for the Replacement, Refinement & Reductions of Animals in Research in the UK; https://www.nc3rs.org.uk and Medical Advances Without Animals in Australia; http://www.mawa-trust.org.au). Many scientists are now actively engaged in further advancing this ethos, by developing improved scientific methods, which serve to reduce the reliance on animals in biomedical research or to completely replace them. A timeline showing key achievements towards the advancement of breast cancer models in biomedical research is shown in Fig. 1. We discuss the various models available and their pros and cons below.

Cell lines

Cell lines have been the workhorses in biomedical research labs for decades. The first and arguably the best known is HeLa, a cervical cancer cell line derived from tissue taken from Henrietta Lacks (Gey et al. 1952). The first breast cancer cell line, BT20, was developed in 1958 from an invasive ductal carcinoma (Lasfargues and Ozzello 1958); however, the most commonly used breast cancer cell line in the world is MCF-7, described in 1973 (Soule et al. 1973) and derived from a pleural effusion from an invasive ductal breast cancer which developed in a 69-year-old Caucasian nun, Frances Mallon. Since

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This article is part of a Special Issue on the ‘IUPAB Edinburgh Congress’ edited by Damien Hall.

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then, a number of different breast cancer cell lines have been developed, and the latter half of the 20th century allowed scientists to use these through in vitro cell culture or in animal experiments using xenografts, in experiments designed to better understand the biology of breast cancer. This research has helped in the development of new diagnostic tests and new treatments, e.g. the presence of HER2 to determine which patients are likely to derive benefit from trastuzumab and the development of tamoxifen for the treatment of breast cancer (Gottardis et al. 1988; Slamon et al. 1989).

While cell lines are convenient research tools to study breast cancer, they are relatively simplistic models, representing a reductionist approach to disease modelling, as they lack the complexity and heterogeneity which characterise human breast tumours. Not only is breast cancer complex with many different subtypes, it know well recognised that the tumour microenvironment can influence breast cancer epithelial cells (Noël and Foidart 1998). Moreover, traditional methods of culturing cells in isolation on plastic substrates further remove this complexity, potentially limiting the translational impact of laboratory findings into the clinic. Given the multi-faceted inter-relationship of cells with their microenvironment in native tumours, scientists have recognised the shortfalls of this reductionist approach, as culturing cells in 2D in tissue culture plastic is not synonymous with this. This was tackled initially in co-culture experiments, where cancer epithelial cells were grown with fibroblasts, the principal cell type within the stromal microenvironment, leading to important insights into how stromal fibroblasts could influence tumour epithelial cells (van Roozendaal et al. 1996; Dong-Le Bourhis et al. 1997; Smith et al. 2015). A News Feature and accompanying Editorial entitled “Goodbye, flat biology?” published in Nature (Abbott 2003a, b) was a rallying call to scientists to consider adopting more relevant 3D models, with due consideration of the microenvironment. This was the first time 2D culture was officially challenged by a high-impact journal. Since then, the number of papers reporting 3D cell culture has overtaken that of 2D culture and continues to grow exponentially (Fig 2).

3D culture using cell lines

Three-dimensional spheroids were first generated using Chinese hamster V79 cells growing in spinner flasks to study the effects of irradiation (Sutherland et al. 1970). Since then, the use of spheroids in cancer research has advanced greatly. The classification of a spheroid is poorly defined but is generally thought of as the formation of a rounded 3D structure composed of multiple cells. Spheroids are good models of cancer as they develop pH, hypoxic and proliferative gradients akin to the avascular stages of solid tumours (Nederman et al. 1984; Rotin et al. 1986). This arrangement is mirrored in native tumours, where the outer cells are the only ones with sufficient contact to a blood supply containing nutrients needed for growth.

There are several ways in which breast cancer spheroids have been cultured. Initial approaches involved plating cell suspensions on an agar–base medium as a means of restricting cell–substrate adhesion (Yuhas et al. 1978), the so-called liquid overlay technique. Other cell types, notably fibroblasts, were added (Seidl et al. 2002). Subsequently, the use of low-attachment plastics allowed spheroid formation (Pickl and
subchondral bone discs (Holen et al. 2015). These types of using metastatic breast cancer cell lines seeded onto human pioneering 3D models of breast cancer metastasis to bone replicate the tumour environment in vivo. These also include immune cells (Augustine et al. 2015). Such models more closely and Lu 2011; Pinto et al. 2014) and the incorporation of im-

recapitulate the human bone microenvironment.

been limited to animal xenograft models, yet these do not
demonstrate the human bone microenvironment.

Nevertheless, spheroids do have their limitations. Different cell types have varying abilities to form spheroids; for example, the BT-474 HER2 overexpressing cell line forms tightly packed rounded spheroids, whereas the SKBR3 HER2 over-expressing cell line forms loose, grape-like aggregates (Froehlich et al. 2016; Roberts et al. 2016). Also, it can be challenging to control the size of spheroids formed and, therefore, the reproducibility of experiments for high-throughput drug screening is limited. This has been examined recently, where 42 different experimental methods were evaluated to test how well spheroid formation was induced using three commonly used breast cancer cell lines; MCF-7, MDA-MB-231 and SKBr3 (Froehlich et al. 2016). Further work in addressing these limitations could make them stronger tools for cancer research in the future.

Animal models

The significance of using animal models in breast cancer research has recently been reviewed comprehensively (Holen et al. 2017), and the reader is directed to this article for up-to-date information. While there is no doubt that these models have contributed to some of the success in translating laboratory findings to the clinic, they have limitations as pre-clinical models. This is exemplified by the high attrition rates of promising pre-clinical agents when entered into clinical trials (Kola and Landis 2004). Scientists are now applying lateral thought to implement better ways of modelling breast cancer and models developed from human clinical material are starting to gain traction. These are discussed below.

Primary cell culture

Recognition that breast cancer was classified into at least four major molecular subgroups (Perou et al. 2000) allowed scientists to reclassify existing cell lines into representative examples (Neve et al. 2006; Holliday and Speirs 2011). However, use of the panel of cell lines available tends to be skewed in favour of the most common Luminal subgroup, exemplified by the ‘workhorse’ of breast cancer research, MCF-7. This is shown in Fig. 3, where the number of papers in PubMed which have used MCF-7 far exceeds those using the second most common breast cancer cell line, MDA-MB-231, often used to model the more aggressive triple negative breast cancer, while aggregate publications of other less commonly used breast cancer cell lines, e.g. to represent HER2-positive breast cancer, is lower still. This has led scientists to consider alternative models using human clinical material.

Generating primary cells from tissue biopsies or resections is regarded by many as a step up from cell lines, moving towards achieving greater clinical relevance in biomedical
Western nations has resulted in the increased detection of DCIS. This can be a precursor of invasive breast cancer in some women, but is an issue for doctors in terms of identifying who should receive treatment, which may turn out to be unnecessary in some cases, as not all DCIS will develop into invasive breast cancer (Marmot et al. 2013). Consequently, there is much interest in better understanding its biology, so a robust in vitro model is critical.

Two groups used cells from the BCNTB to develop physiologically relevant models to better understand the processes that underlie the transition of normal breast to DCIS and DCIS to invasive cancer. The first, a partially humanised 3D tri-culture model of normal breast, comprised luminal epithelial cell lines, primary human fibroblasts from the BCNTB and immortalised human myoepithelial cells growing in 3D in a collagen I matrix (Nash et al. 2015). More recently, a fully humanised 3D in vitro model using material from the BCNTB was developed to study the relationship between luminal and myoepithelial cells, the disruption of which is a critical first step towards the development of DCIS into an invasive phenotype (Carter et al. 2017). These models are important, as research into the biology of DCIS has previously relied on animal models, notably the MIND model, which involves intra-ductal transplantation of either DCIS-like cell lines or fragments of xenografts derived from human DCIS into immunocompromised mice to functionally test molecular events occurring in the initial changes in premalignant progression (Behbod et al. 2009). With increased uptake of the use of the BCNTB cell culture programme by the research community, it is highly likely that additional humanised models will be developed to help scientists work towards reducing reliance on the use of animal models in biomedical research.

**Patient-derived organoids**

Further technical advances towards more advanced disease modelling is the development of the patient-derived organoid (PDO) model. Although organoid modelling per se is not new, the way this is now being applied to human tissues is opening up new opportunities to study and understand disease processes. Organoids are generated from small fragments of tissue from human tumours by mechanical and enzymatic disaggregation and plating in basement membrane extract, which can be maintained in culture. Because cells are maintained in 3D and retain critical cell–cell and cell–matrix interactions, these organoid models can be perceived as an intermediary between in vitro cell lines and animal xenograft models. While still a relatively new method, organoid cultures have enormous potential, with PDOs now derived from...
a number of different types of primary and metastatic human tumours with good success (van de Wetering et al. 2015; Bruna et al. 2016). By using patient tissue for research, the translational impact could increase greatly, with the possibility of advancing personalised medicine. Furthermore, this will certainly reduce, potentially even eliminating, the need for animals as pre-clinical models in the longer term. The use of patient tissue is possible through tissue banks such as the BCNTB mentioned above and others.

**Conclusions**

Models to study breast cancer have evolved in the last few decades, gradually increasing in complexity to reflect native tissue architecture. Complementary to this, research is gradually moving away from 2D culture and in using animals to model breast cancer, towards developing humanised systems using human tissue samples from biobanks. In this era of precision medicine, this has real potential to revolutionise pre-clinical drug testing, offering an intermediate step, which could reduce or may even eventually replace the use of animals. Whilst it is unlikely that a single model alone will be used to recapitulate native tumour biology, using a combinatorial approach could impact on drug efficacy trials, improving translation into patients.

**Acknowledgements**

SR receives a studentship from NC3Rs (grant ref.: NC/N00325X/1). VS receives funding from Breast Cancer Now and is involved in running the Breast Cancer Now Tissue Bank.

**Compliance with ethical standards**

**Conflict of interest** Sophie Roberts declares that she has no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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**References**

Abbott A (2003a) Goodbye, flat biology? Nature 424:861

Abbott A (2003b) Cell culture: biology’s new dimension. Nature 424: 870–872

Augustine TN, Dix-Peek T, Duarte R, Candy GP (2015) Establishment of a heterotypic 3D culture system to evaluate the interaction of TREG lymphocytes and NK cells with breast cancer. J Immunol Methods 426:1–13. doi:10.1016/j.jim.2015.07.003

Beckhove P, Schütz F, Diel IJ, Solomayer E-F, Bastert G, Foerster J, Feuerer M, Bai L, Sinn H-P, Umansky V, Schirmacher V (2003) Efficient engraftment of human primary breast cancer transplants in nonconditioned NOD/Seid mice. Int J Cancer 105:444–453. doi:10.1002/jcc.11125

Behbod F, Kittrell FS, LaMarca H, Edwards D, Kerbawy S, Hestand JC, Young E, Mukhopadhyay P, Yeh H-W, Allred DC, Hu M, Polya K, Rosen JM, Medina D (2009) An intraductal human-in-mouse transplantation model mimics the subtypes of ductal carcinoma in situ. Breast Cancer Res 11:R66. doi:10.1186/bcr2358

Bissell MJ, Bilder D (2003) Polarity determination in breast tissue: desmosomal adhesion, myoepithelial cells, and laminin 1. Breast Cancer Res 5:117–119

Bruna A, Rueda OM, Greenwood W, Batra AS, Callari M, Batra RN, Pogrebniak K, Sandoval J, Cassidy JW, Tufegdzic-Vidakovic A, Sammut SJ, Jones L, Provenzano E, Baird R, Eirew P, Hadfield J, Eldridge M, McLaren-Douglas A, Barthorpe A, Lightfoot H, O’Connor MJ, Gray J, Cortes J, Baselga J, Marangoni E, Welm AL, Aparicio S, Serra V, Garnett MJ, Caldas C (2016) A biobank of breast cancer explants with preserved intra-tumor heterogeneity to screen anticancer compounds. Cell 167:260–274.e22

Buchsbaum RJ, Oh SY (2016) Breast cancer-associated fibroblasts: where are we and where we need to go. Cancers (Basel) 8:E19. doi:10.3390/cancers8020019

Cardiff RD, Kenney N (2011) A compendium of the mouse mammary tumor biologist: from the initial observations in the house mouse to the development of genetically engineered mice. Cold Spring Harb Perspect Biol 3:a003111. doi:10.1101/cshperspect.a003111

Carter EP, Gopsill JA, Gomm JJ, Jones JL, Grose RP (2017) A 3D in vitro model of the human breast duct: a method to unravel myoepithelial–luminal interactions in the progression of breast cancer. Breast Cancer Res 19:50. doi:10.1186/s13058-017-0843-4

Debnath J, Muthuswamy SK, Brugge JS (2003) Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basal membrane cultures. Methods 30:256–268

Deome KB, Faulkin LJ Jr, Bern HA, Blair PB (1959) Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. Cancer Res 19:515–520

Dong-Le Bourhis X, Berthois Y, Millot G, Degeorges A, Sylvi M, Martin PM, Calvo F (1997) Effect of stromal and epithelial cells derived from normal and tumorous breast tissue on the proliferation of human breast cancer cell lines in co-culture. Int J Cancer 71:42–48

Eccles SA, Aboagye EO, Ali S, Anderson AS, Armes J, Berditchevski F, Blaydes JP, Brennan K, Brown NJ, Bryant HE, Buddend NJ, Burchell JM, Campbell AM, Carroll RB, Coles CE, Cook GJ, Cox A, Curtin NJ, Dekker LV, Silva Idos S, Duffy SW, Easton DF, Eccles DM, Edwards DR, Edwards J, Evans D, Fenlon DF, Flanagan JM, Foster C, Gallagher WM, Garcia-Closas M, Gee JM, Gescher AJ, Goh V, Groves AM, Harvey AJ, Harvie M, Hennessy BT, Hiscox S, Holen I, Howell SJ, Howell A, Hubbard G, Hulbert-Williams N, Hunter MS, Jasani B, Jones LJ, Key TJ, Kirwan CC, Kong A, Kunkler I, Landgon SP, Leach MO, Mann DJ, Marshall JF, Martin L, Martin SG, Macdougall JE, Miles DW, Miller WR, Morris JR, Moss SM, Mullan P, Natrajan R, O’Connor JP, O’Connor R, Palmieri C, Pharoah PD, Rakha EA, Reed E, Robinson SP, Sahai E, Saxton JM, Schmid P, Smalley MJ, Speirs V, Stein R, Stingl J, Streuli CH, Tutt AN, Velikova G, Walker RA, Watson CJ, Williams KJ, Young LS, Thompson AM (2013) Critical research gaps and translational priorities for the successful prevention and treatment of breast cancer. Breast Cancer Res 15:R92. doi:10.1186/bcr3493

Ether SP, Mahacek ML, Gullick WJ, Frank TS, Weber BL (1993) Differential isolation of normal luminal mammary epithelial cells
and breast cancer cells from primary and metastatic sites using selective media. Cancer Res 53:627–635

Froehlich K, Haeger JD, Heger J, Pastushcek J, Photini SM, Yan Y, Lupp A, Pfarre C, Mrowka R, Schleufner E, Markert UR, Schmidt A (2016) Generation of multicellular breast cancer tumor spheroids: comparison of different protocols. J Mammary Gland Biol Neoplasia 21:89–98

Gey GO, Coffman WD, Kubicek MT (1952) Tissue culture studies of proliferative capacity of cervical carcinoma and normal epithelium. Cancer Res 12:264–265

Gottardis MM, Robinson SP, Jordan VC (1988) Estradiol-stimulated growth of MCF-7 tumors implanted in athymic mice: a model to study the tumorigenic action of tamoxifen. J Steroid Biochem 30:311–314

Hass R, Bertram C (2009) Characterization of human breast epithelial cells (HBCEC) derived from long term cultured biopsies. J Exp Clin Cancer Res 28:127. doi:10.1186/1756-9966-28-127

Holien I, Nutter F, Wilkinson JM, Evans CA, Avgousti P, Otewell PD (2015) Human breast cancer bone metastasis in vitro and in vivo: a novel 3D model system for studies of tumour cell–bone cell interactions. Clin Exp Metastasis 32:689–702. doi:10.1007/s10585-015-9737-y

Holien I, Speirs V, Morrissey B, Blyth K (2017) In vivo models in breast cancer research: progress, challenges and future directions. Dis Model Mech 10:359–371. doi:10.1242/dmm.028274

Holliday DL, speirs V (2011) Choosing the right cell line for breast cancer research. Breast Cancer Res 13:215. doi:10.1186/bcr2889

Holliday DL, Brouilette KT, Markert A, Gordon LA, Jones JL (2009) Novel multicellular organotypic models of normal and malignant breast: tools for dissecting the role of the microenvironment in breast cancer progression. Breast Cancer Res 11:R3. doi:10.1186/bcr2218

Ivascu A, Kubbies M (2007) Diversity of cell-mediated adhesions in breast cancer spheroids. Int J Oncol 31:1403–1413

Kola I, Landis J (2004) Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov 3:711–715

Lasfargues EY, Ozzello L (1958) Cultivation of human breast carcinoma. J Natl Cancer Inst 21:1131–1147

Lee GY, Penny PA, Lee EH, Bissell MJ (2007) Three-dimensional culture models of normal and malignant breast epithelial cells. Nat Methods 4:359–365

Li L, Lu Y (2011) Optimizing a 3D culture system to study the interaction between epithelial breast cancer and its surrounding fibroblasts. J Cancer 2:458–466

Marmot MG, Altman DG, Cameron DA, Dewar JA, Thompson SG, Wilcox M (2013) The benefits and harms of breast cancer screening. Br J Cancer 108:2205–2240. doi:10.1038/bjc.2013.177

Nagelkerke A, Bussink J, Sweep FC, Span PN (2013) Generation of multicellular tumor spheroids of breast cancer cells: how to go three-dimensional. Anal Biochem 437:17–19. doi:10.1016/j.ab.2013.02.004

Nash CE, Mavria G, Baxter EW, Holliday DL, Tomlinson DC, Treanor D, Novitskaya V, Berditchevski F, Hanby AM, Speirs V (2015) Development and characterisation of a 3D multi-cellular in vitro model of normal human breast: a tool for cancer initiation studies. Oncotarget 6:13731–13741

Nederman T, Norling B, Glimelius B, Carlsson J, Brunk U (1984) Demonstration of an extracellular matrix in multicellular tumor spheroids. Cancer Res 44:3090–3097

Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fevr T, Clark L, Bayani N, Coppe JP, Tong F, Speed T, Spellman PT, DeVries S, Lippman M, Ethier S, Gazdar A, Gray JW (2006) A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. Cancer Cell 10:515–527

Noël A, Foidart JM (1998) The role of stroma in breast carcinoma growth in vivo. J Mammary Gland Biol Neoplasia 3:215–225

Olsen CJ, Moreira J, Lukandin EM, Ambartsoumin NS (2010) Human mammary fibroblasts stimulate invasion of breast cancer cells in a three-dimensional culture and increase stroma development in mouse xenografts. BMC Cancer 10:444

Pereou CM, Sarthi T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Barresen-Dale AL, Brown PO, Botstein D (2000) Molecular portraits of human breast tumours. Nature 406:747–752

Petersen OW, Rønnov-Jessen L, Howlett AR, Bissell MJ (1992) Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. Proc Natl Acad Sci U S A 89:9064–9068

Pickl M, Ries CH (2009) Comparison of 3D and 2D tumor models reveals enhanced HER2 activation in 3D associated with an increased response to trastuzumab. Oncogene 28:461–468. doi:10.1038/onc.2008.394

Pinto MP, Dye WW, Jacobsen BM, Horwitz KB (2014) Malignant stroma increases luminal breast cancer cell proliferation and angiogenesis through platelet-derived growth factor signaling. BMC Cancer 14:735

Roberts GC, Morris PG, Moss MA, Malby SL, Palmer CA, Nash CE, Smart E, Holliday DL, Speirs V (2016) An evaluation of matrix-containing and humanised matrix-free 3-dimensional cell culture systems for studying breast cancer. PLoS One 11:e0157004. doi:10.1371/journal.pone.0157004

Roth D, Robinson B, Tannock IF (1986) Influence of hypoxia and an acidic environment on the metabolism and viability of cultured cells: potential implications for cell death in tumors. Cancer Res 46:2821–2826

Russ A, Louderbough JM, Zamescu D, Schroeder JA (2012) Hugl1 and Hugl2 in mammary epithelial cells: polarity, proliferation, and differentiation. PLoS One 7:e47734

Russell WMS, Burch RL (1959, reprinted 1992) The principles of humane experimental technique. Universities Federation for Animal Welfare, UK

Rygaard J, Povsen CO (2007) Heterotransplantation of a human malignant tumour to “nude” mice. 1969. APMS 115:604–606

Sadlonova A, Novak Z, Johnson MR, Bowe DB, Gault SR, Page GP, Thottassery JW, Welch DR, Frost AR (2005) Breast fibroblasts modulate epithelial cell proliferation in three-dimensional in vitro culture. Breast Cancer Res 7:R46–R59 Available from: http://breast-cancer-research.com/content/7/1/R46

Seidl P, Huetttinger R, Kneuehl R, Kunz-Schughart LA (2002) Three-dimensional fibroblast-tumor cell interaction causes downregulation of RACK1 mRNA expression in breast cancer cells in vitro. Int J Cancer 102:129–136

Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udoe J, Ullrich A, Press MF (1999) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244:707–712

Smith L, Baxter EW, Chambers PA, Green CA, Hanby AM, Hughes TA, Nash CE, Milican-Slater RA, Stead LF, Vergheze ET, Speirs V (2015) Down-regulation of miR-92 in breast epithelial cells and in normal but not tumour fibroblasts contributes to breast carcinogenesis. PLoS One 10:e0139698. doi:10.1371/journal.pone.0139698

Soule HD, Vazguez J, Long A, Albert S, Brennan M (1973) A human cell line from a pleural effusion derived from a breast carcinoma. J Natl Cancer Inst 51:1409–1416

Speirs V, Green AR, Walton DS, Kerin MJ, Fox JN, Carleton PJ, Desai SB, Atkin SL (1998) Short-term primary culture of epithelial cells derived from human breast tumours. Br J Cancer 78:1421–1429

Stampfer M, Hallowes RC, Hackett AJ (1980) Growth of normal human mammary cells in culture. In Vitro 16:415–425

Springer
Sutherland RM, Inch WR, McCredie JA, Kruuv J (1970) A multi-component radiation survival curve using an in vitro tumour model. Int J Radiat Biol Relat Stud Phys Chem Med 18(5):491–495

Thompson A, Brennan K, Cox A, Gee J, Harcourt D, Harris A, Harvie M, Holen I, Howell A, Nicholson R, Steel M, Streuli C (2008) Evaluation of the current knowledge limitations in breast cancer research: a gap analysis. Breast Cancer Res 10:R26. doi:10.1186/bcr1983

van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A, van Houdt W, van Gorp J, Taylor-Weiner A, Kester L, McLaren-Douglas A, Blokker J, Jaksani S, Bartfeld S, Volckman R, van Sluis P, Li VS, Seepo S, Sekhar Pedamallu C, Cibulskis K, Carter SL, McKenna A, Lawrence MS, Lichtenstein L, Stewart C, Koster J, Versteeg R, van Oudenaarden A, Saetz-Rodriguez J, Vries RG, Getz G, Wessels L, Stratton MR, McDermott U, Meyerson M, Garnett MJ, Clevers H (2015) Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 161:933–945. doi:10.1016/j.cell.2015.03.053

van Roozendaal KE, Klijn JG, van Ooijen B, Claassen C, Eggermont AM, Henzen-Logmans SC, Foekens J (1996) Differential regulation of breast tumor cell proliferation by stromal fibroblasts of various breast tissue sources. Int J Cancer 65:120–125

Wang F, Hansen RK, Radisky D, Yoneda T, Barcelos-Hoff MH, Petersen OW, Turley EA, Bissell MJ (2002) Phenotypic reversion or death of cancer cells by altering signaling pathways in three-dimensional contexts. J Natl Cancer Inst 94:1494–5103

Whittle JR, Lewis MT, Lindeman GJ, Visvader JE (2015) Patient-derived xenograft models of breast cancer and their predictive power. Breast Cancer Res 17:17. doi:10.1186/s13058-015-0523-1

Wilson H, Botfield B, Speirs V (2015) A global view of breast tissue banking. Adv Exp Med Biol 864:69–77

Yuhas JM, Tarleton AE, Molzen KB (1978) Multicellular tumor spheroid formation by breast cancer cells isolated from different sites. Cancer Res 38:2486–2491