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Review

Three-dimensional epithelial cultures: a tool to model cancer development and progression

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Summary. Loss of cell polarity is a hallmark of cancer, and although this feature is commonly observed in advanced tumors; growing evidence indicates that loss of cell-cell adhesion and cell polarity may also be important in early stages of cancer. Despite recent important advances, much remains unclear about the molecular and biophysical mechanisms involved in phenotypic changes observed in epithelial architecture during carcinogenesis. Over the past decade the use of three dimensional cultures (3D) has emerged as a valuable tool to study the functions of cancer genes and pathways in an adequate polarized context. 3D cultures are an outstanding tool to understand the morphologic consequences of molecular alterations. In other words, 3D cultures allow a much better understanding of the pathological features of tumours, with the microscope. In this review we will focus on how 3D models have provided unique insights into how basic cell biological processes impact in higher-order tissue architecture and how these models have enhanced our understanding of carcinoma biology.

Key words: Cell polarity, Epithelial tissues, Glandular epithelium

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Importance of 3D cultures in biomedical research.

The goal of using 3D cultures in biomedical research is to bridge the gap between traditional 2D cell culture and *in vivo* settings with an approach that places cultured cells in an environment that more closely represents the complex 3D structures of native tissues. Cultures on plastic, malignant and non-malignant epithelial cells do not essentially show behavioral and morphological differences (Adissu et al., 2007). For instance, some endometrial adenocarcinoma cell lines (which are classified as well to moderately well differentiated) cannot be distinguished from normal cells cultured in 2D whereas the same cells can be identified based on morphologic criteria when they are placed in a 3D culture (Boyd et al., 1990). Moreover, endometrial tumors show age-dependent differences in polarity based on parameters such as the localization of cell nucleus and glandular convolution formation (Saegusa et al., 2002), with abundance of non-polarized neoplasm in older patients (Adissu et al., 2007). Those observations suggest that apical polarity might be an important feature to study the classification of endometrial neoplasias, and a detailed analysis of protein polarity complexes could allow a better classification of adenocarcinomas. 3D culture systems have elucidated the role of the microenvironment in the modulation of drug resistance in solid tumors. Early observations showed that neoplastic cells display a higher drug resistance compared with monolayers when they are part of multicellular spheroids (Miller et al., 1985, 1990; Hartman et al., 2009). More recently, it has been demonstrated that 3D context confers resistance to

chemotherapy on small cell lung cancer (Sethi et al., 1999) and on uveal melanoma cell line (Bérubé et al., 2005). These data emphasize the role of tissue architecture in cell survival and the development of new efficient therapeutic treatment. Individualized therapy would also be beneficial for screening drug sensitivity performed on 3D cultures of malignant cells obtained from each patient.

The scaffolds employed in 3D on *in vitro* studies can also be used in tissue engineering strategies as a potential cell and protein delivery vehicle, being administrated in a minimally invasive manner (Huang and Fu, 2010). Additional advantages of hydrogels are that they may preserve drugs, peptides and proteins against the potentially severe environment in the region area of the liberation site; they enable enhanced residence times, sustained delivery and/or targeted drug delivery and they have notable potential in wound healing applications, although pore size and degradation properties must be optimized (Garg et al., 2012). For instance, injectable poly (N-isopropylacrylamide) hydrogels have been prepared for encapsulating cells and are used as cartilage and nerve regeneration (Park and Yun, 2004). Moreover, bone morphogenic protein introduced into the hydrogel material has been effective in promoting the novo bone and cartilage formation *in vivo* (Chenite et al., 2000). Photo cross linked poly(ethylene glycol) (PEG)-based hydrogels have been utilized for delivery of chondrocytes and osteoblast (Bryant and Anseth, 2003; Williams et al., 2003). Furthermore, Pluronic/heparin composite hydrogels carrying growth factors such as bFGF have been demonstrated to induce angiogenesis (Park and Yun, 2004; Yoon et al., 2007)

3D cultures as systems to study the role of cell polarity in cancer develpment

Most human tumors are of epithelial origin and metastases originating from these tumors lead to up to 80% of all cancer deaths (Jechlinger et al., 2002). For decades, pathologists have identified certain human malignances evaluating the cellular complexity and histopathological architecture of biopsies.

The organization of epithelial tissues depends on three main attributes (Jechlinger et al., 2002), cell-cell and cell-matrix specialized contacts to form a super-cellular structure (Azueta et al., 2010); apical/basal polarity to distinguish the outside surface from the inside, and a mitotic spindle orientation (Clement and Young, 2002) to ensure the formation of the glands and epithelial sheet rather than a cellular lump (McCaffrey and Macara, 2011). Epithelial cells differ from mesenchymal cells because daughter cells do not separate from each other, and proliferation can continue despite cell contact with adjacent cells (Kim et al., 2009). Furthermore, polarity is retained during cellular division (McCaffrey and Macara, 2011). These features ensure that epithelial lamina can grow during development, retaining their intercellular contacts, which prevent leakage of luminal contents, or fragmentation of the lamina (Kim et al., 2009; McCaffrey and Macara, 2011). Therefore, it is obvious that epithelial tissues need other mechanisms to control cell proliferation and ensure the correct organ size. For example, loss of adhesion proteins, such as FAK, can induce an increased susceptibility to apoptosis, and a concomitant failure to upregulate epithelial proliferation (Owen et al., 2011). Another pathway that has been implicated in epithelial cell proliferation control is Hippo (Hpo) pathway. Inactivation of Hpo pathway induces accelerated proliferation, a delay in cell-cycle arrest and resistance to apoptosis in epithelial cells (Genevet and Tapon, 2011). Hence, failure of these controls may contribute to carcinogenesis.

Loss of cell polarity is a hallmark of cancer (Wodarz and Nathke, 2007; Etienne-Manneville, 2008; Lee and Vasioukhin, 2008; Tanos and Rodriguez-Boulan, 2008), and although this feature is commonly observed in advanced tumors growing evidence indicates that loss of cell-cell adhesion and cell polarity may also be important in early stages of cancer (Hedrick et al., 1993). Despite recent important advances, much is still unclear about the molecular and biophysical mechanisms involved in phenotypic changes observed in epithelial architecture during carcinogenesis. Studies using mouse models of epithelial tumors are crucial for a better understanding of epithelial tumor behavior. However, they are relatively unmanageable for studying the biochemical and cell-biological processes involved in tumor formation. Cell culture models, where cells are cultured as monolayers on tissue culture plastic (referred to as 2D culture) are easy to handle and to introduce genetic alterations; however, they do not recapitulate the structural organization or functional differentiation of the epithelium *in vivo* (Hanahan and Weinberg, 2000), and sometimes signaling pathways are fundamentally differently regulated than in polarized structures (Bissell et al., 2005).

Three-dimensional (3D) epithelial culture systems (which allow cells to organize into polarized structures) are an alternative *ex vivo* approach, which reduces the gap between 2D cell cultures and physiological tissues. Over the past decades the use of these cultures has emerged as a valuable tool for studying the functions of cancer genes and pathways in an adequate polarized context. 3D culture allows understanding of the role of some genes in altering morphology. For that reason in this review we will focus on how 3D models have provided unique insights into how basic cell biological processes impact on higher-order tissue architecture and how these models have enhanced our understanding of carcinoma biology.

Organization of epithelial tissues

The archetypes of polarized tissues are the epithelial tissues, which mainly present as distinguishing features

cell-cell and cell-matrix specific contacts, acquisition of apico-basolateral polarity and lumen morphogenesis.

Cell-cell and cell-matrix contacts

Cell adhesions play an important role during the establishment and the maintenance of epithelial glandular structures and tissue organization. Adhesions between adjacent cells include Tight Junctions (TJ), Adherent Junctions (AJ) and desmosomes. These junctions are essential for the modulation of paracellular permeability in various epithelial tissues. Polarized epithelial cells exhibit TJ that lie apical to AJ, these TJ have an organizing role in epithelial polarization and establish an apico-lateral barrier to the diffusion of solutes through the intracellular space. They also restrict the movement of lipids and membrane proteins between the apical and basolateral membrane and are highly ordered membrane contact sites, containing a network of intramembrane fibrils (Aijaz et al., 2006). Tight junctions compromise at least four types of transmembrane proteins, including occludins, claudins, JAMs (Junctional Adhesion Molecules) and CRB (Crumb), and a number of cytoplasmic peripheral proteins. Whereas the transmembrane proteins mediate cell-cell adhesions, the cytosolic tight junction plaque contains various types of proteins (PDZ proteins, such as the ZO (Zona Ocludens) family) that link TJ transmembrane proteins to the underlying cytoskeleton. These adapters also recruit other scaffolding proteins that facilitate the assembly of highly ordered structures, such as junctional complexes or signaling patches that regulate epithelial cell polarity, proliferation and differentiation (Matter et al., 2005).

In addition to cell-cell adhesions and intracellular signaling pathways mediating effects on tissue morphogenesis, the impact from distinct adhesions that forms between cells and the extracellular matrix (ECM) has quickly emerged as a mediator of cell growth, differentiation and homeostasis (Adams, 2001; Hebner et al., 2008). A great variety of different cell-matrix contacts has been described. These include focal adhesions (FAs), focal complexes and fibrillar adhesions (Burridge and Fath, 1989; Cukierman et al., 2001). FAs are stable complexes that form through different interactions of various signaling adaptors and structural molecules the link between ECM and integrin receptors to the cytoskeleton (Webb et al., 2002). These matrix-cell unions are the central component of the microenvironmental cell sensing and support cell motility.

Many signaling proteins are located at cell-matrix contacts, including the Src family kinases and the focal adhesion Kinases (FAK) (Felsenfeld et al., 1999; Volberg et al., 2001; Wang et al., 2001). Because these protein junctions are involved in signaling cascades; their presence strongly suggests that signaling through these adhesions coordinates integrin and growth factor signaling. Moreover, they may translate some stimuli to trigger changes in cellular morphology and function (Lee and Juliano, 2004).

Acquisition of apico-basolateral polarity

Apico-basolateral polarity is characterized by the existence of non-coalescent apical and basolateral plasma membrane domains. The asymmetrical distribution of the protein, lipid and junctional complexes makes possible the differentiation between two distinct epithelial cell surfaces (Adissu et al., 2007). Functional analysis of the polarity proteins in a wide number of model organisms, such as yeast, worm and flies has resulted in the identification of three functional polarity complexes (Betschinger et al., 2003 Martin-Belmonte and Mostov, 2008). The three main polarity complexes identified at the moment are: PAR polarity complex, which is comprised of PAR3, PAR6, atypical protein kinase C (aPKC) and the cell division control protein 42 (CDC42). This complex promotes the establishment of the apico-lateral membrane border. The second complex is Crumbs complex (CRB), it is required for the establishment of the apical surface and is formed by transmembrane protein Crumbs (CRB) and the associated cytoplasmic protein PALS1 (Protein associated with lin-7; also known as MPP5) and the PALS1-associated tight junction protein (PATJ; also known as INADL). This second complex is required for establishing the apical membrane (Bazellieres et al., 2009). The basolateral domain is thought to be specified by the Scribble complex, consisting of signaling adaptors Scribble (Scr), Disc large (Dlg) and lethal giant larvae (Lgl) (Yamanaka and Ohno, 2008). These three complexes have antagonistic interactions and regulate in a spatiotemporal way the epithelial polarity. At initial stages of cell polarization, PAR3 binds to atadin, and this primitive adhesion matures to form the belt-like adhesion junctions and tight junctions that localize at the apical-basal border. At intermediate stages of polarization there is an increased aPKC activity and exclusion of PAR3 from primordial adhesions. This is followed by PAR3 exclusion from both the PAR6-aPKC and CRB complex to establish the apico-lateral border and the apical membrane, whereas the SCRIB complex defines the basolateral plasma membrane domain (Martin-Belmonte and Perez-Moreno, 2012) (Fig.1).

Generation of luminal space

Once polarized cells have recognized the (ECM) and their neighbors, luminal space can be generated. Molecular instructions for whether, where and how lumens will be generated are provided from integrating signals from ECM.

Three main mechanisms have been associated with the generation of luminal space: hollowing (directional vesicle trafficking), cavitation (luminal cell death) and focalized contact (formation of cell-cell contact and repulsion) (Datta et al., 2011).

In the hollowing process lumen is formed by membrane separation. Clusters of cell contacting with ECM but without a luminal space, through a mechanism of traffic endocytic vesicles, generate a central lumen (Tawk et al., 2007; Striliç et al., 2009; Eritja et al., 2010).

In the second process, cavitation, the generation of the central lumen is due to cluster central cells that do no contact with ECM which undergo apoptosis, leading to the generation of the luminal space (Mailleux et al., 2008).

The localized contact lumen formation process is a variant of the hollowing process, which describes combined focalized cell-cell contacts with active membrane repulsion. The combined actions of focalized adhesive and repulsive cues allows the generation of the intercellular luminal space (Martin-Belmonte and Perez-Moreno, 2012). Once lumens are formed they have to expand until mature functional size. In this process different parameters are involved, such as hydrostatic pressure, which is regulated by activation/inactivation of several pumps and channels, or pro-inflammatory cytokine levels, which are implicated in lumen formation and, more importantly, in maintenance of the central lumen.

However, little is known about the extracellular factors that regulate or influence on lumen formation process. In a recent study, our lab has described that pro-inflammatory cytokines, such TNF-α and IL-1α, contribute to lumen formation and maintenance and that this effect is ERα-dependent. In the same work, it is shown that the maintenance of the single lumen is not a passive process, and that the ERK/MAPK signaling pathway drives the effects of TNF-α and IL-1α on lumen polarization and cell polarity (Eritja et al., 2012) (Fig. 2).

Modeling glandular epithelium using 3D cultures

In 3D cultures, the control of the microenvironment is indispensable. Multiple microenvironmental parameters such as cellular origin, composition of extracellular matrix and media, and cell-cell interactions are crucial to interpret experimental results.

Matrices and scaffolds in 3D cultures

The ECM is a network of fibrillar molecules (including collagen, glycoproteins such as fibronectin, laminin and hydrophilic proteoglycans, fibrin, hyaluronic acid, elastin, etc.) which play a central role in differentiation of many organs by controlling the expression of specific genes.

Currently, different scaffolds for cell growth or drug

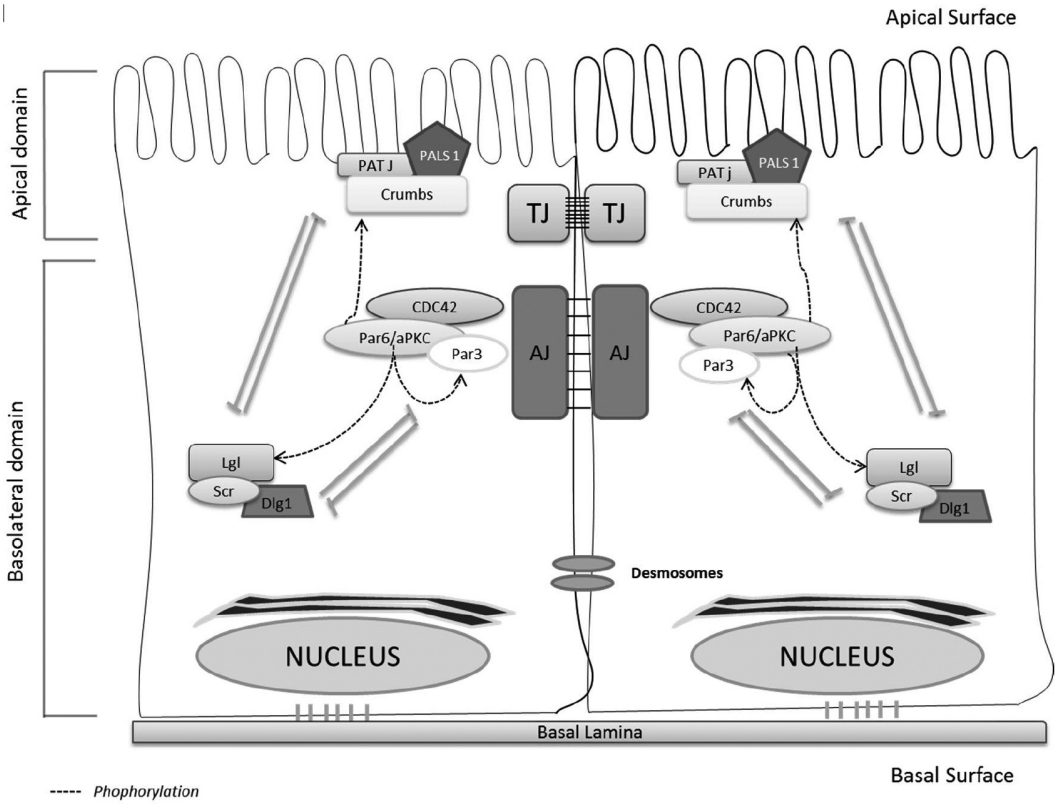

Fig. 1. Schematic model of interaction protein complexes involved in the genesis of cell polarity in epithelial cells. Once initial cell-cell contacts are established Cdc42 activates and enhances aPKC activity, phosphorylating Lgl. This phosphorylation segregates Lgl from the Par6/aPKC/Par3 that promotes TJ formation.

delivery are available: 1) a typical 3d porous matrix, which is a highly porous and well interconnected open pore structure that allows high cell seeding density and tissue in-growth (Garg et al., 2012); 2) a nanofibrous matrix that is prepared by electrospinning of self-assembly that provides a better resemblance of the physiological environment (Shea et al., 1999); 3) thermosensitive sol-gel transition Hydrogel-Based systems which are the most used in 3D epithelial gland reconstructions (Debnath et al., 2003; Debnath and Brugge, 2005; Fischbach et al., 2007) and 4) porous microspheres (Garg et al., 2012).

Hydrogel matrices are appealing for biological applications because of their high water content and biocompatibility (Hoffman et al., 1984). The different types of Hydrogel matrices used for 3D epithelial cell cultures can be divided into purified hydrogel molecules (e.g.: collagen, laminin and fibronectrin), mixtures of hydrogel components (e.g.: BM materials from natural origin such as Matrigel™) and natural molecules combined with biopolymers such as alginate, poly(ethylene glycolide) and poly(N-isopropylacrylamide) (Kreeger et al., 2003; Benton et al., 2009; Garg et al., 2012). The choice of the appropriate ECM might take into consideration functional and morphological epithelial gland aspects. Although some ECM components may permit the formation of multicellular spheroids/glands, they do not allow the cells to develop the baso-apical polarity axis, critical for epithelial differentiation.

A commonly used material to study morphogenesis as well as epithelial cellular transformation and carcinogenesis is Matrigel™, reconstituted basement membranes (rBMs) from Engelbreth-Holm-Swarm tumor-derived basement membrane matrix. Matrigel™ is composed primarily of basement membrane components, such as laminin-1 (60%), IV collagen (30%), sulphate proteoglycan (85%) and entactin or nidogen (1%) (Grant et al., 1985; Arnold et al., 2001). Endometrial differentiation into well-organized glandular structures (Eritja et al., 2010) or cuboidal-columnar monolayers (Schatz et al., 1990; Bentin-Ley et al., 1994) occurs in the presence of Matrigel™. However, because Matrigel™ is isolated from mouse tumor xenograft, it has a complex, poorly defined composition and huge lot-to-lot variability. It represents a significant disadvantage that increases experimental heterogeneity and interferes with experimental reproducibility.

Collagen is a better defined natural ECM and can be easily manipulated through changes in concentration and biochemical modification (Martin et al., 1996; Roeder et al., 2002). Certain epithelial cells, most notably MCDK epithelial cells (Yu et al., 2003) and human colon tumor cells (Dolznig et al., 2011), develop into polarized glands or cysts with a central lumen when grown embedded in matrices composed of collagen. Unfortunately, numerous epithelial cell lines (Emerman et al., 1980; Roeder et al., 2002; Gudjonsson et al., 2002) and primary cultures (Eritja et al., 2010) fail to form polarized structures when cultured in collagen gels. Overall, a great variety of matrixes can be used for 3D

Fig. 2. Multiple lumen gland formation is caused by a decrease of cytokine levels present in the medium. Contrast Phase images of endometrial epithelial glands, from murine origin, grown with medium containing glucocorticoids, which reduce cytokines expression and results in multi-lumened glands. Glands were grown 14 days with growing medium (which contains basically EGF and Insulin) supplemented or not with glucocorticoids. Scale bars: 20 μm.

cultures systems. Consequently, analysis of results from such experiments needs to be interpreted taking into account the limitations of each experimental design. For that reason, nowadays there is an increasing interest to develop new scaffolds, techniques and applications, for instance, the use of microfluid technology and specialized bioreactors.

Microfluid technology and devices used - specialized bioreactors based on the principle of simultaneous growth and dialysis pioneered by G.G. Rose (Rose, 1966)- for 3D cell culture, offer considerable advantages over standard culture techniques. For example, there is an important reduction in size and in reagent use and cells can be cultured with more realistic cell-to-fluid volume ratios that do not dilute paracrine and autocrine factors (Wikswo et al., 2006; Markov et al., 2010). Using this microfluid technology it has been possible to grown 3D osteogenic tissue (Mastro and Vogler, 2009), differentiate and maturate hepatic cells (Sivertsson et al., 2012) or determine drug toxicity (Magrofuoco et al., 2012) among other applications.

3D systems

The heterogeneity and low transparency of whole animals and organs is challenging for data collection. 3D cell cultures represent a simplified reductionist model. Numerous experimental 3D systems that mimic living tissues, as accurately as possible, have emerged during the past last years. One important difference between the multiple models available is the level to which they accurately mimic living tissues. Summarized below are the most used currently available models for studying 3D cellular morphology and behavior classified from the simplest to the more complex in relation to their integration level.

Cellular spheroids are simple 3D systems, which take advantage of the natural affinity of many cell types to aggregate. Spheroids from a broad range of cell types are basically produced by 2 different techniques: the hanging drop (Kelm et al., 2003; Timmins et al., 2005) and the rotating wall vessel (Castañeda and Kinne, 2000). In the first technique, the method relies on gravity enforced self-assembly to produce spheroids (Kelm et al., 2003). To make spheroids, small volumes of cell suspension are pipetted onto the inside lid of a tissue culture plate. The lid is inverted and drops stay attached to the lid due to surface tension. Gravity causes the cells to settle and concentrate at the bottom of the drop, and a single spheroid is formed (Kelm et al., 2003; Achilli et al., 2012). A variety of cell types have formed spheroids using this method including cell lines as well as primary cultures (Kelm and Fussenegger, 2004). The second technique, the rotating wall vessel, creates a microgravity environment. A cell suspension in a rotatory was vessel is slowly rotated about an x-axis, maintaining the cells in continuous free fall. Initially, rotation is very slow, but as spheroids start to form and the mass of the aggregates increase, rotation is increased to keep the aggregates in suspension. Heterotypic spheroids can be formed by co-culture of different cell types with this technique (Ingram et al., 1997). The most obvious advantages of spheroids are that they do not require external scaffolds to aggregate.

Models of polarized epithelial tissues are obtained by culturing non-transformed immortalized epithelial cell lines or primary cells under 3D conditions. There are three main systems to develop 3D cultures from epithelial cells using hydrogel matrices. In the first method, cells are completely embedded in a reconstituted base membrane and they grow in the presence of culture media that contain different growth factors and hormones (Petersen et al., 1998). In the second system, cells are seeded on a solidified layer of Matrigel™ (measuring approximately 1-2mm thickness) and dipped in medium containing 2% of rBM, 5% of horse serum and different amounts of growth factors and hormones (Debnath et al., 2003). In the third method, glandular structures are developed in a defined medium containing only 3% of Matrigel™ without the need for an underlying bed of Matrigel™ (Eritja et al., 2010) (Fig. 3).

Finally, more complex epithelial structures, such as

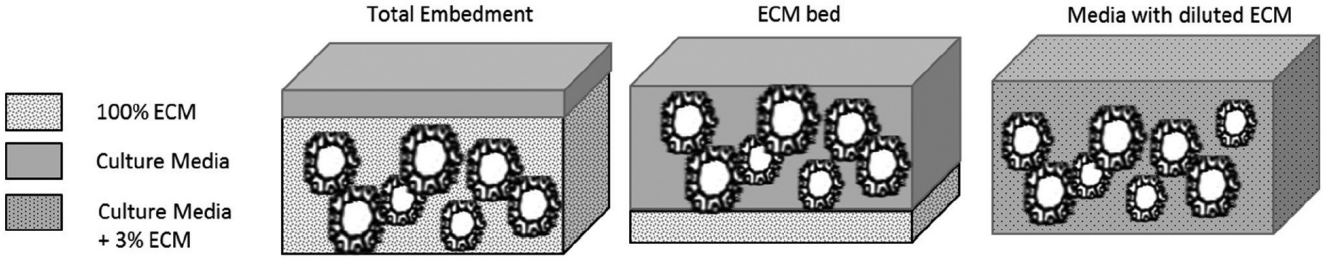

Fig. 3. Schematic model of three common methods used to generate glandular structures. In the first method (left), epithelial cells grow completely embedded within the ECM. Over the ECM, epithelial cells grown in the presence of culture media containing growth factors and hormones that are necessary for proliferation and survival. In the second method (middle) the ECM is first cast to form a bed and epithelial cells are seeded onto this bed as suspension in culture media. In the third method (right), epithelial cells are seeded directly as a cellular suspension in a culture media that contains 3% ECM.

human skin, have been developed from 3d cultures on membrane inserts or by using microscale materials such as supporting fibre meshes (Horch et al., 2005).

3D cultures as a model to study cell polarity

In developmental biology enormous progress has been made in understanding the mechanism and genes involved in glandular morphogenesis. This process is an essential event in gland and organ formation, and implicates a large number of transcription factors, growth factors and receptors (Patel et al., 2006; Monte et al., 2007). The use of 3D culture has provided new information that could not have been established without 3D models. For example, in MDCKII cells a significant reduction in PALS-1 and PATJ expression resulted in a decreased interaction between CRB3 and members of the Par6/Par3/aPKC complex. That complex interaction reduction results in a recruitment failure of aPKC in the tight junctions, which leads to an impaired development of luminal cyst (Straight et al., 2004).

Using 3D systems, nowadays we know that an intriguing relationship exists between epithelial mitosis and morphogenesis, which couples cell proliferation to tissue architecture. Some groups have reported that Cdc42 controls mitotic spindle orientation to correctly position the nascent apical surface in a growing cyst (Jaffe et al., 2008; Bray et al., 2011; Sakamori et al., 2012). In non-polarized cells, cdc42 orients the mitotic spindle, parallel to the substratum, through two different pathways. Cdc42 controls actin cytoskeleton, through PAK2 and ß Pix, and also activates PI3K. Activation of both complexes leads to correct spindle orientation (Mitsushima et al., 2009). However, in polarized cells the correct spindle orientation is PI3K independent (Toyoshima et al., 2007) indicating that different mechanisms are involved depending on polarized cell status. In mitotic MDCK cells aPKC has been implicated in the apical exclusion of LGN (Leu-Gly-Asn Repeat-Enriched Protein), which is an important spindle orientation determinant in symmetrical divisions (Zheng et al., 2010). In this work, the authors show that depletion of LGN expression results in spindle misorientation and defective cytogenesis, resulting in the formation of multiple lumened glands. A recent report describes that Par6B and aPKC (two downstream effectors of Cdc42) work interdependently to control mitotic spindle orientation and, furthermore, that aPKC activity is also indispensable for epithelial cell survival under basal conditions (Durgan et al., 2011).

Using real-time organotypic imaging techniques, 3D cultures have been described to study branching morphogenesis in different organs, such as salivary glands (Larsen et al., 2006; Wei et al., 2007), kidney (Srinivas et al., 1999; Chi et al., 2009) and pancreas (Puri and Hebrok, 2007). Basically, all the studies carried out with different organotypic assays have revealed a surprising degree of dynamic cell movements and rearrangements (Larsen et al., 2006; Chi et al., 2009), which testifies the importance of local mitosis, cell migration and cell shape change in end bud formation (Gray et al., 2010). Future research may explain a unique pattern of all branched organs because at the moment there appear to be fundamental differences in branching patterns of distinct mammalian organs (Nelson et al., 2008).

3D cultures as a model to study cancer

Hence with the role for polarity proteins in glandular morphogenesis, mislocalized or loss of polarity proteins can initiate tumorogenesis. For example, downregulation of Scr is sufficient to induce initiation of mammary tumors in a mouse epithelial cell line that presents mutant allele for gene p53 (Zhan et al., 2008)). The other components of the Scr complexes, Lgl and DLG, are also tumor suppressors that could promote the acquisition of invasiveness when their expression is modified (Spaderna et al., 2008).

Loss of polarity proteins can directly deregulate the cell adhesion process, which in turn disrupts polarity and promotes tumorogenesis. For instance, loss of E-cadherin disrupts 3D citoarquitectural epithelial glands (Eritja et al., 2010) and that alteration could cooperate with other oncogenes to induce tumor progression. Several polarity proteins have been recognized as a target of multiple viral oncoproteins. For example E4-ORF1 interacting cell polarity protein (which interacts with Dlg1, PATJ and ZO-2 (Javier, 2008) is a common target for the high-risk human papillomavirus (HPV) E6 or human T-cell leukemia virus type 1 (HTLV-1) Tax oncoprotein (Ellenbroek et al., 2012).

On the other hand, multiple oncogenes such as SRC or RAS and activated ERBB2 (Warren et al., 1988; Schoenenberger et al., 1991; Muthuswamy et al., 2001) have been shown to disrupt cell polarity in 3d glandular models or polarized monolayers. For instance, studies with Eph4 mouse mammary cells transformed with oncogenic variants of HRAS1 and ERBB2 have revealed that 3D microenvironment controls proliferation rates (Janda et al., 2002). In this study, the authors showed that HRAS1 and ERBB2 transformed cells grown under 3D conditions manifest a significant increase of proliferation compared with the same cells grown in 2D. This hyperproliferation in 3D cultures depends on PI3K activity (Janda et al., 2002). In another mammary 3D model using MCF-10A cells, it has been shown that activation of ERBB2 and CSF1R (colony-stimulating factor 1 receptor) increases proliferation and enhances cell survival (Muthuswamy et al., 2001). Activation of ERBB2 during MCF-10A morphogenesis elicits a complex multiacinar phenotype. These altered structures exhibit many of the properties of early stage cancers, including high levels of proliferation and filling of the luminal space. Protection from apoptosis, combined with changes in polarization, seems to contribute to this complex phenotype (Muthuswamy et al., 2001). By contrast, autocrine activation of CSRF1R in 3

dimensional cultures induces hyperproliferation and a progressive disruption of union integrity in glandular structures. This phenotype is accompanied by a change in E-Cadherin localization (Wrobel et al., 2004).

Transforming growth factor ß (TGF-ß) collaborates with several oncogenes to induce epithelial mesenchymal transition (EMT) (Zavadil and Böttinger, 2005; Wendt et al., 2009). Loss of polarity and disruption of cell contacts is associated with cells undergoing EMT and is a critical step in mediating the acquisition of metastatic phenotypes by localized carcinomas. It is well characterized that TGF-ß promotes and induces EMT (Miyazono, 2009; Wendt et al., 2009). In mouse epithelial cell line, NMuMG, TGF-ß induces cell-cell junctions disassemble. Upon TGF-ß stimulation, TGF-ßRII is recruited to a complex where Par6 is phosphorylated at Ser_{345}. This phosphorylation is required for the ability of TGF-ß to disrupt TJs (Ozdamar et al., 2005). In rat proximal epithelial cells, TGF-ß disrupts polarity through downregulation of Par3 and mislocation of the Par6/aPKC complex (Whiteman et al., 2008). Interestingly, the transcriptional repressor ZEB1, another inducer of EMT, inhibits the transcription of multiple polarity protein complexes such as Crb3, PATJ and Lgl2 (Aigner et al., 2007; Spaderna et al., 2008).

In addition to growth factor receptors, other signaling proteins have been described to be implicated in cancer influence 3D morphogenesis. For instance, Akt activation during MCF-10A morphogenesis causes the formation of large and misshapen structures (Debnath et al., 2003). These effects are all prevented by rapamycin (a highly specific pharmacological inhibitor of the AKT effector of mTor (Bjornsti and Houghton, 2004), these data are in accordance with those observed on transgenic overexpression activated Akt mouse prostate, which presents hyperplastic glands with increased individual cell size that reverts on treatment with the rapamycin analogue (Majumder et al., 2003, 2004).

3D culture of endometrial glands

There is a strong need to develop valuable tools to study endometrial carcinogenesis because this epithelium is thought to be the source of most endometrial tumors, manly via the instability that may arise from continuous proliferation and repair (Rose, 1996).

Similar to other epithelial cells grown in 3D culture systems, endometrial differentiation cells into glandular well polarized structures occurs in the presence of different gel matrices (Schatz et al., 1990; Uchima et al., 1991; Bentin-Ley et al., 1994). Different 3D epithelial endometrial cell cultures have been developed to study different criteria; for instance, several studies have developed three dimensional co-culture systems with stromal and epithelial cells (from different origins) to analyze blastocyst implantation (Bentin-Ley et al., 1994, 1995; Wang et al., 2012). Other groups have established 3D systems as a tool to compare biological features between 2D and 3D of endometrial carcinoma cell lines. Those studies have revealed that cell response can be distinct depending on microenvironment, because 3D structures have higher resistance to diverse anticancer drugs than 2D monolayers (Grun et al., 2009; Chitcholtan et al., 2012).

Finally, a 3D culture of normal endometrial cells of murine origin has been developed. This culture may provide a good instrument to study morphological changes associated with endometroid carcinogenesis (Eritja et al., 2010) and probably constitutes the ideal model to study early events on tumor progression, since cells are not modified/transformed as in human primary endometrial cells and established cell lines. However, this system also presents some weaknesses, as knowledge gained from it may not necessary be translated to human tumor progression. For instance, recent unpublished results from our lab have shown that a combination of EGF, Insulin and chronic estradiol exposure leads PTEN +/- glands to exacerbated growth that resembles an endometrial hyperplasia.

Conclusions

The studies overviewed in this revision illustrate that 3D culture systems have more biological information because they are a living assembly of cells that signal one another and actively respond to the signals that they generate. These models maximize cell-cell contacts and form numerous adhesions that will exert biomechanical forces that change the shape, cytoskeleton and function of the cells and establish 3D gradients of nutrients, metabolites and cell signals, as well as barriers to the transport of molecules. For all these reasons, they have provided a unique platform to discover previously unappreciated mechanical influences on glandular epithelial architecture and homeostasis. Nevertheless, current *in vitro* 3D culture models have inherent limitations in modeling *in vivo* tissue behavior. Further improvement of 3D culture systems, particularly the development of innovative heterotypic co-culture strategies and tunable biomaterial scaffolds (because most of the scaffolds studied are still in the investigation stage and are yet to be approved for clinical use or because the current commercial available hydrogels are extracted from animals or cultured cells and the amount of undesired soluble components varies between batches), will be invaluable in modeling cancer progression and testing novel therapeutic strategies in a biologically relevant context.

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