MODELLING GLANDULAR EPITHELIAL CANCERS IN THREE-DIMENSIONAL CULTURES

Jayanta Debnath[*‡] *and Joan S. Brugge*[*]

Abstract | Little is known about how the genotypic and molecular abnormalities associated with epithelial cancers actually contribute to the histological phenotypes observed in tumours *in vivo*. 3D epithelial culture systems are a valuable tool for modelling cancer genes and pathways in a structurally appropriate context. Here, we review the important features of epithelial structures grown in 3D basement membrane cultures, and how such models have been used to investigate the mechanisms associated with tumour initiation and progression.

Most human cancers arise from epithelial cells and tissues. Throughout the body, epithelial cells form well-ordered sheets that are anchored to basement membranes. These serve as a barrier between the interior of the body and the outside world, or comprise the glandular tissue of organs, such as the breast and the prostate gland. Epithelial tissues have several distinguishing microscopic features, such as a polarized morphology, specialized cell–cell contacts and attachment to an underlying basement membrane. All of these features are necessary for the proper control of cellular proliferation, survival, differentiation and secretion[1–3]. This intact, well-ordered architecture is disrupted during the pathogenesis of epithelial tumours. Indeed, for decades pathologists have evaluated architectural patterns to both diagnose and classify epithelial cancers.

BOX 1 illustrates several histological patterns of both invasive and pre-invasive tumours that are associated with breast carcinoma, one of the most common types of epithelial cancers. These patterns often harbour valuable prognostic information. For example, both tubular carcinomas and invasive lobular carcinomas of the breast are associated with a better clinical outcome than the most commonly observed pattern, invasive ductal carcinoma[4,5]. In some cases, there is a clear relationship between a defined molecular abnormality and histological phenotype, as illustrated by two breast carcinoma subtypes. The first, invasive lobular carcinoma, is commonly associated with losses in *CDH1*, which encodes E-cadherin, an important epithelial cell–cell adhesion molecule[6]. The second, a type of ductal carcinoma *in situ* (DCIS) called comedo DCIS, often exhibits amplification in the *HER2/NEU* oncogene, which encodes the epidermal growth factor (EGF) family receptor tyrosine kinase ERBB2 (REF. 7). However, for the most part, little is known about how the genotypic abnormalities associated with cancer actually elicit the phenotypic changes that are observed in carcinomas. A better understanding of these mechanisms should provide biological insights into carcinoma progression and aid in the discovery of new diagnostic markers and therapies for epithelial cancers.

Because of the large quantity of information that has been gleaned from diverse experimental approaches, cancer biologists are well poised to address the challenging problem of how cancer genes and pathways influence glandular epithelial architecture[8–10]. Studies of primary human tumour tissue and mouse models of epithelial tumours have been crucial for understanding epithelial tumours. However, they are relatively intractable for studying the biochemical and cell-biological processes involved in tumour formation. Cell culture models are better suited to studying the cellular processes and signalling pathways involved in oncogenic transformation. However, such studies have

*Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts 02115, USA.
‡Department of Pathology, University of California San Francisco, San Francisco, California 94143, USA.
Correspondence to J.S.B.
e-mail: joan_brugge@hms.harvard.edu
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Summary

- Epithelial cells grown in 3D cultures recapitulate numerous features of the glandular epithelium *in vivo*. These include the formation of cyst-like spheroids with a hollow lumen, apicobasal polarization of the cells comprising these structures, tight control of cell growth and proliferation, and the establishment of a basement membrane.
- Filling of the lumen with cells is a salient feature of early glandular epithelial cancers that remains poorly understood. Studies in 3D cultures have uncovered multiple processes and regulatory molecules that are involved in the formation and maintenance of luminal space.
- Known cancer genes give rise to a diverse array of morphogenetic phenotypes in 3D cultures that resemble important histopathological features observed in epithelial cancers *in vivo*.
- Apicobasal cell polarity is a fundamental characteristic of the glandular epithelium both *in vivo* and *in vitro*. Recent studies in 3D systems illustrate the importance of this process for an intact architecture.
- Various invasive properties have been observed in 3D systems, including invasion through the basement membrane. Future efforts directed towards co-culture systems that more faithfully represent the histological complexity of epithelial tissue *in vivo* are crucial for our understanding of epithelial cancers.

primarily been conducted using cells that are cultured as monolayers on tissue culture plastic or in soft agar assays, neither of which recapitulate the structural organization or functional differentiation of the glandular epithelium *in vivo*[11]. Three-dimensional (3D) epithelial culture systems, which allow epithelial cells to organize into structures that resemble their *in vivo* architecture, have emerged as tractable cell-based models that allow investigations of the functions of cancer genes and pathways in a biologically relevant context, and in a manner that allows a relatively high throughput[12].

3D models of glandular epithelium

Under 3D culture conditions, normal epithelial cells typically proliferate and organize into spheroids, commonly called 'acini' (a purely operational term with no anatomical connotation), which are notable for the presence of a centrally-localized, hollow lumen, and the polarization of cells surrounding this lumen[13–15] (FIG. 1). Such 3D epithelial culture systems display two characteristics that address some of the limitations of either monolayer (that is, two-dimensional (2D)) cell cultures, mouse models or human tissues. First, they are readily amenable to experimental manipulation and detailed microscopic and biochemical analyses. Second, they recapitulate certain essential structural features of glandular epithelium *in vivo*. BOX 2 describes the types of matrix proteins and cell lines that are used in these models.

Early studies revealed that human breast tumour cell lines did not form acini when grown in 3D culture; rather, they develop into nonpolarized clusters with limited differentiation[14]. These seminal experiments illustrated the stark behavioural contrast between normal and tumour cells in 3D culture, even though subtle phenotypic differences were evident when the same cells were grown as monolayers. They also broached several important questions with regard to the architecture of normal versus cancerous epithelia. First, what are the mechanisms and signalling pathways involved in organizing individual cells into a well-ordered architecture? Second, does disruption of these pathways influence the architectural progression of cancer? Two complementary approaches have been used to examine the cellular processes and pathways responsible for the disruption of epithelial architecture during tumorigenesis. One approach involves using strategies that are designed to revert the disorganized, unpolarized phenotype of tumorigenic cells, whereas the other involves using strategies that are designed to 'reconstruct' tumour phenotypes through genetic manipulations of normal cells — this review will highlight results from both approaches.

Reversion of tumour phenotypes in 3D culture

Studies of phenotypic reversion in the HMT-3522 model of human breast cancer progression have highlighted the complex interplay of cell-adhesion-signalling pathways and growth-factor-receptor signalling pathways in breast tumours[16]. The HMT-3522 cell series consists of a set of mammary epithelial cell populations that progressively evolved from a line of immortalized human mammary epithelial cells. They range in phenotypes from S2 cells, which are non-transformed and able to form normal acini, to T4-2 cells, which are tumorigenic and exhibit highly disordered growth in 3D culture[17–19]. Further analysis of T4-2 cells indicated that they exhibit high surface expression of the extracellular matrix (ECM) receptor β1-integrin when compared with the non-malignant S2 counterparts. However, blocking β1-integrin signalling with a function-blocking antibody causes these cells to behave like normal cells in 3D culture, forming growth-arrested acini that are similar to those formed by S2 cells[19].

Examination of the mechanisms responsible for this reversion revealed that neutralizing β1-integrin function caused downregulation of both β1-integrin expression and the EGF receptor (EGFR). Conversely, inhibiting the function of EGFR elicited the downregulation of β1-integrin levels and signalling[20]. Overall, these results poignantly illustrated the reciprocal crossregulation of the β1-integrin and EGFR signalling pathways. Importantly, these findings were only observed in 3D cultures; no reciprocal downregulation occurred when neutralizing antibodies were added to T4-2 cells cultured as monolayers[20]. In follow-up studies, the tumorigenic potential of T4-2 cells was also reversed by the pharmacological inhibition of extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) or phosphatidylinositol 3-kinase (PI3K) pathways; further studies indicated that the combined inhibition of multiple signalling pathways was able to restore more normal phenotypes to aggressive breast cancer cell lines in 3D cultures[21].

The HMT-3522 series has also facilitated the identification of novel molecules with potential tumour suppressor functions. T4-2 cells have reduced levels of

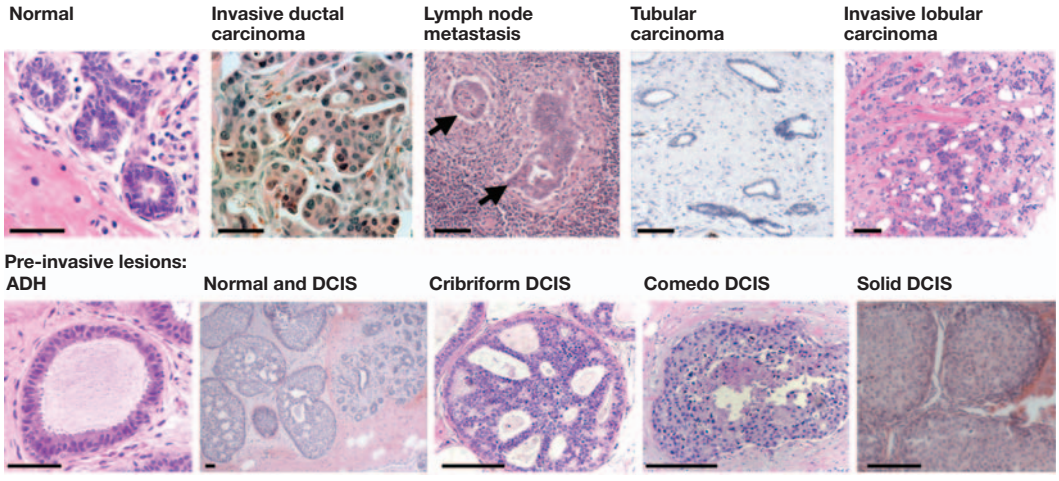
Box 1 | **Histological diversity of pre-invasive and invasive breast cancers**

For pathologists, two important measures for establishing a diagnosis and a prognosis for an epithelial tumour, such as breast carcinoma, are the level of architectural disorder, and cellular and nuclear pleomorphisms (variability in size and shape). Most pre-invasive and invasive epithelial lesions (see figure) are notable for such changes to varying degrees, and recognizing specific architectural and cytological patterns is crucial for predicting the future course of a neoplasm.

The normal breast consists of ducts and lobules with a dual-layered architecture. Luminal secretory cells surround a hollow lumen, and in turn are surrounded by a layer of myoepithelial cells that lie in direct contact with the basement membrane. Evidence of disruption of the myoepithelial cell layer and basement membrane is required for the diagnosis of invasive carcinoma in the breast. Over 70% of human breast cancers are **invasive ductal carcinomas**, in which malignant cells form solid nests, poorly formed tubules, and anastomosing cords and sheets[120]. The tumour infiltrates the surrounding fibrous and adipose tissue as collections of cells with well-preserved cell–cell junctions. In fact, even metastases in lymph nodes and distant organs are notable for epithelial nests that exhibit rudimentary gland formation (indicated by the arrows). Several other breast carcinoma subtypes also have prognostic importance. Tubular carcinomas consist exclusively of well-formed tubules that are in direct contact with the stroma due to the absence of a myoepithelial layer[5]. Invasive lobular carcinoma, which makes up 5–10% of breast carcinomas, is notable for single files or small nests of poorly cohesive cells, loosely dispersed throughout a fibrous stroma[120]. Both tubular and lobular carcinomas are associated with a better prognosis than invasive ductal carcinoma, making their correct diagnosis crucial[5].

The frequency of diagnosing pre-invasive lesions (where the tumour does not invade the basement membrane or myoepithelial layer), including ductal carcinoma *in situ* (DCIS), has significantly increased due to radiological screening by mammography[121]. There are several architectural subtypes of DCIS and a single patient can have multiple patterns[122]. In cribriform DCIS, the tumour cells are organized in a 'cookie cutter' pattern within the gland and are notable for the presence of multiple lumens. In solid DCIS, tumour cells completely fill the lumens of the gland. Solid sheets of highly atypical, pleomorphic (high grade) cancer cells with intraluminal necrosis characterize comedo DCIS[123].

An early precursor lesion in the breast that is being detected more frequently is atypical ductal hyperplasia or ADH[123]. ADH is notable for increased numbers of abnormal cells within a duct or lobule, but overall the lesion does not meet criteria for the diagnosis of DCIS; in some cases, the lumens of these glands remain hollow[124]. The ability to both effectively diagnose these early lesions and confidently predict the future outcome for these patients has assumed a greater significance in breast cancer diagnosis and treatment[125]. The development of model systems that recreate the architectural features observed in these early tumours should further contribute to our understanding of these intermediate stages in epithelial cancer progression. Please note that the scale bars approximate the size of a normal breast lobule (40 μm).



dystroglycan 1 (DG1), a basement membrane receptor. On restoration of DG1 expression in T4-2 cells, polarized structures that are growth arrested form in 3D cultures; these cells also exhibit reduced tumorigenicity when grafted into immunocompromised mice[22]. Finally, analyses of multiple carcinoma cell lines indicated that higher levels of DG1 correlated with the increased ability of these cells to form polarized structures in 3D culture, and examination of human breast and prostate cancers have revealed loss of DG1 expression[22,23]. Although the exact role of DG1 in breast cancer remains unclear, these initial results obtained from 3D culture assays strongly intimate that it functions as a tumour suppressor and serves as a crucial mediator of the polarization effects induced by the basement membrane[24]

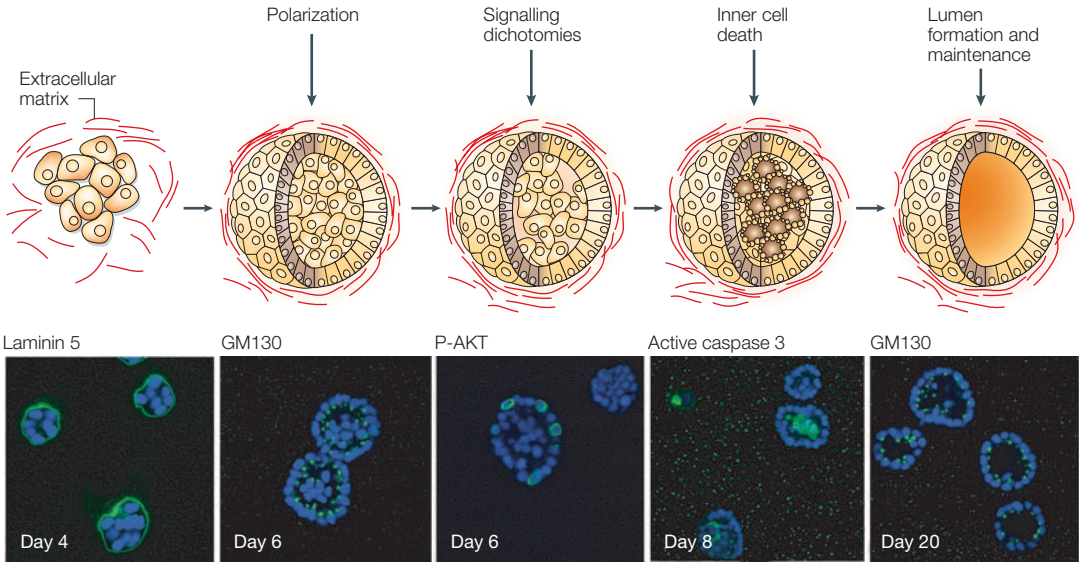


Figure 1 | **Events in MCF-10A acinar morphogenesis.** An ordered sequence of biological events is observed during the development of MCF-10A mammary epithelial acini cultured on Engelbreth–Holm–Swarm (EHS) tumour-derived basement membrane[34]. During the early stages of 3D culture, apicobasal polarization becomes evident within cell clusters before evidence of bona fide lumen formation. At day 5–8 in culture, two distinct populations of cells become evident within each acinus — an outer layer of cells in direct contact with the matrix (marked by laminin 5 staining on day 4) and an inner subset of cells lacking matrix contact. Throughout morphogenesis, this outer cell layer remains polarized with respect to the acinus centre (delineated by the apical orientation of the Golgi protein, GM130, on day 6 and day 20). Evidence of signalling dichotomies between these two populations is also observed; increased AKT survival signalling is observed in the outer cell populations compared with the central cells, as shown by staining of phosphorylated AKT (P-AKT) on day 6. Starting at day 8, the centrally located cells undergo cell death, which involves apoptosis, characterized by the expression of active caspase 3. This cell death contributes to the formation of a hollow lumen. The acinus remains hollow thereafter. The photomicrographs are reproduced with permission from REF. 34 © 2002 Elsevier Science.

Studies in other models have further substantiated the importance of cell-adhesion proteins in modulating the tumour phenotype. α2-integrin is expressed in normal differentiated mammary epithelial cells and frequently lost in undifferentiated carcinomas. Re-expression of α2-integrin in the Mm5MT mouse adenocarcinoma cell line causes a dramatic reversion from disorganized clusters of spindle-shaped cells to organized gland-like structures in 3D culture[25]. In addition, re-expression of the cell-adhesion molecule CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1)[14] in the MCF-7 human breast cancer cell line, and the restoration of GAP JUNCTIONS in MDA-435 breast tumour cells, have both been shown to promote the formation of organized spheroid structures[26,27]. Overall, these studies indicate that modulating cell-adhesion proteins that are aberrantly expressed in tumour cells can dominantly interfere with the phenotypic expression of the transformed state, even in the context of multiple oncogenic mutations.

Reconstruction of tumour phenotypes in 3D

3D cultures provide a context in which it is feasible to identify cellular genes that induce phenotypic alterations similar to those associated with tumour progression, and to investigate the mechanisms responsible for these phenotypic effects. In the following two sections, we will describe the use of 3D systems to model various biological events that are associated with epithelial cancers, such as filling of the luminal space, loss of polarization, escape from proliferative suppression, invasive behaviour and loss of cell adhesion.

Filling of the lumen. A hallmark of most glandular epithelial tumours is the absence of a hollow lumen (BOX 1). However, little is known about the processes involved in generating the lumen in glandular structures or in filling the lumen during tumorigenesis. Studies in 3D cultures have delineated a number of mechanisms and molecular regulators that contribute to these processes (FIG. 2).

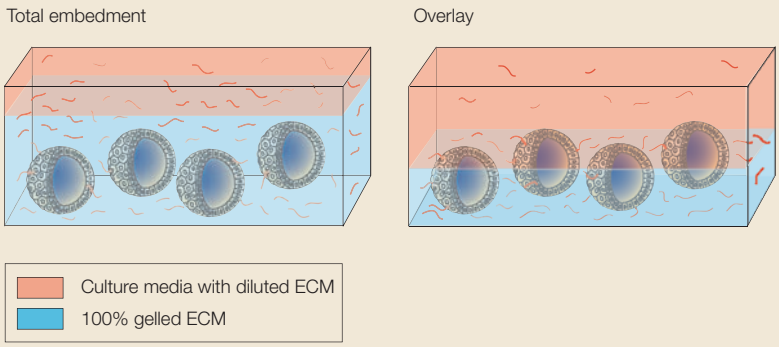
Apoptosis and lumen formation. In several epithelial cell types, lumen formation can spontaneously occur within small epithelial clusters intimating that a hard-wired mechanism, coinciding with the establishment of apicobasal polarity, can generate luminal space in small structures[2,28,29]. In other 3D models, apoptosis also contributes to lumen formation; indeed, apoptotic cells are present in the developing lumen of salivary gland and Madin–Darby canine kidney (MDCK) cysts, as well as mammary spheroids[30–34] (FIGS 1,3). Presumably, the cells that die during lumen formation lack direct contact with the basement membrane, which renders them susceptible to anoikis, a form of programmed cell death that epithelial cells undergo on matrix detachment[35,36]. Indeed, molecules involved in

GAP JUNCTION
A channel between two cells that allows the passage of molecules.

Box 2 | **Commonly used 3D culture techniques**

Two methods are commonly used to generate acinar structures. In the first method (see figure left), epithelial cells are completely embedded within the extracellular matrix (ECM). The gelled bed is grown in the presence of culture media containing growth factors and hormones that are necessary for proliferation and survival. In the second method (see figure right), the ECM is first cast to form a gelled bed measuring 1 mm in thickness. Epithelial cells are seeded onto this bed as a single-cell suspension in culture media. The optimal choice of ECM for 3D culture depends on the cell type. Madin–Darby canine kidney (MDCK) epithelial cells develop into polarized cysts with a hollow lumen when embedded within matrices comprised of type I collagen[126,127]. By contrast, other epithelial cells will not form acini in collagen, but are able to do so in or on a reconstituted basement membrane derived from Engelbreth–Holm–Swarm (EHS) tumours (commercially available as MATRIGEL)[128]. Several non-transformed mammary epithelial cell lines can be induced to undergo acinar morphogenesis[14,15,19,129,130]. In addition, primary mouse and human mammary epithelial cells form polarized structures with a hollow lumen in 3D culture[31,113,131].

Extensive work indicates that the mechanical properties of the 3D microenvironment are crucial for the development of polarized, differentiated acinar structures. This was originally shown in a series of studies involving normal breast epithelia grown on top of floating or attached collagen gels; mammary architecture, metabolic function and differentiation could only be maintained in the malleable, floating type I collagen gel[132,133]. The importance of ECM compliance was corroborated in subsequent studies that showed that epithelial cells cultured within a thick deformable matrix underwent glandular differentiation, whereas most cells cultured on a thin ECM coating on rigid tissue culture plastic did not[130].



anoikis also seem to be important regulators of luminal apoptosis in acini. Recent studies have shown that BIM, a pro-apoptotic BH3-only BCL family protein, is strongly induced after cell detachment in several epithelial cell types and is upregulated during morphogenesis of the MCF-10A cell line. Furthermore, SMALL INTERFERING RNA (siRNA)-mediated reduction of BIM expression inhibits anoikis as well as luminal apoptosis in MCF-10A 3D structures[37,38]. Although these results suggest that the blockade of apoptosis will prevent lumen formation, the ectopic expression of the apoptosis inhibitor BCL2 in MCF-10A cells merely delays lumen formation by a few days; ultimately, these structures still form a hollow lumen[33,34] (FIG. 3). Notably, these results mimic those observed during ductal elongation in mouse mammary gland development[39].

Morphogenetic regulators of lumen formation. The existence of master regulatory molecules that control lumen formation has been broached by studying two molecules: CEACAM1 and epimorphin. CEACAM1, which is downregulated in breast and colon carcinomas, is a cell–cell adhesion molecule expressed on the luminal surface of normal epithelia[40–42]. Recent studies indicate that CEACAM1 is required for lumen formation in acini derived from MCF-10F cells (a non-transformed cell line related to MCF-10A) in 3D culture[32]. In addition, as mentioned above, re-expression of CEACAM1 in MCF-7 cells, which lack CEACAM1 and are unable to form lumens, leads to the formation of morphologically normal structures in 3D culture[26].

Epimorphin, also known as syntaxin 2, is a multifunctional protein in which the extracellular form can operate as a morphogen. Recombinant epimorphin directs distinct morphogenetic pathways depending on how it is presented to mouse mammary cells in 3D collagen cultures. Polar presentation to the basal surface of cell clusters causes branching morphogenesis, whereas apolar presentation to the entire cell surface gives rise to cystic structures with a large hollow lumen[43]. Subsequent studies have demonstrated that the ability of epimorphin to mediate luminal morphogenesis involves controlling the expression of CCAAT/enhancer binding protein (CEBP) β-isoforms[44]. How molecules like epimorphin and CEACAM1 regulate the processes involved in creating a lumen, including cell death, remains an important issue for further study.

Filling of the luminal space. Studies of cancer genes and activated receptor tyrosine kinases in MCF-10A cultures have illustrated some of the mechanisms and pathways that contribute to luminal filling in 3D culture. For example, when proliferation is increased within MCF-10A acini via the ectopic expression of cyclin D1 or the inactivation of the retinoblastoma protein (RB) tumour suppressor pathway by the E7 oncoprotein from human papilloma virus (HPV) 16, a hollow architecture is maintained by the increased apoptosis of excess cells that occupy the lumens of these structures[34] (FIGS 2,3). However, luminal filling does occur when increases in proliferation are combined with the inhibition of apoptosis via the overexpression of anti-apoptotic BCL2 proteins or expression of oncoproteins with anti-apoptotic activities, including ERBB2, colony-stimulating factor 1 receptor (CSF1R), SRC, and IGF1R (Y. Irie and J.S.B., unpublished data; REFS 34,37,45,46) (FIG. 3). Decreased expression of BIM, observed in MCF-10A cells expressing activated ERBB2 or SRC, serves as one possible mechanism to promote survival in the lumen[37]. Expression of the matrix metalloproteinase (MMP) inhibitor tissue inhibitor of MMP1 (TIMP1) also inhibits lumen formation through an anti-apoptotic function that is mediated by PI3K and focal adhesion kinase (FAK) but which is independent of the MMP inhibitory activity of TIMP1 (REF. 47).

Notably, in some early human breast precancerous lesions, such as atypical ductal hyperplasia, a hollow architecture is sometimes maintained in the context of hyperproliferation, whereas advanced lesions, such as DCIS, exhibit varying degrees

MATRIGEL
The extracellular matrix secreted by the Engelbreth–Holm–Swarm mouse sarcoma cell-line. It contains laminin, collagen IV, nidogen/entactin and proteoglycans, and so resembles the basement membrane.

SMALL INTERFERING RNA
Short double-stranded RNA sequences that engage a cellular complex that cleaves mRNAs homologous to the short RNA sequences.

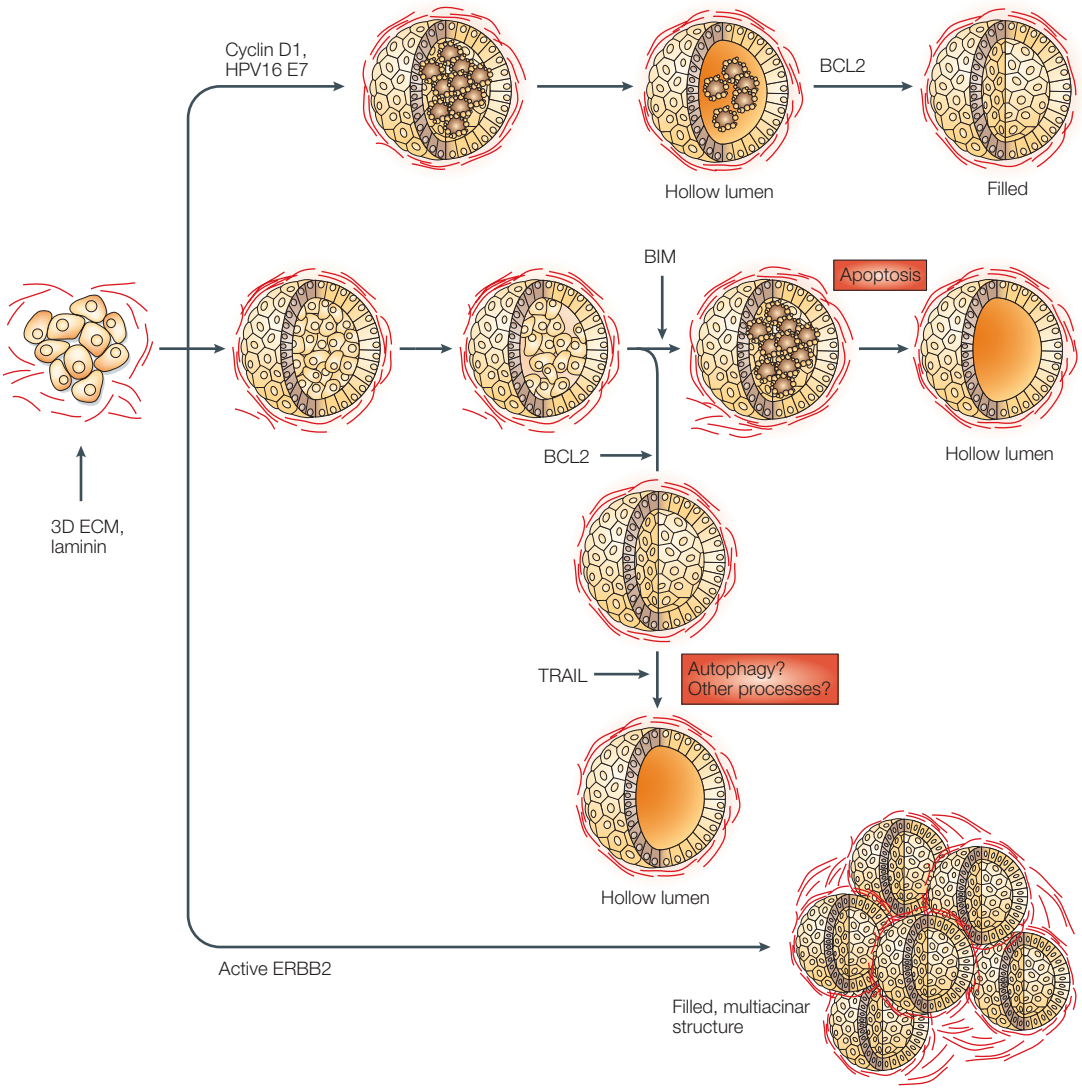


Figure 2 | **Lumen formation and maintenance in epithelial acini grown *in vitro*.** This figure illustrates several conditions in which the process of lumen formation is altered by oncogenes, and it highlights some of the molecules involved in the process. The central images show steps in the process of lumen formation, as illustrated in FIG.1. The pro-apoptotic protein BIM is required for induction of cell death[37]. Surprisingly, blocking classical apoptosis by overexpression of anti-apoptotic proteins, such as BCL2, delays, but does not prevent, the development of a hollow lumen. The inability of BCL2 proteins to prevent lumen formation both *in vitro* and *in vivo* indicates that additional processes participate in the generation and maintenance of the luminal space. Electron microscopy has revealed extensive vacuolation, resembling autophagy, within the central cells of developing mammary acini. Importantly, the central cells in BCL2-expressing acini also display these autophagy-like vacuoles before clearance[34,139]. Recent evidence indicates that the tumour-necrosis factor (TNF) family protein, TNF-related apoptosis-inducing ligand (TRAIL) is induced during lumen formation, and, moreover, might regulate the induction of vacuoles in the lumen[139]. However, loss-of-function studies of known autophagy genes (ATG) are required to establish a functional role for autophagy in lumen formation[140]. Proliferation is not sufficient to cause filling of the lumen (see upper images). For example, overexpression of the oncoproteins cyclin D1 or human papillomavirus (HPV) 16 E7 oncoprotein allows the cells to proliferate constitutively. Although lumen formation might not be affected because the excess cells undergo luminal apoptosis, the combined disruption of proliferation and apoptosis leads to luminal filling again[34,45]. An alternative scenario occurs on activation of the ERBB2 oncoprotein — a filled, multiacinar phenotype results because of hyperproliferation and anti-apoptotic activities in 3D culture[34,45] (lower images). ECM, extracellular matrix.

of luminal filling (BOX 1). So, one might speculate that in early breast lesions, architectural changes induced by proliferative signals are limited because of compensatory factors, such as increased apoptosis; accordingly, more advanced phenotypes could express anti-apoptotic signals that allow the survival of these proliferating cells.

Regulation of apicobasal polarity. The cells comprising glandular epithelial tissues exhibit a characteristic polarity, such that their apical poles point towards the central lumen. This polarity is often disrupted in carcinomas, which is generally considered a poor prognostic sign. Invertebrate models have provided a wealth of information about the role of cell polarity

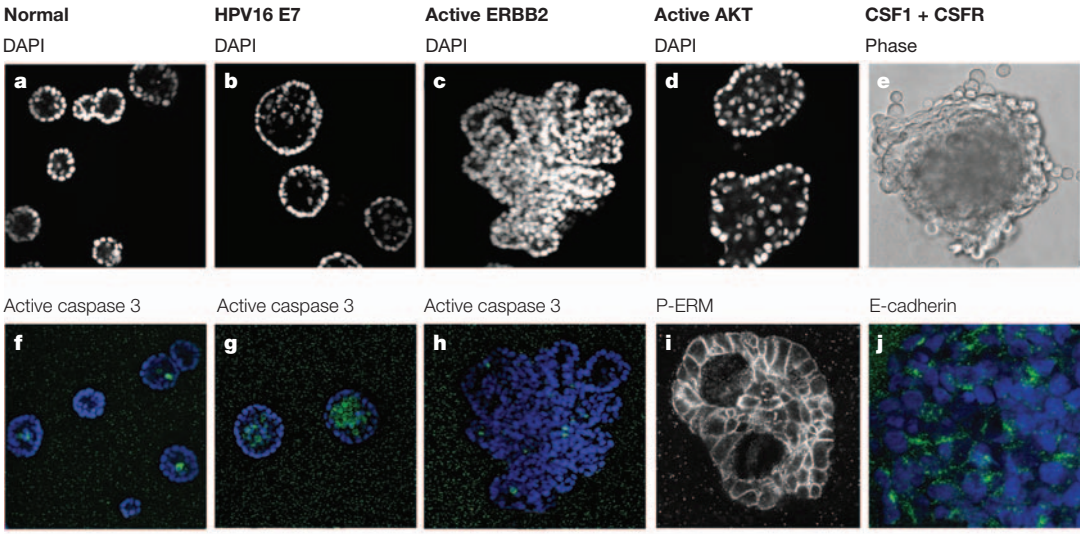


Figure 3 | **The effect of cancer genes on 3D epithelial architecture.** The examination of oncogenes and activated growth factor receptors in MCF-10A cells grown in 3D culture has uncovered a diverse array of morphological phenotypes. In many cases, the 3D phenotype results from specific biological processes and pathways that are modulated by the introduced oncogene. Normal acini exhibit a characteristic hollow spherical structure with a uniform size and shape (**a**,**f**). Acini expressing proliferative oncoproteins, such as human papillomavirus (HPV) 16 E7, exhibit a hollow architecture because the increased proliferation is offset by increased apoptosis (marked by active caspase 3) in the luminal space (**b**,**g**)[34]. By contrast, ERBB2 activation elicits a multiacinar phenotype, which is notable for excess proliferation and luminal filling (**c**); protection from apoptosis, as well as changes in apicobasal polarization, contribute to this complex phenotype (**h**)[34,45]. AKT activation elicits large distorted structures in 3D culture, which is partially due to its effects on the individual size and shape of cells that comprise these structures (**d**,**i**)[64]. The combined expression of colony stimulating factor receptor (CSFR) with its ligand, colony stimulating factor 1 (CSF1), elicits a progressively discohesive structure during 3D culture that is associated with the disruption of E-cadherin at adherens junctions (**e**,**j**)[46]. As discussed in the main text, many of these phenotypes resemble the histological changes observed in human tumours *in vivo*. **a**–**d** are 4′-6-diamidino-2-phenylindole (DAPI)-stained images that mark cell nuclei; **e** is a phase-contrast image illustrating the discohesiveness of cells in this structure; **f**–**h** show dying cells with activated caspase 3 in green, whereas DAPI-stained nuclei are blue; **i** is stained for P-ERM, a protein located at the juxtamembrane cytoskeleton that therefore marks cell borders, which was used to illustrate the size and shape of cells in this structure; **j** shows E-cadherin staining, marked green, whereas DAPI-stained nuclei are marked blue. Panels **a**–**c** and **f**–**h** were reproduced with permission from REF. 34 © 2002 Elsevier Science. Panels **d** and **i** reproduced with permission from REF. 64 © 2003 Rockefeller Univ. Press. Panels **e** and **j** reproduced with permission from REF. 46 © 2004 Rockefeller Univ. Press.

in regulating cell proliferation and the organization of epithelial structures[48,49]. MDCK cyst models have also demonstrated that epithelial cells possess hard-wired mechanisms for generating polarity within the context of a 3D tissue structure. Furthermore, it has been demonstrated that the ECM plays a fundamental instructive role in directing these mechanisms[2] (FIG. 4).

Several oncogenes have been shown to disrupt cell polarity in 3D cyst models or polarized monolayers, including mutant forms of SRC or RAS, and activated ERBB2 (REFS 45,50,51). The PI3K pathway might contribute to polarity disruption by these oncogenes, possibly through effects on RAC, as inhibition of PI3K reverted the phenotype of T4-2 transformed mammary epithelial cells in 3D cultures, and this effect was blocked by an activated form of RAC[52]. Recently, the tumour suppressor LKB1 has been shown to drive polarity in intestinal epithelial cells, and loss of LKB1 disrupted polarity in Caco-2 carcinoma cells. Notably, mutations in the C-terminus of LKB1, which are associated with the cancer prone Peutz–Jeghers syndrome, interfere with the regulation of polarity by LKB1 (REFS 53,54).

In *Drosophila melanogaster*, disruptions of genes that control polarity, such as *scribble* (*Scrib*), *discs large* (*Dlg*) and *lethal giant larvae* (*Lgl*) elicit hyperplastic growth; therefore, the proper regulation of epithelial polarity may directly influence tumor progression[55]. These genes are conserved in higher organisms and, interestingly, mammalian *DLG* and *SCRIB* are common targets for several viral oncoproteins, including the E6 oncoprotein of HPV[48,56–58]. Given the highly suggestive role of SCRIB, DLG and LGL as mammalian tumour suppressors, these molecules warrant further study in mammalian epithelial cells and tissues[48]. 3D culture models should prove useful in investigating this important subject. The mammalian orthologues of three genes involved in epithelial polarity in *D. melanogaster* — *crumbs homologue 3* (*CRB3*), *PALS1*, and *PATJ* — exist in a macromolecular complex that localizes to the tight junction in MDCK cells[59,60] and have been implicated in 3D studies in mammalian polarity and lumen formation[61] (FIG. 4).

The importance of polarity in tumour cell survival has also recently been recognized in 3D culture. The formation of polarized 3D structures confers protection

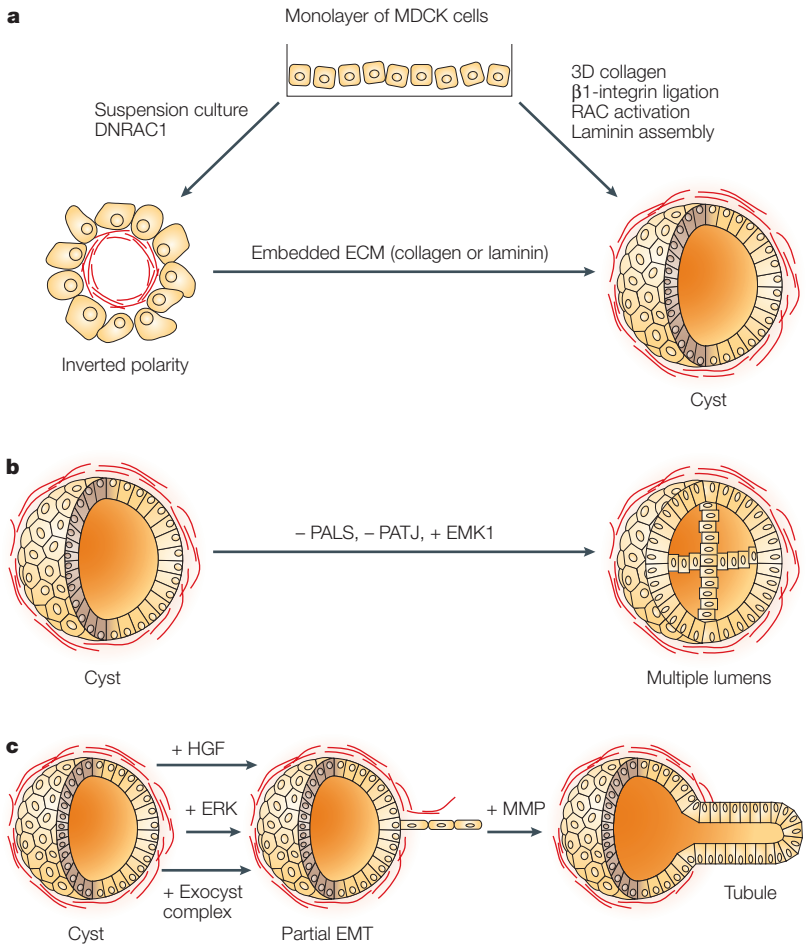


Figure 4 | **Cystogenesis, tubulogenesis and regulation of polarity of MDCK cells.** **a** | Studies using Madin–Darby canine kidney (MDCK) cysts point to the importance of the extracellular matrix (ECM) and integrin engagement as a polarity cue[126,127,141]. MDCK cells grow as monolayers on tissue culture plastic. When grown in liquid suspension, these cells form reversed structures in which the free apical surface points away from the central axis of the sphere and towards the surrounding culture medium; these structures still generate a basal surface by depositing the basement membrane into an internal cavity. When these cysts are embedded in collagen I, thereby providing a strong ECM cue on the outside of these structures, extensive cellular remodelling occurs, resulting in cysts with a central hollow lumen[126,127]. The orientation of apical poles requires β1-integrin and the small GTPase RAC1, which mediate the proper assembly of laminin at the cyst–ECM interface[142,143]. Expression of a dominant negative RAC1 (DNRAC1) or treatment with an antibody to β1-integrin in MDCK cysts inverts the apical pole toward the cyst periphery in 3D, but not 2D, cultures. By providing an exogenous source of laminin to the cyst periphery, the phenotypic effects of β1-integrin- or RAC-inhibition can be rescued[142,143]. **b** | The ablation of PALS1 or PATJ — the mammalian orthologues of two crucial polarity regulators in *Drosophila melanogaster* — disrupts polarity during MDCK cyst formation. The resulting structures do not form a central lumen; instead, they contain multiple small and incomplete lumens, or lack a lumen altogether[61]. In addition, well-established markers of apical polarity in MDCK cells, such as GP135, are completely mislocalized in these structures[61]. Similarly, overexpression of ELKL motif kinase 1 (EMK1), the mammalian orthologue of PAR1, promotes the formation of multiple lumens[144]. Notably, the formation of multiple small lumens in these MDCK structures highly resembles the cribriform patterns observed in breast DCIS and in certain prostate hyperplasias (BOX 1). **c** | Hepatocyte growth factor (HGF) induces the formation of branched tubules in various epithelial cell types grown in collagen gels[85,106]. The formation of tubules in cultured MDCK cysts initially involves an HGF-induced, partial epithelial-to-mesenchymal transition (EMT), followed by redifferentiation[108]. The induction of partial EMT requires the activation of the extracellular regulated kinase (ERK) pathway. Other processes, such as membrane biogenesis, mediated by the components of the exocyst complex, also contribute to tubulogenesis[145]. The late phases of tubule formation, in which cells redifferentiate, require matrix metalloproteinase (MMP) function and are associated with decreased HGF-induced ERK signalling[108].

from apoptosis caused by a variety of chemotherapeutic insults in both normal and malignant mammary epithelial cells from the HMT-3522 progression series. By contrast, non-polarized cells are uniformly sensitive to apoptosis[62]. Accordingly, disrupting the activation of the laminin receptor, $\alpha_6\beta_4$-integrin, perturbs HEMIDESMOSOME organization, disrupts polarity and promotes apoptosis[62]. Interestingly, resistance to apoptosis can be acquired in non-polar cells by the autocrine secretion of laminin 5, which results in the ligation of $\alpha_6\beta_4$-integrin, as well as the activation of RAC and nuclear factor-κB (NF-κB), a known positive modulator of cell survival[63]. The excess secretion of laminin 5 in a non-polarized manner on hyperactivation of growth factor receptors, such as ERBB2, has also been observed during MCF-10A morphogenesis, and might contribute to the ability of cells to survive in the lumen[45]. These results indicate that the pro-survival effects associated with the polarized state in 3D culture can be co-opted by the activation of specific survival signals provided by non-polarized laminin 5 secretion.

Escape from proliferative arrest. Although the mechanisms suppressing proliferation in acini-like structures would be predicted to be distinct from those in 2D cultures, little is known about the processes that induce arrest of proliferation in 3D structures. Nonetheless, several known oncogenes have been shown to escape proliferative arrest in MCF-10A acini, including activated receptor tyrosine kinases and cyclin D1 (REFS 34,45,46). The induction of constitutive proliferation by cyclin D1 is dependent on growth factors; so, signalling pathways stimulated by EGF or other growth factors are required. An oncogenic variant of AKT (also known as protein kinase B), a downstream effector of the PI3K pathway that is often activated in epithelial cancers, increases proliferation during early MCF-10A morphogenesis but cannot overcome proliferative suppression. This leads to arrested structures that contain larger numbers of cells than controls. Interestingly, the co-expression of cyclin D1 and activated AKT leads to constitutive, growth factor independent proliferation, indicating that the processes activated by AKT cooperate with cyclin D1 to promote unregulated proliferation[64].

Studies of Eph4 mouse mammary cells transformed with oncogenic variants of HRAS1 and ERBB2 have also demonstrated unique properties of the 3D microenvironment in the control of proliferation. When cultured in a 3D matrix, transformed Eph4 cells exhibit significantly higher levels of proliferation compared with control cells. This hyperproliferation in 3D culture is dependent on PI3K activity, and is not observed when the same cells are cultured using conventional 2D plastic conditions. Both RAS transformed and ERBB2 transformed Eph4 cells exhibit a strong upregulation of cyclin D1 in 3D, but not in 2D, culture, which might explain why the hyperproliferation induced by these oncogenes is only observed in the 3D context[65].

Recently, the membrane-anchored MMP, MT1MMP (membrane type 1 MMP), has been found to promote cell proliferation through its control of cell geometry in 3D collagen matrices[66]. MMPs have a well-established role in invasion and metastasis[67,68]. The expression of MT1MMP in MDCK cells elicits remodelling of the surrounding ECM and robustly increases proliferation in 3D cultures; this effect is not observed with other soluble MMP family members[66,69]. On expression of MT1MMP in tumour cell lines, a similar increase in proliferation is observed both in 3D culture and *in vivo*. The proliferative advantage attributed to MT1MMP expression depends on its collagenolytic activity because cells grown in protease-resistant collagen do not exhibit increased proliferation. The effects of MT1MMP on cell proliferation in 3D culture seem to arise through its regulation of cell shape and cytoskeletal organization within the confines of the 3D architecture; without MT1MMP-induced proteolysis, cells embedded in a 3D collagen gel are physically constrained by the surrounding ECM[66]. This study of MT1MMP highlights the important role of ECM remodelling in the growth and proliferation of cells in a 3D microenvironment.

The biological activities of cancer genes in 3D culture. Various 3D morphogenesis studies have also illustrated how biological activities provided by specific cancer genes could contribute to the phenotypic heterogeneity observed in epithelial cancers *in vivo* (FIG. 3). Although the activation of two different growth factor receptors — ERBB2 and colony-stimulating factor 1 (CSF1) growth factor receptor (CSF1R) — in MCF-10A acini increases proliferation and enhances cell survival, these molecules each provide additional distinctive biological activities that influence the ultimate morphogenetic phenotype in unexpected ways. Activation of ERBB2 homodimers using a controlled dimerization strategy during MCF-10A morphogenesis elicits a complex multiacinar phenotype (FIG. 3). These altered structures exhibit many of the properties of early stage cancers, including high levels of proliferation, filling of the luminal space and a lack of invasive properties. Protection from apoptosis, combined with changes in polarization, seems to contribute to this complex phenotype[45] (FIG. 2). Similar hyperplastic lesions, notable for the presence of multiacinar clusters, have been observed *in vivo* in tumours on transgenic expression of activated *Erbb2* in the mouse mammary gland, as well as when primary tumour cells isolated from these mice are cultured on Engelbreth–Holm–Swarm (EHS) tumour extracts[70–72].

By contrast, activation of the CSF1R pathway produces a distinct phenotype in MCF-10A morphogenesis. In humans, increased co-expression of CSF1R and its ligand, CSF1, correlates with invasiveness and poor prognosis of various epithelial tumours, but its significance remains unclear[73]. Autocrine activation of CSF1R in 3D culture, via the co-expression of CSF1R and CSF1 in mammary cells, induces hyperproliferation and a progressive disruption of junctional integrity in acinar structures; the discohesive phenotype is accompanied by the change in E-cadherin location from the plasma membrane to intracellular vesicles (FIG. 3). The junctional disruption induced by autocrine CSF1R signalling is completely dependent on the sustained activation of the SRC family tyrosine kinases[46]. These results could be relevant for tumours exhibiting a discohesive phenotype, such as invasive lobular breast carcinoma (BOX 1) or diffuse-type gastric carcinomas. Although loss or silencing of the *CDH1* locus is commonly associated with lobular breast and diffuse gastric carcinomas, loss of membrane-bound E-cadherin is also seen in the absence of a genetic lesion, indicating that other mechanisms might elicit such changes in cell–cell adhesion *in vivo*[6,74–76].

In addition to growth factor receptors, other signalling proteins implicated in cancer influence 3D morphogenesis. Examination of AKT has revealed valuable insights regarding the importance of cell size in 3D culture[77]. AKT activation during MCF-10A morphogenesis causes the formation of large, distorted structures that primarily arise from the combined disruption of cell size and proliferation[64]. The effects of AKT on cell size are particularly interesting from a histological perspective. Individual cells in these structures are about 50% larger on average; moreover, there is a wide variability in both cell size and shape in these cells[64] (FIG. 3). Such pleomorphism is commonly observed in both pre-malignant and invasive epithelial cancers; the potential contribution of the PI3K–AKT and mTOR (mammalian target of rapamycin) pathways to such histological changes *in vivo* remains unknown.

The effects of AKT activation on morphological disruption, individual cell size and shape are all prevented by rapamycin, a highly specific pharmacological inhibitor of the AKT effector mTOR[78]. These results indicate that mTOR, a well-established regulator of cell growth and size, is functionally required for all of the phenotypic changes elicited by AKT during the morphogenesis of mammary epithelial structures, but is not required for the formation of normal acinar structures[64,79]. Notably, these data are in accordance with those observed on transgenic overexpression of activated AKT in the mouse prostate, which exhibit hyperplastic, disorganized glands with increased individual cell size that reverts on treatment with the rapamycin analogue, RAD001 (REFS 80,81).

The effects of rapamycin on the AKT-induced 3D structures, and of SRC inhibitors on the autocrine CSF1R-induced structures, also highlight the usefulness of 3D models in evaluating the phenotypic effects of candidate chemotherapeutic agents. 3D cultures provide the opportunity to assess a larger spectrum of biological effects of candidate drugs compared with typical assays of cell proliferation and cytotoxicity in 2D cultures.

Invasive and migratory behaviour in 3D cultures

Invasion and metastasis *in vivo* are complex processes that involve alterations in both cell fate and behaviour. Moreover, from a histopathological perspective, there are no consistent architectural features associated

HEMIDESMOSOME
Specialized junction between an epithelial cell and its basal lamina that mediates their interactions.

Box 3 | **3D models of cellular invasive behaviour**

Invasion by epithelial tumour cells has been thought to be dependent on extracellular matrix (ECM) proteolysis[68]. Accordingly, when migrating through 3D matrices, highly invasive tumour cell lines display a constitutive, mesenchyme-like movement involving local protease-mediated breakdown of the ECM, creating tube-like degradation tracks in the gel[82]. However, recent studies of the migratory behaviour of tumour cells in 3D collagen I matrices have revealed an alternate, protease-independent mechanism of cell motility, similar to that used by leukocytes[134]. On near total pharmacological inhibition of a wide array of proteases, MDA-MB-231 mammary carcinoma cells exhibit a non-proteolytic 'amoeboid' form of cell migration, propulsively squeezing through gaps and spaces in the surrounding 3D ECM scaffold. In fact, the overall rate of migration remains undiminished in these cells, indicating that amoeboid migration serves as an important compensatory mechanism on inhibition of pericellular proteolysis[134]. Therefore, tumour cells can display significant plasticity in adapting to perturbations in their environment. Moreover, these distinct forms of movement are under the control of different cellular pathways. The amoeboid, protease-independent movement is dependent on the small GTPase RAS homologue gene (*RHO*) family acting through its effectors RHO-associated, coiled-coil containing protein kinase (ROCK) and ezrin (also known as villin 2, VIL2), whereas the elongated cell motility is RAC dependent[135].

Collective movement of cells in 3D cultures has also been described. In these cultures, clusters of 5–100 cells detach from the original explants and migrate through 3D collagen I gels with directional persistence[136]. Tubular branching and cohort migration of tumour cell lines has been shown to require deposition of fibronectin and protease activity[137,138]. Hepatocyte growth factor (HGF)-induced collective migration of colon carcinoma cells on a gelatin matrix involves the specific expression and activation of matrix metalloproteinases in the cells at the leading edge[138] (FIG. 4). So, there are differential responses to mitogens among individual cells that comprise cell sheets or solid spherical masses. Such collective behaviour might contribute to invasion in carcinomas that exhibit glandular differentiation and cell–cell junctions; notably, the invasive component in these tumours usually appear as cell clusters, poorly formed glands or as single files of cells[90] . However, without careful 3D reconstruction and a temporal analysis of cell movement in tumour tissue *in vivo*, the functional significance of these *in vitro* migratory behaviours remains uncertain.

with invasion. Even within the carcinoma of a single individual, the invasive component is comprised of diverse morphologies that include poorly formed glands, clusters, cords, sheets and in some cases isolated single cells (BOX 1). This heterogeneity is not only influenced by the cancer cells themselves but by the surrounding microenvironment of the tumour[11,67]. Given the intrinsic complexity of the invasion process, *in vitro* monotypic 3D cell-culture systems are limited in their ability to model invasion. However, with advances in 3D culture techniques, certain processes associated with invasion can be modelled, such as the breakdown of the basement membrane, disruption of cell junctions and acinar architecture, partial or complete epithelial-to-mesenchymal transition (EMT) and the migratory behaviour of tumour cells. We will highlight a few examples where 3D models have provided unique insights into these processes. Many reviews have dealt more extensively with individual topics associated with invasion[82–84].

Cell migration in 3D microenvironments. The cellular response to pro-migratory cytokines can be profoundly influenced by constraints that are imposed by cell–cell and cell–matrix interactions in 3D structures, and by the physical resistance of matrix components that envelop cells in 3D cultures. For example, treatment of 2D cultures of mammary epithelial cells, such as MCF-10A cells, with EGF induces a strong chemokinetic migratory response; however, similar treatment of such cells within 3D acini does not induce cell movement nor disrupt spheroid structure. In addition, hepatocyte growth factor (HGF) treatment can induce scattering of certain cell types and loss of cell adhesion in 2D cultures, but induces tubular branches of normal adherent cells in 3D cultures[85].

Most normal cell types undergo a multistep sequential mechanism of cell motility in 2D cultures that involves pseudopod extension, adhesion to the ECM, focal breakdown of the ECM, forward movement of the cell body and tail retraction[86,87]. In 3D collagen matrices, tumour cells undergo a similar multistep mechanism of cell motility[88]. However, studies of epithelial tumour cells in 3D matrices have revealed alternative mechanisms of cell motility that are not readily detected in 2D migration models[82,89] (BOX 3).

Collective migratory behaviour. Tumour cell movement, like that of normal cells during development, might also proceed without the complete loss of cell–cell junctions such that cells move as coherent cell sheets, nests or tubular protrusions[90]. Collective movement *in vitro* has been shown to involve a localized disruption of cell–cell junctions and transient loss of polarity, therefore allowing the extension of lamellae[91,92]. While most *in vitro* studies have been performed with immortalized tumour cell lines using 2D cultures, such as WOUND-SCRATCH ASSAYS, these collective movements have also been observed when primary tumour explants are cultured in 3D collagen gels (BOX 3).

Epithelial-to-mesenchymal transition. EMT was originally described as a normal developmental process that mediates many crucial morphogenetic alterations during embryonic development[93]. EMT was found to be associated with the acquisition of a fibroblastoid, migratory morphology, downregulation of epithelial-specific adhesion proteins (for example, E-cadherin) and the concomitant gain of various mesenchymal proteins such as vimentin[93,94]. Although EMT is widely regarded as a mechanism for tumour invasion, epithelial tumours rarely convert to a complete mesenchymal phenotype; indeed, even metastatic carcinoma cells often exhibit cell–cell contacts and show evidence of glandular differentiation[95] (BOX 1). Accumulated evidence raises the possibility that tumour cells might instead undergo reversible EMT-like processes induced by the local environment[95,96]. EMT-like processes have been characterized in 3D basement membrane models. Since EMT has been reviewed extensively, we will highlight a few mechanistic findings emerging from 3D cultures.

The study of the cooperation of transforming growth factor-β (TGFβ) with RAS, as well as receptor tyrosine kinases, in mediating EMT and invasion has provided insights into the mechanisms and molecular players

WOUND-SCRATCH ASSAY
An assay involving the migration of cells into a space generated by scraping a confluent monolayer of cells with a fine tipped device.

associated with EMT[97]. The ability of TGFβ to inhibit the proliferation of normal epithelial cells is well documented; however, this response is context-dependent as TGFβ can also contribute to tumour progression[98,99]. One mechanism underlying the effects of TGFβ on tumour progression was explored using the Eph4 cell line. In 3D collagen gels, this cell line forms acinar structures that undergo growth arrest when treated with TGFβ. However, RAS-transformed Eph4 cells undergo a dramatic phenotypic conversion in 3D collagen, forming invasive cell cords that protrude into the collagen gel in the presence of TGFβ[100]. Subsequent studies have shown that a hyperactive RAF–ERK pathway is required for EMT and invasive behaviour[101,102]. Additional studies have also indicated a cooperative role between ERBB2 and TGFβ in promoting metastasis *in vivo* and invasive and migratory behaviour *in vitro*[71,103,104]. Cells isolated from mouse mammary tumours co-expressing ERBB2 and TGFβ exhibited a branched invasive phenotype in 3D culture that was inhibited by blocking TGFβ function[71]. In 3D culture, MCF-10A cells co-expressing activated ERBB2 and TGFβ also displayed an invasive phenotype. As with RAS, the ability of ERBB2 to cooperate with TGFβ correlated with the sustained, increased activation of the ERK pathway[104] and constitutive activation of ERK could substitute for ERBB2 in cooperating with TGFβ .

MMPs have also been shown to regulate EMT in 3D culture. For example, the induction of an activated form of MMP3 (also called stromelysin 1) in Scp2 cells, a normal mouse mammary epithelial cell line, inhibits lactogenic differentiation and progressively elicits invasive behaviour in both 2D and 3D cultures in reconstituted basement membranes. Furthermore, these cells exhibit both the morphological and molecular hallmarks of EMT[105].

Partial epithelial-to-mesenchymal transition and tubulogenesis. A series of studies on tubulogenesis in MDCK cysts in 3D collagen cultures might explain how EMT could contribute to the invasive behaviour of tumour cells in the absence of an irreversible EMT conversion. As discussed above, HGF induces the formation of tubules in a variety of epithelial cell types grown in collagen gels[85,106]. Detailed analysis of this process in MDCK cysts has revealed that tubulogenesis proceeds though a series of defined stages[107] (FIG. 4c). The early stages involve a partial, transient EMT in which the epithelial cells lose their polarity and migrate and proliferate away from the parent cyst wall to form a chain of cells. In the next stages, these chains repolarize to form a multilayered cord and, ultimately, a cylindrical tubule with a hollow lumen[107,108]. The formation of both extensions and tubules does not involve losses in cell–cell contact[107]. Recent studies have demonstrated that activation of the ERK pathway is necessary for the initial stage of partial EMT, whereas MMP activity is required for the second stage for cells to reorganize as a tubule[108]. One can speculate that this well controlled process of tubulogenesis might also be co-opted as a mechanism for tumour cells to break away from a primary lesion and invade through the stroma. However, because the EMT-like process is transient, clusters of tumour cells can reform at a high rate, possibly explaining why invasive human carcinomas are frequently observed to be collections of cells with varying degrees of gland-like differentiation. A salient feature of partial EMT is the retention of cell–cell contacts — cells possess migratory properties but do not completely break away from their neighbours[108]. This might explain why fibroblastoid morphology, an important feature of traditional EMT, is not commonly observed in human carcinomas.

A phenotype resembling partial EMT has also been observed in invasive mammary cells expressing ID1, an inhibitor of helix–loop–helix transcription factors[109]. In Scp2 mammary cells, ectopic expression of ID1 causes the gradual disintegration of acinar structures; these cells slowly invade the basement membrane and migrate into the 3D matrix. Although the ID1 expressing cells fail to fully differentiate, they continue to express epithelial markers and form loose acinar structures, indicating that they have not undergone a complete EMT[110,111]. Cells expressing ID1 secrete a gelatinase, which seems to be required for the invasive activity in the basement membrane. Both this gelatinase and ID1 are highly expressed during involution of the mouse mammary gland, suggesting that this process might also take place *in vivo*[111].

The transient nature of partial EMT described in 3D culture provides an attractive paradigm for human carcinoma invasion *in vivo*. Although the role of partial, transient EMT needs to be further validated in human tissues, 3D cultures have provided some thought-provoking insights into the morphogenetic and biological complexities of invasive behaviour during both normal tissue development and cancer.

Building better models

One promising area of research is the development of heterotypic 3D culture systems. As mentioned earlier, because both normal epithelial tissues and tumours represent a community of heterotypic cell types, the use of such monotypic 3D cultures has inherent limitations[11,12,67]. So, future efforts demand culture systems that more faithfully represent the histological complexity of epithelial tissues *in vivo*. Important elements of these systems include, but are not limited to, the use of primary human cells and the incorporation of additional stromal components, such as fibroblasts and immune system cells. Progress has already been made in the development of more complex 3D co-culture models and has been a focus of two recent reviews[1,12]. However, certain studies warrant a mention because they highlight the power of 3D systems in analyzing the molecular crosstalk between diverse cell populations in maintaining tissue organization and homeostasis.

The ducts and lobules that make up the normal breast are composed of two layers, and DCIS is notable for the preservation of the outer myoepithelial layer (BOX 1). These cells have been proposed to have a crucial role in preventing tumour invasion[112].

The role of myoepithelial cells in directing the polarization of luminal cells has recently been delineated through the 3D co-culture of these two cell types. In 3D collagen culture, primary human luminal mammary cells fail to polarize. However, on co-culture of primary luminal and myoepithelial cells, a polarized dual-layered acinar organization develops. Secretion of laminin 1 by the myoepithelial cells is crucial for the development of this polarized acinar organization. Remarkably, myoepithelial cells isolated from tumours do not produce high levels of laminin 1 and fail to direct the polarity of luminal cells in 3D culture[113]. These results demonstrate the important role of myoepithelial cells in the maintenance of normal breast tissue architecture. Cell–cell interactions between myoepithelial cells and luminal epithelial cells have also been investigated in 3D cultures. The co-culture of luminal cells and myoepithelial cells in these systems results in cell aggregates with a bilayered organization that resembles a breast acinus. Disruption of myoepithelial-specific desmosomal cadherins disrupts the sorting of myoepithelial cells to the basal surface of luminal cell aggregates, demonstrating the importance of desmosome-mediated interactions in the proper organization of the luminal-myoepithelial bilayer[114].

As abundant evidence now indicates that the stromal microenvironment profoundly influences the behaviour of tumour cells, the development of co-culture systems in which glandular structures are intermingled with stromal elements is also an important requisite for the future[67,115–117]. The importance of the stroma has also been illustrated through studies of human tissue organization and function in mouse xenograft models. These include co-culture models in which human prostate or mammary epithelium, along with stromal cells, are introduced into the renal capsule of immunocompromised mice, and, more recently, the elaboration of a mammary ductal tree on injection of human primary mammary epithelial cells into a 'humanized' fat pad, created by the previous engraftment of human stromal fibroblasts[116,118,119]. Along with *in vitro* 3D co-culture systems, the creation of these xenograft animal systems using human cells will meet a crucial demand for models that faithfully recreate human tissues.

Concluding remarks

The studies overviewed here illustrate the power of 3D epithelial culture models as one approach for modelling the biological activities of cancer genes and pathways in a relatively high-throughput manner. This is because they provide a biologically rich system that can be used to assay the influence of cancer genes and pathways on glandular architecture. The further use and development of these *in vitro* culture systems, including the improvement of heterologous co-culture strategies, will be invaluable in modelling cancer progression and testing therapeutics in a biologically relevant context.

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Competing interests statement

The authors declare no competing financial interests.

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