Modeling disease using three dimensional cell culture: multi-lumen and inverted cyst phenotypes

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TABLE OF CONTENTS

1. ABSTRACT

Three-dimensional cell culture provides a unique system to investigate intrinsic mechanisms and micro environmental cues involved in the morphogenesis of epithelial glandular architectures. While this culture system allows insight into normal tissue development, it is also is readily amenable to manipulations that permit cellular modeling of various disease states. Here, we discuss a range of cellular and genetic alterations that result in two distinct cyst phenotypes, the multi-lumen cyst and the inverted cyst, both of which involve defects in cell polarity and lumen formation. Multi-lumen cyst formation results from disturbances in the mechanisms that regulate cell polarity, apical assembly, and the rate of lumen clearance. In the inverted cyst, the apical domain is oriented adjacent to the matrix, markedly affecting the morphogenic cues the matrix provides for cystogenesis. Both of these abnormal glandular phenotypes are highly reminiscent of histological patterns used to classify a number of diseases. A better understanding of the causes of multi-lumen and inverted cysts will provide insights into the origin and progression of epithelial diseases, potentially leading to the development of new therapies.

2. INTRODUCTION: THREE DIMENSIONAL CELL CULTURE AS A MODEL FOR DISEASE

Though the three-dimensional cell culture system has revealed unique properties for modeling tissue assembly for decades, the field of cell biology has only relatively recently seen an increasing trend towards more common utilization of this culture environment. Several review articles have highlighted the many advantages of three-dimensional (3D) cell culture, such as the ability to investigate the delicately choreographed events in the development of single units of glandular tissues, the functional relevance of these structures in contrast to traditional monolayer cultures, and the relative ease of investigation and specific manipulations when compared to animal models (1-3). The findings generated from experimenting with 3D culture systems have broken new ground in cell biology research, offering models where epithelial cell programs can be studied both spatially and temporally in concert with extracellular matrix. These studies have provided different perspectives on how disease can be modeled and evaluated. The 3D culture system continues to evolve within cell biology and has spread its applications throughout cancer research, pharmacological discovery, and biomaterials engineering.

Table 1. Common MDCK cyst phenotypes, their genetic or epigenetic causes, and the diseases related to these phenotypes

MDCK Cyst Phenotype	Altered gene expression or Culture Condition (Ref)	Related Disease Phenotype
Multiple Lumens	Constitutively Active ARF6 [25], Downregulation of Pals or PatJ [16], Knockdown of Par3 [20]	Breast Cancer- Ductal Carcinoma *In situ,* Pre-invasive Prostatic hyperplasias
Inverted Domain	Dominant Negative Rac1 [41], Dominant Negative and Constitutively Active Cdc42 [44], Dominant Negative Rho A [44], Function Blocking beta1-integrin [42], suspension cultures [7], Biochemically Activated RhoA [43]	Adult Dominant Autosomal Polycystic Kidney Disease, Extrahepatic Obstructive Cholestasis, Invasive Ductal Breast carcinoma- particularly in the metastases in the lymphatic compartment, malignant mesothelioma, Micropapillary breast carcinoma, Rectal adenocarcinoma
Filled Lumens	Overexpression of Crumbs3 [19], Dominant negative Annexin 2 [30], Knockdown of Lgl [54]	Polycystic Kidney Disease, Breast Carcinoma *In situ*
Multivacuolar	PTEN siRNA [30], Cdc42 siRNA [30], Annexin 2 siRNA [30]	Microvillus Inclusion Disease (Davidson's Disease)
Loss of Polarity	Dominant Negative Rab11 [55], Knockdown of Lgl [54]	Lobular mammary carcinoma
Cystogenesis Inhibition	Constitutively active [44], alpha2-integrin siRNA [56]	

The body of work stemming from three-dimensional cultures has yielded a vast amount of information. However, these investigations also present some challenges in organizing and interpreting these data. When characterized tumor cell lines are studied, or when normal cell lines are transformed with oncogenes that are associated with specific diseases, the implications to human physiology can be deduced. The reverse genetics approach to the three-dimensional cell culture system has illuminated multiple phenotypes that depict disease progression in many different tissues, including breast, kidney, prostate, and colon. These studies, while very relevant, have led to a bias against using 3D cultures for forward genetic analysis and against exploration into epigenetic changes that accompany disease related phenotypes. This, in turn, has somewhat diminished what can be learned from the Madin Darby Canine Kidney (MDCK) cell cultures, traditionally seen as the mainstay of three-dimensional cell culture. The phenotypes exhibited by MDCK cysts in 3D culture environments are reminiscent of architectural patterns observed in diseases of the kidney and other tissues as numerated in Table 1. Repeatedly, in the literature, malformed MDCK cysts are described as being filled, apolar, multi-lumen, and inverted. These events are often treated as irregular and unrelated to normal epithelial development or to disease states. However, the similarities among these phenotypes and the frequencies with which they are detected are consistent. As outlined below emerging literature suggests that the reoccurring morphologies of the multi-lumen and inverted MDCK cysts potentially provide vital insight into the pathology of specific diseases.

3. MULTI-LUMEN CYSTS

Epithelial morphogenesis relies on the establishment of polarity within a single epithelial cell and the polarized organization of cells throughout a growing tissue (4, 5). Indeed, cell polarity underlies a number of cell behaviors, including division, intercellular signaling, intracellular communication, cytoskeletal activities and organization, migration, and differentiation (6). Using the MDCK 3D cell culture system, the elements that dictate tissue organization can be investigated and the mechanisms that govern epithelial polarity can be minutely examined. In normal cyst development, a distinct apical domain is established early in cystogenesis, often detectable soon after the first cell division (7). During subsequent cell divisions, the apical domain of each daughter cell that comprises the spherical monolayer of the cyst must be oriented towards the center of the enlarging glandular structure temporally with programmed cell death of cells within the lumen. A disruption in this balance will lead to aberrant cyst phenotypes including the formation of multiple lumens. Cysts are characterized as multi-lumen when, in contrast to normal development of a single and centralized lumen, cysts form more than one lumen. These lumens do not coalesce in development, but remain distinct from each other in maturity, resulting in a glandular structure that often support the survival of cells that are not in contact with basement membrane.

As originally defined in invertebrate model systems, three major protein complexes control epithelial cell polarity: the Par3/Par6/aPKC Polarity Complex, the Scribble complex, and the Crumbs Complex (8-11). These complexes, and their subsequent activities, coalesce to define the apical and basolateral domains of epithelial cells, as well as their demarcating boundary, the tight junction (12-15). A number of studies have shown that a perturbation in individual cell polarity will disrupt MDCK cystogenesis. In many instances, the disruption of the tight junction or the improper assembly of the polarity complexes will result in multi-lumen MDCK cysts. For example, loss of PALS1, the tight-junction associated protein and mammalian orthologue of the Drosophila polarity determinant Stardust, results in the impaired development of a single, centralized lumen (16). When PALS1 is knocked down via a stable small interfering RNA expression system, the resultant cyst phenotype is multiple, small lumen, some of which are incomplete (16). A decrease in PALS expression also led to decreased expression of its binding partner PATJ, a tight-junctional protein and mammalian orthologue of the Drosophila Discs Lost, and resulted in the dissociation of its complex member CRB3, a member of the Crumbs polarity complex, from the known polarity complex Par6/Par3/aPKC (16-18). It has been shown that CRB3 assists in the recruitment of aPKC to the forming apical domain. When CRB3 expression is over expressed, or a dominant negative version of PALS1 that cannot localize CRB3 is introduced, there is a disruption of polarity in MDCK cysts, resulting in nonpolar multicellular aggregates with no recognizable lumina or multiple indistinct lumen (19). Horikoshi et al. further demonstrated the necessity of the Par polarity complex in properly defining the apical domain in epithelial cells (20). They showed that Par3- specifically its ability to interact with aPKC- is essential for apical

membrane formation. Knockdown of Par3 or expression of a Par3 mutant defective in aPKC binding during MDCK cyst formation results in multiple lumens (20). Also, overexpression of Par1, a downstream target of the aPKC/Par3/Par6 complex, stimulates the formation of multiple, ectopic lumens when overexpressed in MDCK cysts or monolayers (21). Additionally, the multi-lumen effect is seen when junctional adhesion molecule A (JAM-A) is misregulated. JAM-A is an integral membrane protein that binds Par3 and localizes the tight junctions of epithelial cells. When a JAM-A mutant, lacking the extracellular domain, is expressed, the protein is mistargeted from its appropriate tight-junctional location to the apical surface, causing a mistargeting of endogenous Par3 and ZO-1 (22). Expression of this JAM-A mutant (JAM-A/MC) impairs the ability of MDCK cells to form cysts in the three-dimensional cell culture environment, and the multicellular aggregate that forms contains multiple small lumina instead of a single lumen. In these studies, the authors believed that these multi-lumen cyst phenomena are due to a delay in the onset of polarity that resulted from a disruption of the tight junction or the Par polarity complex or its Crumbs and Scribble partner complexes (2, 23). It is possible that when the establishment of polarity is delayed beyond a critical point in cyst development, the resultant cyst phenotype will display multiple lumens in MDCK cysts.

However, there are many instances in which apical-basal polarity appears intact in MDCK glandular structures that display the multi-lumen phenotype. In many cases, the cells lining each individual small lumen have tight junctions that are functional, as well as a distinct separation of apical and basolateral components to their proper domains. In these instances, the multi-lumen phenotype is often the result of a delay in the formation or delivery of apical domain components to a single, centralized location rather than a delay in polarity. Meder et al. have demonstrated that the apical component, gp135/podpcalyxin, localizes to a pre-apical domain at an early point in cell polarization that precedes strong adherens junction formation, and is sorted in this way after preliminary attachment of a single cell to a substrate24. Given this early level of organization, gp135 may be considered a polarity regulator, its localization being a putative defining element of the apical domain, possibly acting to recruit other apical components to a primordial apical domain. Further, when gp135 is knocked down in MDCK cells grown on filters, polarity is severely delayed (24). Interestingly, this delay in polarity manifests itself in the three dimensional cell culture systems as a defect in apical membrane formation. The majority of gp135 knockdown cysts displayed multiple lumens, while many cysts in this condition were incapable of forming any lumen. The dynamics of the assembly of the apical domain early in cyst development could be considered a rate-limiting step in cystogenesis. In many studies where cyst phenotypes are multi-lumen, these dynamics are hindered or inhibited.

The multi-lumen phenotype also appears to support the survival of cells that are not in direct contact with the matrix. For instance, sustained activation of the small GTP-binding protein, ARF6, drives a dramatic alteration in MDCK cyst development, and results in a multi-lumen phenotype, although the cellular basis of this phenotype needs further investigation (25). ARF6 is a downstream effector molecule of two widely accepted oncogenes, c-Met and Src (26). Previous work has also demonstrated that the activation of ARF6 promotes the internalization of E-cadherin, and thereby disrupts cell-cell contacts (26). The multi-lumen cysts structures with hyperactive ARF6 exhibit disruptions in normal growth factor receptor trafficking and accumulate in intracellular signaling endosomes, resulting in hyperactive ERK and sustained Bcl-2 stabilization. The overactive pro-survival signal promotes the survival of non-matrix adjacent cells, the presence of which could impair multiple small lumen coalescence.

The multi-lumen phenotype, which contains surviving cells not in contact with matrix, resembles epithelial tissue phenotypes used to classify and establish prognosis for epithelial tumors. In particular, non-invasive breast carcinoma, ductal carcinoma in situ, and certain prostatic hyperplasias are characterized by a histological phenomenon in which tumor cells are organized in a sub-glandular arrangement within the gland and are characterized by the presence of multiple lumens (2). Thus, epithelial architecture corresponding to the multi-lumen cyst phenotype is often considered to be pre-invasive in the glands where it is present. Understanding the delicate progression from in situ to invasive diseases is an important element of cancer biology and advancements in the medicinal management of this progression have the potential impact of greatly improving the prognosis of these carcinomas.

During cyst maturation, a block in the delivery of vesicles carrying apical components to a central, single lumen may also be described as a multi-lumen phenotype. It is important to distinguish between the coalescence of larger intracellular lumens and an accumulation of vacuolar apical compartments (VACs) within the cell. Though morphologically similar, the phenotypes appear to result from distinct cellular alterations. The presentation of multiple lumens coincides with a higher number of cells within a cyst structure, as well as an upregulation of pro-survival signals such as Bcl-2 (25). Accumulation of apical components in vesicles, usually in a subapical region, likely results from a trafficking defect that delays or inhibits the movement of these components to a centrally localized, single lumen, and prevents the coalescence of these components into intracellular lumen (28). This defect does not promote the growth or survival of non-matrix associated cells; here we will describe it as a "multivacuolar" phenotype. The multivacuolar phenotype presents polarity defects distinct from the multi-lumen phenotype. When there is a subapical inclusion of vacuolar apical compartments, the apical domains remain immature, with reduced numbers of microvilli and decreased levels of apical biomarkers (29). In a study of the formation of the luminal space, Martin-Belmonte and colleges have defined a number of critical molecules responsible for the assembly

of the apical domain at the center of the developing cyst structure. These investigators identified a cascade of events involving PTEN enrichment of PIP2 at a preliminary apical domain, which associates with Annexin 2, and recruits Cdc42 and Par6/ aPKC, helping define the apical domain at the nascent lumen (30). A deregulation in any of these players results in an accumulation of vacuolar apical compartments in a subapical distribution. Therefore, the communication of these signaling elements is considered to be critical for apical plasma membrane assembly resulting in an apically lined lumen.

A similar multivacuolar event occurs in Davidson's disease, or microvillus inclusion disease, a rare congenital condition affecting neonates, in which the intestinal lining is underdeveloped (31). The pathological identification lies in a biopsy of the small intestine to reveal an absence of apical microvilli that serve as an important medium for absorption of nutrients. In these tissues, critical apical components are sequestered within a vesicular compartment in the cytoplasm of the cells lining the gut. The condition results in an inability to absorb nutrients, starting in the first few days of life and ultimately leading to metabolic acidosis, severe dehydration, and eventually fatality31. Similarly, this multivacuolar event occurs in the gut of Rab8 deficient mice. Mice bearing a Rab8 conditional knockout present pups with the same digestive symptoms as patients suffering from Davidson's disease (32). In addition to the knockout mouse, a microvillus inclusion disease patient, who presented with an identical phenotype to the Rab-8 deficient mice, showed a reduced expression of RAB8 in the intestine (32). Thus, defects in the localization of the apical proteins in intestinal epithelial cells, caused by little or no expression of Rab8, resulted in epithelial polarity defects and a disease state similar to Davidson's disease, just as suggested by the three-dimensional cell culture model.

There is a general consensus in the literature that multi-lumen/ multivacuolar cysts are the result of a delay in the formation of the tight junction and therefore in cell polarity, or an inability of apical components to be trafficked to a lumen initiation site or a delay in their delivery. The temporal organization of the tight junction corresponds well with the delivery of the apical domain components, which suggests the possibility of a common, yet to be defined trigger (33). However, there is some evidence in MDCK and other cell lines that multi-lumen phenotypes are related to a disruption in the orientation of the mitotic spindle, dictating the location of the daughter cells within a proliferating cyst structure34-37. It has recently been reported that Cdc42, already identified as a major contributor to cystogenesis for its role in the delivery of the apical compartment, affects the orientation of the mitotic spindle in the expanding cyst structure (34). The Cdc42-specific GEF, Intersectin2, localized directly to the centrosomes of dividing cells in the MDCK cyst (34). Intersectin 2 regulates Cdc42 activation locally at the site of the assembling spindle, and silencing either Cdc42 or Intersectin 2 disrupts the proper orientation of the mitotic spindle, as well as the normal formation of the lumen. The result is a multi-lumen cyst phenotype. The causative

relationship between the positioning of the spindle pole and subsequent daughter cells within a growing cyst structure and the delivery of apical components to a single, centralized lumen has yet to be elucidated; however, Cdc42 appears to be a critical component of both of these morphogenic events. It is unclear to what extent the orientation of the spindle dictates cyst phenotype in MDCK cells, as so many morphogenic and environmental factors are also at play. We cannot rule out the possibility that the maintenance of tissue architecture requires proper spindle orientation and that spindle orientation defects may promote disease progression. Continued investigations into the events that result in multiple lumens during the formation of glandular structures will contribute to our understanding of the progression of many epithelial diseases.

4. INVERTED CYSTS

Epithelial cells play a fundamental role in forming and organizing the biological compartments of the body. To complete this role, individual epithelial cells within a tissue must organize structurally, biochemically, and physiologically distinct apical and basolateral plasma membrane domains in concert with their supporting stroma. The proper establishment and maintenance of these specialized surface membrane domains is important for the organization of tissues and a disruption in the integrity of this organization could lead to cell dysfunction and ultimately a pathologic state4. The physiological cues that orient this tissue organization can come under direct investigation by studying the culture effects that result in inverted MDCK cysts.

Inverted MDCK cysts were first described in suspension cultures (7). Individual cells were allowed to proliferate into aggregates without cues from a substrate material to uncouple the roles of cell-cell and cell-matrix cues in establishment of individual cell polarity in a multicellular context. It was found that by approximately the third day of cyst development, 72 hours after initial single cell suspension, the morphology of the aggregates had become distinctly organized, individual cells showing a marked polarity between apical (gp135) and basal-lateral (Na+, K+ -ATPase) domains, with the basal-lateral membrane facing the center of the cyst. At that time-point the apical membrane facing the free, external surface, and assembly of microvilli at the apical domain could be observed. Also, there was a strong localization of the tight junction (ZO-1), as well as an internalized polar organization of the Golgi and nucleus. After three days in suspension, these aggregates are well-formed cysts. The organization of these cysts is "correct" since their apical domains face a cleared space, and their basolateral domains face a core of secreted basement membrane proteins. However, this orientation is the opposite of that seen in glandular tissues in vivo, and are thus "inverted cysts".

Suspension-grown inverted cysts prompted investigations into how the extracellular environment communicates with the cellular machinery that orients polarity. When suspension-grown inverted cysts are

imbedded into a matrix environment, the cysts interpret the extracellular matrix cue, and extensively remodel, resulting in cysts with a central, hollow, and apically lined lumen. This activity requires beta1-integrin activity and activation of the Rac1 GTPase (38-40). The cues orienting cyst polarity are very closely related to the ability of the cell to interact with matrix physically and to convey the information provided by the extracellular environment to cell machinery via distinct Rho-GTPase cell signaling cascades. For instance, expression of the dominant negative Rac1 mutant, Rac1v12, or treatment with a beta1-integrin antibody that blocks its function inverts MDCK cysts, orienting the apical pole toward the cyst periphery (41, 42). In addition, activation of RhoA by treatment with CNFy, Yersinia pseudotuberculosis cytotoxic necrotizing factor, causes a reorientation of the apical domain marker gp135 to the cortex of the cyst structure43. Further, expression of dominant negative RhoA, constitutively active Cdc42, or dominant negative Cdc42 also result in the assembly of the actin-rich apical domain at the periphery of the inverted cyst (44). The orientation of inverted cysts expressing dominant negative Rac1 is rescued by supplying exogenous laminin, whereas inverted cysts resulting from blocking beta1-integrin are rescued by either activating Rac1 or by supplying laminin to reorient cyst organization (41, 42).

Although all of the inverted cysts described above, resulting from blocking beta1-integrin or altering small GTPase activity, are characterized by the assembly of the actin-rich apical domain at the periphery of the cysts, they exhibit some phenotypic differences. For example, when cysts are inverted by the dominant negative expression of Rac1, cysts are regularly spherical but the basolateral markers are nonpolar (41). The cells within these inverted cysts cannot restrict the localization of beta-catenin, p58, or E-cadherin to the basolateral domain (41, 42). On the other hand, when cysts are inverted by treatment with the beta1-intrgrin function-blocking antibody, the cyst structures are irregular with many inverted cells protruding at the surface of the inverted cyst42. Furthermore, neither type of inverted cyst, RacN17 cysts or cysts in which beta1-integrin is functionally blocked, can clear the luminal space; the inverted cysts contain live cells throughout the structure (41, 42).

The relationship of the MDCK inverted cyst to autosomal dominant polycystic kidney disease (ADPKD) is evident. The pathology of ADPKD is characterized by swelling of renal tubules and formation of fluid filled cysts within the kidney, often leading to kidney failure (45). Patients present with abdominal pain, blood in the urine, kidney stones, and/or reoccurring bladder or kidney infections. A histological study of healthy and cystic regions of whole kidneys from ADPKD patients and in confluent primary cultures of micro-dissected renal tubule and cyst adjacent epithelium showed a mislocalization of Na+, K+ -ATPase (46). Diseased tissues revealed Na+, K+ -ATPase localized to the apical domain, at the lumens of the glands, rather than its basolateral orientation in healthy tissues. The mislocalized Na+, K+ -ATPase was shown to be functionally active in these cultures and the inverted

direction of its activity is proposed to be the mechanism for cyst formation and tubule swelling in diseased kidneys. However, there was no reversal of polarity of other basolateral or apical membrane markers detected (46).

The inverted cyst phenotype may also be related to an inversion of domains used as a histological marker for breast cancer. In the lymphovascular region of invasive ductal carcinoma tumors in the breast, there is an observable reversal of glandular polarity (47). In a study to investigate the polarity of breast invasive ductal carcinoma cells, immunohistochemical analysis was done on tumor sections localized within lymphovascular spaces against those located in extravascular compartment (47). Using an apical marker (HMFG-1), and a basolateral marker (AUA-1), it was found that only one of 11 tumors, resected from patients whom were diagnosed as grades 1 or 2 with metastases to lymph nodes, had a focus of reversed glandular polarity in the extravascular tumor space. However six of eleven tumors showed a reversal of glandular polarity in the lymphovascular space. Given that the inverted polarity allows for direct interaction between apical domain-type molecules at the periphery and lymphovascular endothelium, it was hypothesized that a reversal of domains is a mechanism for intravasation into the lymph and could affect the establishment of metastatic disease (47).

Although the formation of MDCK cysts is controlled by intrinsic genetic programs, the influence of the ECM receptors and the matrix environments demonstrate that the microenvironment in which epithelial cells proliferate and differentiate also contributes to the morphogenesis of glandular structures (48). For instance, it has been demonstrated that the mechanism of lumen formation in MDCK epithelial cysts is dependent on the composition of the extracellular matrix environment (49). The proper matrix compliance and physical and chemical polarizing cues are critical for the survival and appropriate cyst morphogenesis in MDCK and have been well characterized in a number of breast epithelial cell lines (50-52). Specifically, when the three-dimensional matrix environment does not supply the appropriate polarizing cues, MDCK cystogenesis is severely inhibited causing multicellular aggregates of an ambiguous or inverted polarity (50). Further, when the compliance of the matrix is adjusted via modulating collagen concentration, epithelial cyst morphology changes dramatically (52). When epithelial cysts are grown in increasingly stiffer microenvironments, mechanoregulatory cell signaling is altered and malignant behaviors are promoted (52). Investigating the properties of the matrix within three-dimensional cell culture systems will begin bridging the gap between cellular signaling biochemistry and the changes in tissue morphologies observed *in vivo* with the advancement of disease. Notably, it has been recently demonstrated that enhancing the rigidity of the tissue microenvironment can induce invasion of premalignant epithelium *in vitro* and tumor progression *in vivo* (53). Given the role matrix has in orienting cyst polarity, and how this role is perturbed in inverted cysts, the importance of gaining an understanding not only cell driven behaviors

but also matrix biology and the transformation of the tissue microenvironment is becoming increasingly evident.

5. CONCLUDING REMARKS AND PERSPECTIVES:

We have discussed how 3D MDCK cell culture have been valuable in demonstrating the importance of proper cellular organization in a multicellular context and how these experimental systems recapitulate the architecture of epithelial tissues that have been disrupted in disease states. Disruption of cell polarity in 3D cultures leads to two prominent phenotypes-multi-lumen and inverted cysts- both of which have implication for understanding disease. Multi-lumen phenotypes are reminiscent of the pathology of less advanced disease states and may provide insight into the transformation from pre-invasive to invasive disease. Future studies need to address how the sub-glandular multi-lumen phenotype contributes to carcinomas *in situ* and how these structures may be involved in the progression from *in situ* to invasive diseases. The inverted cyst phenotype is also indicative of the establishment and progression of varied diseases. Although it is well established that cell polarity relies on membrane transport, whether the cues controlling this transport are intrinsic to the cell or are matrix-based remain to be defined. Since inverted cysts present their apical domains adjacent to the matrix, the inverted domain may adversely affect the interaction between the epithelial cells and their extracellular environments and may even have a transforming effect on the extracellular matrix. Although invasion has been thought to be a distinctly basolateral event, epithelial cells have been shown to reorganize into an inverted conformation once free from the confines of extracellular matrix. It could also be extrapolated that when suspension-grown inverted cysts reorganize after being imbedded into matrix, these events could model a disease state in which cells that were previously suspended in the bloodstream or the lymph have extravasated and begun to proliferate at a secondary site to form metastases.

The establishment and maintenance of polarity in epithelial tissues is complex and alterations in this organization play a role in the etiology of a number of specific diseases. A number of cues that are involved in the establishment and maintenance of polarity in epithelial cultures have been identified. Further study of 3D epithelial cell cultures will provide a greater understanding of the intracellular and extracellular cues that regulate cell polarity in epithelial tissues and assist in the design of specific molecular targets to combat disease.

6. ACKNOWLEDGMENTS

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