Biochemical Society Transactions (2019) **47** 1689–1700
https://doi.org/10.1042/BST20190131

PORTLAND PRESS

Review Article

3D multicellular models to study the regulation and roles of acid–base transporters in breast cancer

Dominika Czaplinska, Line O. Elingaard-Larsen, Michala G. Rolver, Marc Severin and Stine F. Pedersen

Section for Cell Biology and Physiology, Department of Biology, University of Copenhagen, Universitetsparken 13, 2100 Copenhagen, Denmark

Correspondence: Dominika Czaplinska (dominika.czaplinska@bio.ku.dk) or Stine F. Pedersen (sfpedersen@bio.ku.dk)

As a result of elevated metabolic rates and net acid extrusion in the rapidly proliferating cancer cells, solid tumours are characterized by a highly acidic microenvironment, while cancer cell intracellular pH is normal or even alkaline. Two-dimensional (2D) cell monocultures, which have been used extensively in breast cancer research for decades, cannot precisely recapitulate the rich environment and complex processes occurring in tumours *in vivo*. The use of such models can consequently be misleading or non-predictive for clinical applications. Models mimicking the tumour microenvironment are particularly pivotal for studying tumour pH homeostasis, which is profoundly affected by the diffusion-limited conditions in the tumour. To advance the understanding of the mechanisms and consequences of dysregulated acid–base homeostasis in breast cancer, clinically relevant models that incorporate the unique microenvironment of these tumours are required. The development of three-dimensional (3D) cell cultures has provided new tools for basic research and pre-clinical approaches, allowing the culture of breast cancer cells under conditions that closely resemble tumour growth in a living organism. Here we provide an overview of the main 3D techniques relevant for breast cancer cell culture. We discuss the advantages and limitations of the classical 3D models as well as recent advances in 3D culture techniques, focusing on how these culture methods have been used to study acid–base transport in breast cancer. Finally, we outline future directions of 3D culture technology and their relevance for studies of acid–base transport.

Introduction

Breast cancer is the most frequent type of carcinoma, and the second leading cause of cancer-related death in the global female population. According to the International Agency for Research on Cancer (IARC), in 2018 alone over 2 million new breast cancer cases were diagnosed, and nearly 630 000 women died of the disease [1]. Despite the development of new early detection methods and improvements in the clinical management of the disease (e.g. targeted therapies), breast cancer remains a major public health problem [2].

The tumour microenvironment is a critical participant in breast cancer progression and therapeutic responses. However, the focus has been mainly on the impact of the stroma, i.e. the non-cancer cells in the tumour, whereas the role of the physico-chemical microenvironment in disease progression remains relatively poorly understood. Due to elevated metabolic rates, and a shift toward anaerobic glycolysis, highly proliferative tumour cells produce and extrude copious amounts of acid. Such tumours are therefore characterized by profound extracellular acidification, as low as pH 6–6.8, while intracellular pH (pH_i) remains normal or alkaline [3,4]. We and others have demonstrated that net acid extruders such as the Na^+/H^+-exchanger NHE1 (SLC9A1) and the Na^+,HCO_3^- cotransporter NBCn1 (SLC4A7) are frequently overexpressed or post-translationally activated in breast cancer cells and play key roles in their pH_i regulation [5–7], three-dimensional (3D) growth [8–10] and cell cycle progression [11].

Received: 5 September 2019
Revised: 1 November 2019
Accepted: 12 November 2019

Version of Record published:
5 December 2019

PORTLAND PRESS

Biochemical Society Transactions (2019) **47** 1689–1700
https://doi.org/10.1042/BST20190131

While acidosis is a recognized hallmark of cancer with a huge clinical potential, it remains understudied in breast cancer, and ongoing research aiming at developing new treatments generally does not consider the acidic microenvironment. Clearly, to better understand the role of acid–base transport in breast cancer, we need to employ clinically relevant cellular models that include acidic extracellular pH (pH_e) as a microenvironmental factor participating in cancer progression. It is well recognized that compared with traditional two-dimensional (2D) cell cultures, 3D models better mimic *in vivo* cell–cell and cell–extracellular matrix (ECM) interactions [12], thereby enabling more accurate recapitulation of *in vivo* gene expression [13,14] and cell polarization [15,16]. However, it is less widely recognized that such models are also essential because of the key role of dysregulated acid–base homeostasis in cancer progression.

This review focuses on classical 3D models as well as recent advances in 3D culture techniques, and their potential use in breast cancer research. We provide an overview of the commonly used 3D experimental systems and of how these culture models have been used to study acid–base transport in cancer, and we discuss future directions of 3D cell culture technology.

BOX 1: Definitions

- Co-culture: A cell culture containing two or more different types of cells.
- Spheroids: Cell lines grown at high density (generally ~0.5–2 × 10^4 cells/ml) in suspension, forming tight aggregates often in the shape of a sphere. Multicellular spheroids can be composed of a single cell type or a co-culture of multiple cell types.
- Cysts: Cell lines grown on top of or embedded into a layer of the reconstituted basement membrane, causing them to spontaneously form a polarized sphere of cells with a hollow lumen reminiscent of a duct or other tubular structures.
- Organoids: Patient- or animal-derived stem cells grown in a dome of the matrix. Each organoid is derived from one or a few cells, which can self-organize and differentiate into multiple organ-specific cell types exhibiting the spatial organization and functions similar to the organ of origin.
- Organotypic: Methods involving culturing of cells — usually several different cell types - in a manner that recapitulates the basic architecture and function of the tissue of origin.
- Microfluidic system: Cells, including co- and 3D cultures, grown in a microfluidic device that allows precise control of biochemical and physical parameters.

For further information and references, please see the respective sections in the text.

3D culture models

Numerous methods for 3D cell culture models of cancer exist (Figures 1 and 2). Below, we describe the most important such methods and outline their use and advantages in the context of studying tumour acid–base homeostasis and its consequences for tumour development. An overview of the main 3D cell culture methods, their advantages and disadvantages is provided in Table 1.

Choices of ECM materials and scaffolds for 3D cultures

As the reciprocity between tumours and their microenvironment is instrumental in understanding the development of cancer [17], the ability to change the composition of the ECM is important when studying the interactions between tumour cells and their surrounding environment [18]. Purified ECM proteins such as collagens and laminin, as well as extracts from Engelbreth-Holm-Swarm mouse sarcoma cells [19] such as Matrigel, are inherently cytocompatible and have long been used in 3D culture [20]. Importantly, Matrigel and mammary gland have a very similar stiffness and tensional homeostasis [21]. However, large variation between batches and poorly defined composition [22] with limited ability to modulate chemical and physical properties have led to the development of a wide range of synthetic polymers (e.g. polyethylene glycol (PEG), poly(vinyl alcohol (PVA)) as well as natural biomaterials such as chitosan and alginate for 3D matrices [23]). The main advantage of matrices derived from engineered biomaterials is the ability to finely tune their properties, including matrix

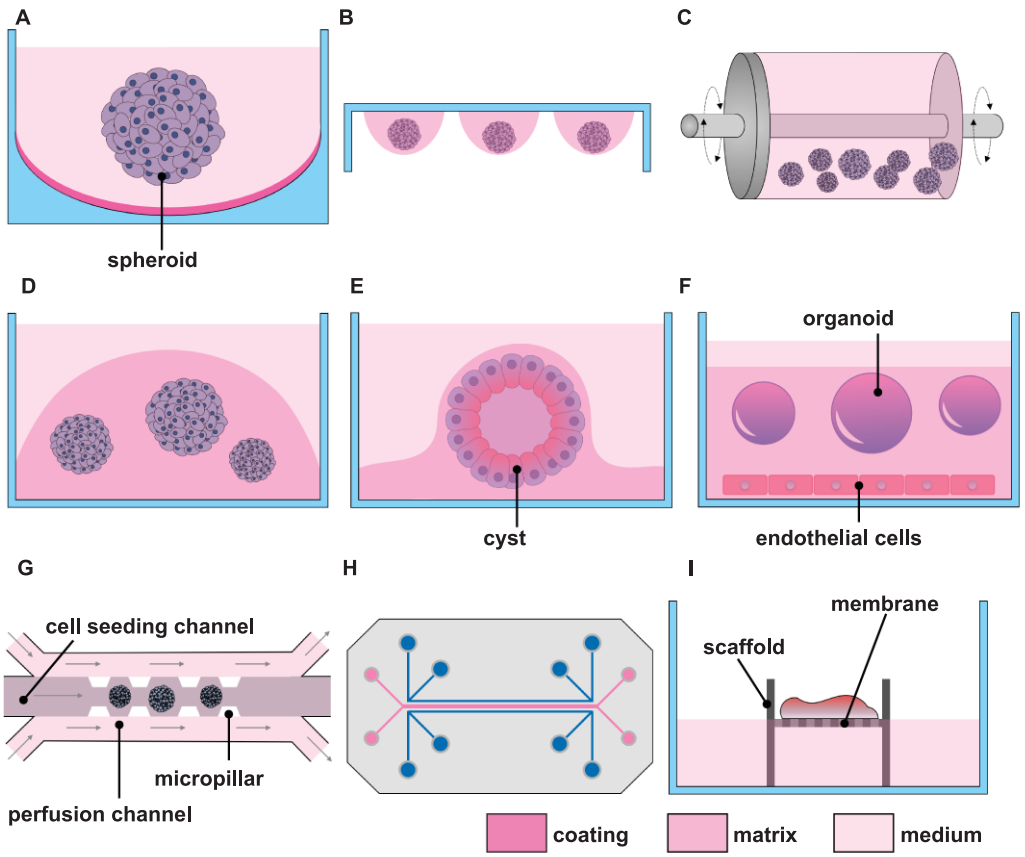

Figure 1. 3D culture methods.

(**A**) Suspension culture in low-adhesion wells. (**B**) Hanging drop culture. (**C**) Rotational bioreactor (spinner flask) culture. (**D**) Matrix-embedded culture; can be used for spheroids as well as organoids. (**E**) 3D-on-top culture, here used for cyst generation. (**F**) Example of an organotypic culture, consisting of organoids cultured on a layer of endothelial cells. (**G** and **H**) Microfluidic systems for spheroid growth (**G**) or microenvironmental control, e.g. as organ-on-a-chip culture (**H**). (**I**) Organ explant culture. Several of the culture methods shown are relevant for other 3D preparations than those shown in the examples. See Table 1 and text for further details.

stiffness, pore size, degradation and the inclusion of ECM proteins, all factors demonstrated to be vital for tumour progression, invasion and metastasis [24–26]; see also [27] and [28] for recent reviews on biomaterials. Another relatively recent development is the ability to produce intact decellularized matrices from *in vivo* tumours and use them as scaffolds for preparing new *in vitro* tumours [29] or for analyzing the complexity of the ECM [30]. Such studies will enhance the detailed understanding of the composition and importance of the ECM in different cancers, and guide future efforts in designing *in vitro* 3D models.

Spheroids

The multicellular spheroid model (Figure 2A) is several decades old [31] (Figure 3), yet is still used extensively in cancer research. This likely reflects the many advantages this model offers in terms of providing a micro-environment mimicking that of *in vivo* tumours, yet still being relatively affordable and high-throughput [32]. Cells grown as spheroids not only exhibit cell–cell and cell–ECM interactions, influencing intra- and intercellular signalling, they furthermore develop chemical gradients of nutrients, oxygen, pH and waste products when spheroid diameter reaches 200–500 μm [33–35]. As a result, their gene expression profile differs from that of 2D cultures, reflecting clinical expression profiles more closely [32,34,36], as well as affecting growth rates and drug responses [32,36,37]. Cancer cell spheroids can be prepared using several different techniques. A simple method involves the seeding of cells into round-bottom plates with a non-adhesive coating, forcing cell–cell adhesion (Figure 1A). This spontaneous spheroid formation is often mediated by E- and N-cadherins, however

Biochemical Society Transactions (2019) **47** 1689–1700
https://doi.org/10.1042/BST20190131

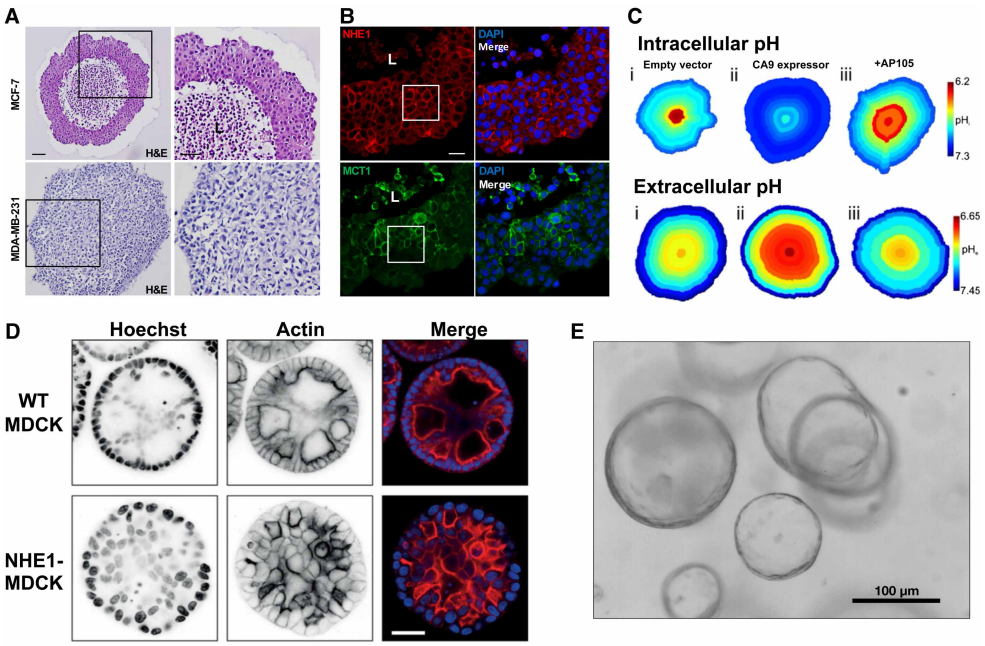

Figure 2. Examples of experiments involving 3D culture methods.

(**A**) Spheroids grown from MCF-7 and MDA-MB-231 breast cancer cells (9 day culture). Spheroids were embedded, sectioned, and stained using hematoxylin and eosin (H & E) to illustrate cell organization. (**B**) Immunohistochemical analysis of the localization of NHE1 (upper panel) and MCT1 (lower panel) in MCF-7 spheroids. Scale bars: 20 μm. L: Indicates lumen of spheroid. Note the localization of NHE1 throughout the viable part of the spheroid, and MCT1 only in the hypoxic core. **A**, **B** are reproduced from [8]. (**C**) Maps of pH_i and pH_e in HCT116 spheroids transfected with empty vector (i), a plasmid vector with cDNA for human carbonic anhydrase 9 (ii) or pretreated with a carbonic anhydrase inhibitor (AP105) (iii). Note that CAIX overexpression increases pH_i and acidifies pH_e, while CA inhibition has the opposite effect. Reproduced from [48]. (**D**) MDCK cells were grown as cysts for 8 days. Cysts of WT MDCK cells were organized as spheres with apical actin strands and cavities. MDCK cysts over-expressing NHE1 were less organized with no or few cavities and no clear apical actin bands. Reproduced from [64] with permission. (**E**) Light microscopic images of an organoid model of mouse pancreatic ductal adenocarcinoma (PDAC).

some cell types need an ECM supplement for compact spheroid formation, often facilitated by collagen I and integrin interactions [38]. Similarly, the hanging drop method (Figure 1B), exploits the combination of gravity and lack of attachment surfaces in a drop of medium to force spheroid formation. Finally, the spinner flask technique (Figure 1C) uses constant stirring of a cell suspension to induce cell–cell collisions creating multiple spheroids in suspension.

The observed change in anti-cancer therapy treatment response when moving from 2D to 3D cultures, has resulted in a rather large literature on spheroid drug screening assays in 3D spheroids [37,39–41]. In general, treatments are expected to lose efficacy when applied in a 3D tumour microenvironment compared with 2D, at least in part reflecting a combination of reduced drug uptake and altered growth/survival signalling in the 3D setting [32,37]. The spheroid model is applicable to co-cultures with e.g. fibroblasts, adipocytes or immune cells further increasing the relevance of this model for drug screening and anti-cancer resistance evaluation [42–44]. Other abilities facilitating cancer progression, such as invasion, can likewise be examined using the spheroid model. Finally, by embedding spheroids into a gel mimicking the ECM (Figure 1D), and monitoring invasion into this gel, effects of pharmaceutical or biological manipulation on metastatic potential can be evaluated [45–47].

In the study of acid–base homeostasis, multicellular spheroids are a very relevant model because, as discussed above, the pH environment created by the cells in the tumour is short-circuited when cells are grown in a buffered 2D monolayer. The localization, expression and activity of acid–base transporters is highly affected by the 3D structure and surrounding tumour microenvironment. For example, Andersen et al. [8] found the spatial

Biochemical Society Transactions (2019) **47** 1689–1700
https://doi.org/10.1042/BST20190131

Table 1 Advantages and disadvantages of different 3D culture methods Part 1 of 2

Method	Model(s)	Advantages	Limitations
Low adhesion plates	Spheroids	Simple and inexpensive ECM microenvironment Applicable for most cancer cells Amenable to high-throughput testing Automated quantification possible (e.g. IncuCyte® systems)	Passaging is difficult Not practical for applications requiring large amounts of mRNA/protein
Hanging drops	Spheroids	Simple and inexpensive External scaffolds not required for aggregation	Cell number and drop volume limited (volume ~ 40 μl) Long-term culture/passaging difficult Medium exchange difficult/ impossible
Gel embedded cultures	Spheroids Cysts Organoids	Incorporate ECM Automated quantification possible Long-term culture/passaging possible Two or more cell types to mimic real tumour niches	Expensive materials Sensitive to variations in ECM components, growth factors etc. Biochemical analysis complicated (additional steps required to separate cells from the matrix)
Stem cell-based 3D culture	Organoids	Patient-specific organoids possible Genetic modification possible (transfection, lenti-/retroviral transduction, CRISPR/Cas9) Long-term culture and passaging Possible to transplant into mice Can be stored cryopreserved Long-term stem/progenitor cell culture without loss of characteristics	Expensive materials and additives Involve surgical procedures and human patients or lab animals Less amenable to high-throughput screening
Rotational bioreactor/spinner flask	Spheroids	The liquid flow prevents cell adhesion to culture flask and equally distributes nutrients and oxygen Produces a large number of spheroids	Requires expensive equipment Mechanical stress/damage to spheroids can occur Not possible to precisely control spheroids size and composition No ECM microenvironment
Microfluidic systems	Spheroids Organotypic culture Organoids	Mimic fluid circulation in living organisms Spheroids size and composition may be controlled very precisely Well-defined flow, nutrients, etc Can incorporate ECM	Requires expensive and complicated equipment Analysis options are limited (mostly microscopy)
Organ-on-a-chip	2- and 3D co-cultures	Reproduces architectural complexity of tissues and organs Mimics *in vivo* fluid circulation Can be equipped with optical, physical and biochemical sensors for automated continual measurements of various parameters	Microengineering is complicated and expensive Less amenable to high-throughput screening
Non-ECM 3D scaffold culture	Spheroids Cysts Organoids	Use of synthetic polymers or biomaterials (e.g. alginate) increases versatility, reproducibility, and stability Processed more easily than mammalian ECM-based matrices Mimics 3D tissue architecture Ability to tailor protein content	Expensive for large scale culture Synthetic polymers are not inherently bioactive Difficult dissociation of the cells from the scaffold

Continued

Biochemical Society Transactions (2019) **47** 1689–1700
https://doi.org/10.1042/BST20190131

Table 1 Advantages and disadvantages of different 3D culture methods Part 2 of 2

Method	Model(s)	Advantages	Limitations
Organ/tissue explant cultures	Organ/tissue explants	Possible to culture tissue from both embryos and adult organisms Inherently multiple cell types and interactions Incorporate ECM microenvironment	Involve surgical procedures and human patients or lab animals Relatively expensive Complicated 3D imaging and analysis Require tightly controlled temperature and oxygenation to maintain viability Culture and passaging is limited to <3 weeks

organization and expression of the major acid-extruding transporters NHE1, NBCn1 and the monocarboxylate transporters, MCT1 and MCT4, to differ between cell types, when evaluated in breast cancer spheroids (Figure 2B), and described a cell-type specific dependence of these transporters on 3D growth. Studies of pH_i and pH_e regulation in cancer cell spheroids by Swietach et al. [48] demonstrated how the carbonic anhydrase IX (CAIX) contributes to acidifying the extracellular space of spheroids, while alkalizing the intracellular space, pointing to CAIX as a potential therapeutic target (Figure 2C). In a more recent study, the expression and inhibition of NHE1, CAIX and the vacuolar H^+-ATPase (V-ATPase) were evaluated in hypoxic breast cancer spheroids, revealing a correlation between hypoxia and CAIX or NHE1 expression depending on the cell type, as well as a reduced invasion in 3D in response to CAIX inhibition [49].

Cysts

Developed as a model four decades ago [50], epithelial cell lines grown in 3D culture form growth-arrested cyst-like acini with a spherical epithelial monolayer featuring an internal apical lumen and apicobasal polarity. The cyst model has consequently been widely used for studying polarity and cell–cell and cell–ECM interactions, and the dysregulation of these processes in cancer cells [51].

To form cysts, individual cells are seeded either directly into a gel (embedded cysts) or on top of a polymerized gel (cysts on top) [52,53] (Figure 1E). Once cultured, individual acinar cells have been found to rotate in a manner similar to embryonal development. After the first mitosis cells continue to rotate and divide in a

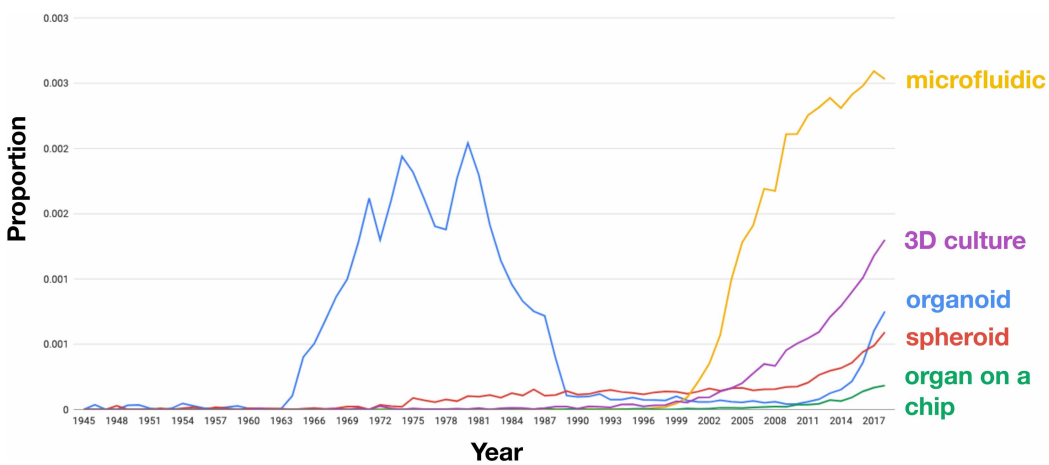

Figure 3. PubMed entries involving 3D culture methods.

Proportion for each search in PubMed by year from 1945 to 2018. The graph uses proportions instead of raw numbers, as the increase in the biomedical literature over time makes absolute values less illustrative of changes. Graph generated with PubMed by Year [86].

synchronized manner as the multicellular architecture is established [54]. Blocking E-Cadherin, PAR3 or actin dynamics disrupt this synchronised rotation and the formation of polarized acini [54].

Cysts are highly amenable to confocal imaging and can also be extracted from the gel for molecular and protein analysis [52]. The morphology and expression profiles of breast cancer cell lines in 3D culture has been shown to correlate well with their invasiveness [55].

In cyst models, morphogenesis of mammary acini can be studied in detail [20,56], illuminating factors crucial to their formation [57] and to the development of malignant phenotypes [58–60]. Cysts can be co-cultured with tumour-derived epithelial cells as shown by Spink et al. [61], where MCF-7 cells together with MCF10A cells appear to form a luminal and myoepithelial layer respectively. In a similar experiment, Ivers et al. [62] showed that MDA-MB-231 cells engulfed and destroyed MDCK cysts when present in sufficient numbers. Gudjonsson et al. [63] co-cultured human primary luminal and myoepithelial cells to form double-layered acini and demonstrated that normal polarity could be formed by luminal cells alone with the addition of laminin-1 to collagen gels.

The cyst model has not yet been widely used for studies of acid–base regulation. In a recent study however, NHE1 overexpression in MDCK cysts was found to cause polarity disruption and epithelial disorganization with lack of lumen formation [64] (Figure 2D). While no such studies are, to our knowledge, published so far, cysts should be amenable to live imaging of pH_i and transporter activity using small-molecule or genetically encoded pH sensors, and this would be an interesting future development of this model in the context of breast cancer.

Organoids

Organoids are simplified and miniaturized organ models that mimic endogenous cell structure and interactions (Figs. 1F and 2E). According to Lancaster and Knoblich [65] an organoid exhibits the following properties: (i) is composed of multiple organ-specific cell types, (ii) recapitulates specific function of the particular organ (e.g. endocrine secretion, neural activity, contraction), (iii) its cells are spatially organized in three dimensions, similar to an organ. Organoids are derived from one or a few stem cells (e.g. embryonic stem cells, induced pluripotent stem cells, adult stem cells extracted from the target organ), which have the unique abilities of self-renewal and differentiation [65]. The use of organoids started as a major technological breakthrough in the 1960's [66,67]. This model was seemingly forgotten for decades (Figure 3), but its popularity has significantly increased since early 2010s paralleling the widespread use of stem cell cultures and reflecting its great potential as a tool in biological research and potential clinical use [68]. Illustrating this, the organoid culture technique was named by The Scientist as one of the biggest scientific advancements of 2013 [69] and by Nature Methods as the Method of the Year 2017 [70]. To date, scientists have produced organoids derived from the brain, intestine, liver, pancreas (Figure 2D), kidney and stomach, and many others are on the way [65].

A major advantage is that organoids can be grown from a limited amount of material, e.g. biopsies and used to develop personalized therapies. Sachs et al. have generated a biobank of 95 organoids representing major breast cancer subtypes derived from patients. These organoids have maintained the mutational signature as well as the genetic and histological profiles of their tissue of origin (e.g. over 90% of ER/PR-negative tumours gave rise to organoids with the same hormone receptor status) and were used for *in vitro* drug screening that was consistent with *in vivo* xeno-transplantations and patient response [71]. Walsh et al. have tested the potential value of patient-derived breast cancer organoids for drug screening. They found that the *in vitro* organoid drug response(s) to a panel of breast cancer therapeutics mirrored the result of animal studies *in vivo* [72]. Furthermore, exemplified by Duarte et al. [73] who studied cancer-drug resistance employing a BRCA-deficient mouse mammary tumour organoid model, organoids are also amenable to gene editing and can be used for discovery or validation of possible drug targets.

While there are, to our knowledge, no such studies published yet, we consider organoid cultures highly relevant tools for acid–base transport studies in breast cancer. Thus, contrary to conventional *in vitro* models, organoids preserve *in vivo* tumour microenvironment properties such as the environmental heterogeneity, cell–cell and cell–ECM signalling. Organoid models have the potential to advance the understanding of acid–base transport in breast cancer not only because they faithfully recapitulate and preserve cell–ECM interactions found *in vivo*. Organoid culture techniques allow precise control and real-time analysis of microenvironmental factors relevant to acid–base transport and tumour progression — e.g. pH (using pH-sensitive fluorescent dyes for tracking cytosolic and luminal pH over time [74]), O_2 concentration (with optical sensor probes [75]) and metabolism (e.g using Seahorse XF technology [76]). Importantly, organoid cultures can be established with a

Biochemical Society Transactions (2019) **47** 1689–1700
https://doi.org/10.1042/BST20190131

very limited amount of material (e.g. from biopsies) and employing spinning bioreactors will allow for mass production of patient-derived organoids, opening for the prospect of making the analysis of dysregulated pH homeostasis a personalized medicine approach.

Microfluidic 3D systems

Microfluidic systems are increasingly being used to study cancer cell 3D cultures (Figure 3; for reviews, see [77–79]). Combining 3D cell culture with microfluidic systems has several advantages that are highly relevant to the study of acid–base transport. First of all, microfluidic systems with chambers of confined size for growing of spheroids can be used to make and analyze very homogenous spheroids [80,81] (Figure 1G) While mimicking the complex, superimposed gradients of the tumour microenvironment is currently beyond the limits also of this technique, microfluidic systems can nevertheless be used to precisely control gradients of, e.g. pH, lactate, and oxygen pressure and to combine multiple cell types and environments, creating an organ-on-a-chip (Figure 1H), and monitor such gradients using e.g. optical techniques or thin-film microelectrodes [82]. The technique can also be used to co-culture cancer cells with other cell types in a 3D setting. In an elegant example of the latter, Ayuso et al. [83] used a microfluidics device to culture ductal carcinoma *in situ* cells inside a model mammary duct, followed by the analysis of metabolites and gene expression.

While not yet a standard method in the cancer field, the ability of using microfluidics to very precisely control microenvironmental parameters relevant to acid–base transport should be much more widely exploited. Among several other measured parameters, Weltin et al. [82] employed pH sensor electrodes based on iridium oxide and lactate biosensor electrodes based on lactate oxidase, to monitor glycolytic status in cultured human glioblastoma multiforme cells. The microfluidics technique can be combined with co- and 3D culture. Spheroids can be retrieved and analyzed off-chip, by qPCR, sequencing, and other relevant assays [81] or microfluidics can be used for selection of single cells with specific properties, followed by on-chip clonal expansion and analysis [84].

Other organotypic models

Several other organotypic models have been employed for studies of breast cancer. These include the use of decellularized matrices as a scaffold for the culture of breast cancer cells, e.g. in studies of the interaction between the specific ECM of the metastatic niche and breast cancer cell colonization [85], or in establishing models of the primary breast tumour microenvironment. Another important 3D culture technique is the *in vitro* maintenance of organ tissue explants (Figure 1I, [83,84]), which allows, for instance, testing of drug candidates in a preparation in which native cell–cell interactions are maintained. To our knowledge, none of these models have so far been employed for the study of acid–base homeostasis in breast cancer, except for very brief experiments not involving *in vitro* growth *per se* [5]. To the extent that such models faithfully recapitulate the *in vitro* tumour and are amenable to optical analyzes and/or to mRNA and protein extraction, all of such models are likely to be of great interest in the field of pH in cancer.

Conclusions

Cell behaviour is profoundly influenced by cell–cell and cell–matrix interactions and the physico-chemical environment, making use of 3D cell culture imperative in studies of cancer. This is particularly true for studies of tumour-specific dysregulation of acid–base homeostasis, now recognized to play a key role in cancer development. Hence, increasing the use of 3D models is essential for driving further advances in understanding how this key component of the tumour microenvironment contributes to cancer progression.

Perspectives

Despite substantial advances in treatment options, breast cancer remains the leading cause of cancer-related mortality in women globally, and new therapies are urgently needed, especially targeting treatment-resistant subtypes. Breast cancers, like most other solid tumours, are characterized by increased metabolic acid production and up-regulation of acid extrusion from the cancer cells. In conjunction with poor diffusion, this creates a heterogeneous microenvironment with marked spatiotemporal gradients of extracellular acidity, which contributes to treatment resistance and favours development of a highly aggressive cancer cell phenotype.

- Most of the current understanding of the importance of the acidic microenvironment in cancer development comes from conventional 2D cell culture models, which are unable to mimic the complexity of the tumour microenvironment in the patient.
- To drive progress in this field and take the understanding of dysregulated acid–base homeostasis in tumours to a level where it can be exploited therapeutically, it is essential to more accurately reflect these conditions.
- Recent rapid advances in microfluidics techniques, 3D printing, biomaterials, and stem cell techniques have increased the repertoire and sophistication of *in vitro* 3D culture models mimicking the heterogeneous, acidic 3D environment of patient tumours, enabling new insights and successful development and assessment of new therapies. A more widespread use of models such as breast cancer spheroids in high-throughput testing of novel therapeutics, and patient biopsy-derived organoids as part of a personalized medicine scheme, would facilitate dissection of how tumour acidity contributes to cancer development and how this can be exploited in cancer therapeutics.

Abbreviations

CAIX, carbonic anhydrase IX; ECM, extracellular matrix; IARC, International Agency for Research on Cancer; PEG, polyethylene glycol; PVA, poly vinyl alcohol.

Author Contribution

The concept for this review was developed by all the authors. D.C. wrote the introduction, the section on organoids, and prepared Table 1 and all figures; L.E.L. and M.R. wrote the section on spheroid culture; M.R. the definitions box, M.S. the sections on matrix choice and cysts; and S.F.P. the microfluidics and other organotypic models sections, conclusion and perspectives. All authors read, commented and approved the final submitted version.

Funding

Related work in our laboratory is supported by The Danish Cancer Society [grant no. A12359] and the European Union [H2020-MSCA-ITN-2018, grant no. 813834].

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

References

1 Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. and Jemal, A. (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **68**, 394–424 https://doi.org/10.3322/caac.21492
2 Siegel, R.L., Miller, K.D. and Jemal, A. (2019) Cancer statistics, 2019. *CA Cancer J. Clin.* **69**, 7–34 https://doi.org/10.3322/caac.21551
3 Andersen, A.P., Moreira, J.M. and Pedersen, S.F. (2014) Interactions of ion transporters and channels with cancer cell metabolism and the tumour microenvironment. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **369**, 20130098 https://doi.org/10.1098/rstb.2013.0098
4 White, K.A., Grillo-Hill, B.K. and Barber, D.L. (2017) Cancer cell behaviors mediated by dysregulated pH dynamics at a glance. *J. Cell Sci.* **130**, 663–669 https://doi.org/10.1242/jcs.195297
5 Boedtkjer, E., Moreira, J.M., Mele, M., Vahl, P., Wielenga, V.T., Christiansen, P.M. et al. (2013) Contribution of Na^+,HCO_3^--cotransport to cellular pH control in human breast cancer: a role for the breast cancer susceptibility locus NBCn1 (SLC4A7). *Int. J. Cancer* **132**, 1288–1299 https://doi.org/10.1002/ijc.27782
6 Gorbatenko, A., Olesen, C.W., Morup, N., Thiel, G., Kallunki, T., Valen, E. et al. (2014) Erbb2 upregulates the Na^+,HCO_3^--cotransporter NBCn1/SLC4A7 in human breast cancer cells via Akt, ERK, Src, and Kruppel-like factor 4. *FASEB J.* **28**, 350–363 https://doi.org/10.1096/fj.13-233288
7 Lee, S., Mele, M., Vahl, P., Christiansen, P.M., Jensen, V.E. and Boedtkjer, E. (2015) Na^+,HCO_3^--cotransport is functionally upregulated during human breast carcinogenesis and required for the inverted pH gradient across the plasma membrane. *Pflugers Arch.* **467**, 367–377 https://doi.org/10.1007/s00424-014-1524-0
8 Andersen, A.P., Flinck, M., Oernbo, E.K., Pedersen, N.B., Viuff, B.M. and Pedersen, S.F. (2016) Roles of acid-extruding ion transporters in regulation of breast cancer cell growth in a 3-dimensional microenvironment. *Mol. Cancer* **15**, 45 https://doi.org/10.1186/s12943-016-0528-0

9 Lee, S., Axelsen, T.V., Andersen, A.P., Vahl, P., Pedersen, S.F. and Boedtkjer, E. (2016) Disrupting Na^+, HCO_3^--cotransporter NBCn1 (Slc4a7) delays murine breast cancer development. *Oncogene* **35**, 2112–2122 https://doi.org/10.1038/onc.2015.273
10 Andersen, A.P., Samsoe-Petersen, J., Oernbo, E.K., Boedtkjer, E., Moreira, J.M.A., Kveiborg, M. et al. (2018) The net acid extruders NHE1, NBCn1 and MCT4 promote mammary tumor growth through distinct but overlapping mechanisms. *Int. J. Cancer* **142**, 2529–2542 https://doi.org/10.1002/ijc.31276
11 Flinck, M., Kramer, S.H., Schnipper, J., Andersen, A.P. and Pedersen, S.F. (2018) The acid-base transport proteins NHE1 and NBCn1 regulate cell cycle progression in human breast cancer cells. *Cell Cycle* **17**, 1056–1067 https://doi.org/10.1080/15384101.2018.1464850
12 Ravi, M., Paramesh, V., Kaviya, S.R., Anuradha, E. and Solomon, F.D. (2015) 3D cell culture systems: advantages and applications. *J. Cell Physiol.* **230**, 16–26 https://doi.org/10.1002/jcp.24683
13 Bissell, M.J., Hall, H.G. and Parry, G. (1982) How does the extracellular matrix direct gene expression? *J. Theor. Biol.* **99**, 31–68 https://doi.org/10.1016/0022-5193(82)90388-5
14 Ghosh, S., Spagnoli, G.C., Martin, I., Ploegert, S., Demougin, P., Heberer, M. et al. (2005) Three-dimensional culture of melanoma cells profoundly affects gene expression profile: a high density oligonucleotide array study. *J. Cell Physiol.* **204**, 522–531 https://doi.org/10.1002/jcp.20320
15 O'Brien, L.E., Jou, T.S., Pollack, A.L., Zhang, Q., Hansen, S.H., Yurchenco, P. et al. (2001) Rac1 orientates epithelial apical polarity through effects on basolateral laminin assembly. *Nat. Cell Biol.* **3**, 831–838 https://doi.org/10.1038/ncb0901-831
16 Zegers, M.M., O'Brien, L.E., Yu, W., Datta, A. and Mostov, K.E. (2003) Epithelial polarity and tubulogenesis in vitro. *Trends Cell Biol.* **13**, 169–176 https://doi.org/10.1016/S0962-8924(03)00036-9
17 Ronnov-Jessen, L. and Bissell, M.J. (2009) Breast cancer by proxy: can the microenvironment be both the cause and consequence? *Trends Mol. Med.* **15**, 5–13 https://doi.org/10.1016/j.molmed.2008.11.001
18 Wei, S.C., Fattet, L., Tsai, J.H., Guo, Y., Pai, V.H., Majeski, H.E. et al. (2015) Matrix stiffness drives epithelial-mesenchymal transition and tumour metastasis through a TWIST1-G3BP2 mechanotransduction pathway. *Nat. Cell Biol.* **17**, 678–688 https://doi.org/10.1038/ncb3157
19 Orkin, R.W., Gehron, P., McGoodwin, E.B., Martin, G.R., Valentine, T. and Swarm, R. (1977) A murine tumor producing a matrix of basement membrane. *J. Exp. Med.* **145**, 204–220 https://doi.org/10.1084/jem.145.1.204
20 Barcellos-Hoff, M.H., Aggeler, J., Ram, T.G. and Bissell, M.J. (1989) Functional differentiation and alveolar morphogenesis of primary mammary cultures on reconstituted basement membrane. *Development* **105**, 223–235 PMID:2806122
21 Paszek, M.J., Zahir, N., Johnson, K.R., Lakins, J.N., Rozenberg, G.I., Gefen, A. et al. (2005) Tensional homeostasis and the malignant phenotype. *Cancer Cell* **8**, 241–254 https://doi.org/10.1016/j.ccr.2005.08.010
22 Hughes, C.S., Postovit, L.M. and Lajoie, G.A. (2010) Matrigel: a complex protein mixture required for optimal growth of cell culture. *Proteomics* **10**, 1886–1890 https://doi.org/10.1002/pmic.200900758
23 Vining, K.H., Stafford, A. and Mooney, D.J. (2019) Sequential modes of crosslinking tune viscoelasticity of cell-instructive hydrogels. *Biomaterials* **188**, 187–197 https://doi.org/10.1016/j.biomaterials.2018.10.013
24 Sodek, K.L., Brown, T.J. and Ringuette, M.J. (2008) Collagen I but not Matrigel matrices provide an MMP-dependent barrier to ovarian cancer cell penetration. *BMC Cancer* **8**, 223 https://doi.org/10.1186/1471-2407-8-223
25 Levental, K.R., Yu, H., Kass, L., Lakins, J.N., Egeblad, M., Erler, J.T. et al. (2009) Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* **139**, 891–906 https://doi.org/10.1016/j.cell.2009.10.027
26 Taubenberger, A.V., Bray, L.J., Haller, B., Shaposhnykov, A., Binner, M., Freudenberg, U. et al. (2016) 3D extracellular matrix interactions modulate tumour cell growth, invasion and angiogenesis in engineered tumour microenvironments. *Acta Biomater.* **36**, 73–85 https://doi.org/10.1016/j.actbio.2016.03.017
27 Beri, P., Matte, B.F., Fattet, L., Kim, D., Yang, J. and Engler, A.J. (2018) Biomaterials to model and measure epithelial cancers. *Nat. Rev. Mater.* **3**, 418–430 https://doi.org/10.1038/s41578-018-0051-6
28 Gu, L. and Mooney, D.J. (2016) Biomaterials and emerging anticancer therapeutics: engineering the microenvironment. *Nat. Rev. Cancer.* **16**, 56–66 https://doi.org/10.1038/nrc.2015.3
29 Li, W., Hu, X., Yang, S., Wang, S., Zhang, C., Wang, H. et al. (2018) A novel tissue-engineered 3D tumor model for anti-cancer drug discovery. *Biofabrication* **11**, 015004 https://doi.org/10.1088/1758-5090/aae270
30 Mayorca-Guiliani, A.E., Madsen, C.D., Cox, T.R., Horton, E.R., Venning, F.A. and Erler, J.T. (2017) ISDot: in situ decellularization of tissues for high-resolution imaging and proteomic analysis of native extracellular matrix. *Nat. Med.* **23**, 890–898 https://doi.org/10.1038/nm.4352
31 Sutherland, R.M. (1988) Cell and environment interactions in tumor microregions: the multicell spheroid model. *Science* **240**, 177–184 https://doi.org/10.1126/science.2451290
32 Hirschhaeuser, F., Menne, H., Dittfeld, C., West, J., Mueller-Klieser, W. and Kunz-Schughart, L.A. (2010) Multicellular tumor spheroids: an underestimated tool is catching up again. *J. Biotechnol.* **148**, 3–15 https://doi.org/10.1016/j.jbiotec.2010.01.012
33 Kunz-Schughart, L.A. (1999) Multicellular tumor spheroids: intermediates between monolayer culture and in vivo tumor. *Cell Biol. Int.* **23**, 157–161 https://doi.org/10.1006/cbir.1999.0384
34 Pampaloni, F., Reynaud, E.G. and Stelzer, E.H. (2007) The third dimension bridges the gap between cell culture and live tissue. *Nat. Rev. Mol. Cell Biol.* **8**, 839–845 https://doi.org/10.1038/nrm2236
35 Rodriguez-Enriquez, S., Gallardo-Perez, J.C., Aviles-Salas, A., Marin-Hernandez, A., Carreno-Fuentes, L., Maldonado-Lagunas, V. et al. (2008) Energy metabolism transition in multi-cellular human tumor spheroids. *J. Cell Physiol.* **216**, 189–197 https://doi.org/10.1002/jcp.21392
36 Gaedtke, L., Thoenes, L., Culmsee, C., Mayer, B. and Wagner, E. (2007) Proteomic analysis reveals differences in protein expression in spheroid versus monolayer cultures of low-passage colon carcinoma cells. *J. Proteome Res.* **6**, 4111–4118 https://doi.org/10.1021/pr0700596
37 Friedrich, J., Seidel, C., Ebner, R. and Kunz-Schughart, L.A. (2009) Spheroid-based drug screen: considerations and practical approach. *Nat. Protoc.* **4**, 309–324 https://doi.org/10.1038/nprot.2008.226
38 Ivascu, A. and Kubbies, M. (2007) Diversity of cell-mediated adhesions in breast cancer spheroids. *Int. J. Oncol.* **31**, 1403–1413 PMID:17982667
39 Vinci, M., Gowan, S., Boxall, F., Patterson, L., Zimmermann, M., Court, W. et al. (2012) Advances in establishment and analysis of three-dimensional tumor spheroid-based functional assays for target validation and drug evaluation. *BMC Biol.* **10**, 29 https://doi.org/10.1186/1741-7007-10-29
40 Breslin, S. and O'Driscoll, L. (2016) The relevance of using 3D cell cultures, in addition to 2D monolayer cultures, when evaluating breast cancer drug sensitivity and resistance. *Oncotarget* **7**, 45745–45756 https://doi.org/10.18632/oncotarget.9935

41 Ivanov, D.P. and Grabowska, A.M. (2017) Spheroid arrays for high-throughput single-cell analysis of spatial patterns and biomarker expression in 3D. *Sci. Rep.* **7**, 41160 https://doi.org/10.1038/srep41160
42 Bochet, L., Lehuede, C., Dauvillier, S., Wang, Y.Y., Dirat, B., Laurent, V. et al. (2013) Adipocyte-derived fibroblasts promote tumor progression and contribute to the desmoplastic reaction in breast cancer. *Cancer Res.* **73**, 5657–5668 https://doi.org/10.1158/0008-5472.CAN-13-0530
43 Amann, A., Zwierzina, M., Koeck, S., Gamerith, G., Pechriggl, E., Huber, J.M. et al. (2017) Development of a 3D angiogenesis model to study tumour—endothelial cell interactions and the effects of anti-angiogenic drugs. *Sci. Rep.* **7**, 2963 https://doi.org/10.1038/s41598-017-03010-6
44 Kuen, J., Darowski, D., Kluge, T. and Majety, M. (2017) Pancreatic cancer cell/fibroblast co-culture induces M2 like macrophages that influence therapeutic response in a 3D model. *PLoS ONE* **12**, e0182039 https://doi.org/10.1371/journal.pone.0182039
45 Berens, E.B., Holy, J.M., Riegel, A.T. and Wellstein, A. (2015) A cancer cell spheroid assay to assess invasion in a 3D setting. *J. Vis. Exp.* **105**, e53409 https://doi.org/10.3791/53409
46 Vinci, M., Box, C. and Eccles, S.A. (2015) Three-dimensional (3D) tumor spheroid invasion assay. *J. Vis. Exp.* **99**, e52686 https://doi.org/10.3791/52686
47 Cisneros Castillo, L.R., Oancea, A.D., Stullein, C. and Regnier-Vigouroux, A. (2016) Evaluation of consistency in spheroid invasion assays. *Sci. Rep.* **6**, 28375 https://doi.org/10.1038/srep28375
48 Swietach, P., Patiar, S., Supuran, C.T., Harris, A.L. and Vaughan-Jones, R.D. (2009) The role of carbonic anhydrase 9 in regulating extracellular and intracellular pH in three-dimensional tumor cell growths. *J. Biol. Chem.* **284**, 20299–20310 https://doi.org/10.1074/jbc.M109.006478
49 Meehan, J., Ward, C., Turnbull, A., Bukowski-Wills, J., Finch, A.J., Jarman, E.J. et al. (2017) Inhibition of pH regulation as a therapeutic strategy in hypoxic human breast cancer cells. *Oncotarget* **8**, 42857–42875 https://doi.org/10.18632/oncotarget.17143
50 Hall, H.G., Farson, D.A. and Bissell, M.J. (1982) Lumen formation by epithelial cell lines in response to collagen overlay: a morphogenetic model in culture. *Proc. Natl Acad. Sci. U.S.A.* **79**, 4672–4676 https://doi.org/10.1073/pnas.79.15.4672
51 Hebner, C., Weaver, V.M. and Debnath, J. (2008) Modeling morphogenesis and oncogenesis in three-dimensional breast epithelial cultures. *Annu. Rev. Pathol.* **3**, 313–339 https://doi.org/10.1146/annurev.pathmechdis.3.121806.151526
52 Lee, G.Y., Kenny, P.A., Lee, E.H. and Bissell, M.J. (2007) Three-dimensional culture models of normal and malignant breast epithelial cells. *Nat. Methods* **4**, 359–365 https://doi.org/10.1038/nmeth1015
53 Elia, N. and Lippincott-Schwartz, J. (2009) Culturing MDCK cells in three dimensions for analyzing intracellular dynamics. *Curr. Protoc. Cell Biol.* **Chapter 4**, Unit 4.22 https://doi.org/10.1002/0471143030.cb0422s43
54 Tanner, K., Mori, H., Mroue, R., Bruni-Cardoso, A. and Bissell, M.J. (2012) Coherent angular motion in the establishment of multicellular architecture of glandular tissues. *Proc. Natl Acad. Sci. U.S.A.* **109**, 1973–1978 https://doi.org/10.1073/pnas.1119578109
55 Kenny, P.A., Lee, G.Y., Myers, C.A., Neve, R.M., Semeiks, J.R., Spellman, P.T. et al. (2007) The morphologies of breast cancer cell lines in three-dimensional assays correlate with their profiles of gene expression. *Mol. Oncol.* **1**, 84–96 https://doi.org/10.1016/j.molonc.2007.02.004
56 Petersen, O.W., Rønnov-Jessen, L., Howlett, A.R. and Bissell, M.J. (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 https://doi.org/10.1073/pnas.89.19.9064
57 Seifert, A. and Posern, G. (2017) Tightly controlled MRTF-A activity regulates epithelial differentiation during formation of mammary acini. *Breast Cancer Res.* **19**, 68 https://doi.org/10.1186/s13058-017-0860-3
58 Weaver, V.M., Petersen, O.W., Wang, F., Larabell, C.A., Briand, P., Damsky, C. et al. (1997) Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. *J. Cell Biol.* **137**, 231–245 https://doi.org/10.1083/jcb.137.1.231
59 Pearson, G.W. and Hunter, T. (2007) Real-time imaging reveals that noninvasive mammary epithelial acini can contain motile cells. *J. Cell Biol.* **179**, 1555–1567 https://doi.org/10.1083/jcb.200706099
60 Miroshnikova, Y.A., Rozenberg, G.I., Cassereau, L., Pickup, M., Mouw, J.K., Ou, G. et al. (2017) α5β1-integrin promotes tension-dependent mammary epithelial cell invasion by engaging the fibronectin synergy site. *Mol. Biol. Cell* **28**, 2958–2977 https://doi.org/10.1091/mbc.e17-02-0126
61 Spink, B.C., Cole, R.W., Katz, B.H., Gierthy, J.F., Bradley, L.M. and Spink, D.C. (2006) Inhibition of MCF-7 breast cancer cell proliferation by MCF-10A breast epithelial cells in coculture. *Cell Biol. Int.* **30**, 227–238 https://doi.org/10.1016/j.cellbi.2005.11.006
62 Ivers, L.P., Cummings, B., Owolabi, F., Welzel, K., Klinger, R., Saitoh, S. et al. (2014) Dynamic and influential interaction of cancer cells with normal epithelial cells in 3D culture. *Cancer Cell Int.* **14**, 108 https://doi.org/10.1186/s12935-014-0108-6
63 Gudjonsson, T., Ronnov-Jessen, L., Villadsen, R., Rank, F., Bissell, M.J. and Petersen, O.W. (2002) Normal and tumor-derived myoepithelial cells differ in their ability to interact with luminal breast epithelial cells for polarity and basement membrane deposition. *J. Cell Sci.* **115**, 39–50 PMID:11801722
64 Jensen, H.H., Pedersen, G.A., Morgen, J.J., Parsons, M., Pedersen, S.F. and Nejsum, L.N. (2019) The Na^+/H^+ exchanger NHE1 localizes as clusters to cryptic lamellipodia and accelerates collective epithelial cell migration. *J. Physiol.* **597**, 849–867 https://doi.org/10.1113/JP277383
65 Lancaster, M.A. and Knoblich, J.A. (2014) Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* **345**, 1247125 https://doi.org/10.1126/science.1247125
66 Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E. et al. (2009) Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**, 262–265 https://doi.org/10.1038/nature07935
67 Unbekandt, M. and Davies, J.A. (2010) Dissociation of embryonic kidneys followed by reaggregation allows the formation of renal tissues. *Kidney Int.* **77**, 407–416 https://doi.org/10.1038/ki.2009.482
68 Clevers, H. (2016) Modeling development and disease with organoids. *Cell* **165**, 1586–1597 https://doi.org/10.1016/j.cell.2016.05.082
69 https://www.the-scientist.com/research-round-up/2013s-big-advances-in-science-38220
70 (2018) Method of the year 2017: organoids. *Nat. Methods* **15**, 1 https://doi.org/10.1038/nmeth.4575
71 Sachs, N., de Ligt, J., Kopper, O., Gogola, E., Bounova, G., Weeber, F. et al. (2018) A living biobank of breast cancer organoids captures disease heterogeneity. *Cell* **172**, 373–86.e10 https://doi.org/10.1016/j.cell.2017.11.010
72 Walsh, A.J., Cook, R.S., Sanders, M.E., Aurisicchio, L., Ciliberto, G., Arteaga, C.L. et al. (2014) Quantitative optical imaging of primary tumor organoid metabolism predicts drug response in breast cancer. *Cancer Res.* **74**, 5184–5194 https://doi.org/10.1158/0008-5472.CAN-14-0663
73 Duarte, A.A., Gogola, E., Sachs, N., Barazas, M., Annunziato, S., RdR, J. et al. (2018) BRCA-deficient mouse mammary tumor organoids to study cancer-drug resistance. *Nat. Methods* **15**, 134–140 https://doi.org/10.1038/nmeth.4535

Biochemical Society Transactions (2019) **47** 1689–1700
https://doi.org/10.1042/BST20190131

74 McCracken, K.W., Aihara, E., Martin, B., Crawford, C.M., Broda, T., Treguier, J. et al. (2017) Wnt/β-catenin promotes gastric fundus specification in mice and humans. *Nature* **541**, 182–187 https://doi.org/10.1038/nature21021
75 Hill, D.R., Huang, S., Nagy, M.S., Yadagiri, V.K., Fields, C., Mukherjee, D. et al. (2017) Bacterial colonization stimulates a complex physiological response in the immature human intestinal epithelium. *eLife* **6**, e29132 https://doi.org/10.7554/eLife.29132
76 Bas, T. and Augenlicht, L.H. (2014) Real time analysis of metabolic profile in ex vivo mouse intestinal crypt organoid cultures. *J. Vis. Exp.*, e52026 https://doi.org/10.3791/52026
77 Valente, K.P., Khetani, S., Kolahchi, A.R., Sanati-Nezhad, A., Suleman, A. and Akbari, M. (2017) Microfluidic technologies for anticancer drug studies. *Drug Discov. Today* **22**, 1654–1670 https://doi.org/10.1016/j.drudis.2017.06.010
78 Shang, M., Soon, R.H., Lim, C.T., Khoo, B.L. and Han, J. (2019) Microfluidic modelling of the tumor microenvironment for anti-cancer drug development. *Lab. Chip* **19**, 369–386 https://doi.org/10.1039/C8LC00970H
79 Sontheimer-Phelps, A., Hassell, B.A. and Ingber, D.E. (2019) Modelling cancer in microfluidic human organs-on-chips. *Nat. Rev. Cancer* **19**, 65–81 https://doi.org/10.1038/s41568-018-0104-6
80 Kwapiszewska, K., Michalczuk, A., Rybka, M., Kwapiszewski, R. and Brzozka, Z. (2014) A microfluidic-based platform for tumour spheroid culture, monitoring and drug screening. *Lab. Chip* **14**, 2096–2104 https://doi.org/10.1039/C4LC00291A
81 Sakai, Y., Hattori, K., Yanagawa, F., Sugiura, S., Kanamori, T. and Nakazawa, K. (2014) Detachably assembled microfluidic device for perfusion culture and post-culture analysis of a spheroid array. *Biotechnol. J.* **9**, 971–979 https://doi.org/10.1002/biot.201300559
82 Weltin, A., Slotwinski, K., Kieninger, J., Moser, I., Jobst, G., Wego, M. et al. (2014) Cell culture monitoring for drug screening and cancer research: a transparent, microfluidic, multi-sensor microsystem. *Lab. Chip* **14**, 138–146 https://doi.org/10.1039/C3LC50759A
83 Ayuso, J.M., Gillette, A., Lugo-Cintron, K., Acevedo-Acevedo, S., Gomez, I., Morgan, M. et al. (2018) Organotypic microfluidic breast cancer model reveals starvation-induced spatial-temporal metabolic adaptations. *EBioMedicine* **37**, 144–157 https://doi.org/10.1016/j.ebiom.2018.10.046
84 Mocciaro, A., Roth, T.L., Bennett, H.M., Soumillon, M., Shah, A., Hiatt, J. et al. (2018) Light-activated cell identification and sorting (LACIS) for selection of edited clones on a nanofluidic device. *Commun. Biol.* **1**, 41 https://doi.org/10.1038/s42003-018-0034-6
85 Xiong, G., Flynn, T.J., Chen, J., Trinkle, C. and Xu, R. (2015) Development of an ex vivo breast cancer lung colonization model utilizing a decellularized lung matrix. *Integr. Biol. (Camb)* **7**, 1518–1525 https://doi.org/10.1039/c5ib00157a
86 Sperr, E. (2019) Available from: https://esperr.github.io/pubmed-by-year/