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A Computational Study of the Development of Epithelial Acini: II. Necessary Conditions for Structure and Lumen Stability

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Abstract

Simple epithelial tissues are organized as single layers of tightly packed cells that surround hollow lumens and form selective barriers separating different internal compartments of the body. The maintenance of epithelial structure and its function requires tight coordination and control of all the life processes of epithelial cells via cell-to-cell communication and signaling. These well-balanced cellular systems are, however, quite often disturbed by genetic or environmental cues that may lead to the formation of epithelial tumors (carcinomas). In fact, more than a half of all diagnosed tumors are initiated from epithelial cells. It is, therefore, important to gain a greater understanding of the factors that form and maintain the epithelial structure, as well as the features of the acinar structure that are modified during cancer development as observable in experimental and clinical research. We address these questions using the bio-mechanical model of the developing hollow epithelial acini introduced in Rejniak and Anderson (*Bull. Math. Biol.* 70:677–712, 2008). Here, we propose several scenarios involving various bio-mechanical interactions between neighboring cells that result in abnormal acinar development. Whenever possible, we compare our computational results with known experimental cases of mutant acini.

Keywords

Development of epithelial tumors; Formation of mutant acini; Single cell based model; Immersed boundary method

1. Introduction

Normal epithelial tissues form very selective barriers lining different internal compartments of the body, such as the bronchi and alveoli of the lungs, the breast ducts and lobules, the gastrointestinal crypts, the reproductive and urinary tracts, or the endocrine glands. Simple epithelial tissues are organized in single layers composed from tightly packed cells surrounding the hollow lumen. All life processes of epithelial cells, such as cell proliferation, apoptotic death, epithelial polarization, and cell-to-cell signaling are strictly coordinated and controlled within the tissue to ensure its integrity and proper functionality. However, even such well-organized cellular systems are exposed to disturbances, either of a genetic or environmental nature that can lead to the formation of carcinomas (epithelial tumors). Recent studies in the UK indicate that such events are not sporadic. In contrast, the majority of diagnosed cancers are of epithelial origin including breast, lung, bowel, and prostate tumors that together account for over half of all new cases of cancer (Cancer Research UK, 2008). Typically, carcinomas originate from individual epithelial cells that have accumulated certain genetic mutations and gained the ability to escape from the tight

control of cell growth and death observed in normal epithelia. This leads to the overgrowth of normal ductal cells by the tumor cells and to their further development into histologically distinct forms of intraductal carcinomas. These are characterized by different architectural and cytological patterns of tumor cells incorporating the partially or fully filled lumen (Debnath and Brugge, 2005; Winchester et al., 2000; Mallon et al., 2000). The level of architectural disorder of the epithelium, cytological abnormalities of epithelial and intraductal cells, and the variability in cell shape and size are key factors used by pathologists to classify epithelial tumors and to provide prognosis about their clinical outcome and treatment strategy. Various architectural patterns of intraductal carcinomas, such as cribriform, micropapillary, trabecular, comedo, or solid tumors are observed in clinical biopsy samples, (Winchester et al., 2000; Mallon et al., 2000; Bostwick et al., 1993; Che and Grignon, 2002; Tavassoli, 2001). However, little is known about how the genotypic abnormalities associated with cancers actually lead to such histologically distinct tumors (Debnath and Brugge, 2005). The pre-invasive carcinomas in situ may progress to the invasive form, if they undergo the epithelial-mesenchymal transformation that can lead to breach of the basal membrane surrounding the epithelial duct and to active cell invasion throughout the extracellular matrix (Savagner, 2001; Thiery, 2002; Yamaguchi et al., 2005). By acquiring such an invasive phenotype tumor cells can then migrate to distant parts of the body and colonize them resulting in the formation of secondary tumors.

It is important to know what factors maintain epithelial homeostasis and ensure that the lumen is completely empty of viable cells, as well as what processes cause such well-organized and tightly controlled cells to lose their epithelial characteristics. Understanding what mechanisms drive alterations in the acinar structure and lead to the development of different tumor morphologies, observed in experimental and clinical research, will ultimately aid our ability to treat cancer. To address these issues experimentally, three-dimensional culture systems have been introduced that mimic the natural epithelial environment and enable the growth of hollow epithelial acini, such as acini derived from Madin–Darby canine kidney (MDCK) cells, mammary epithelial MCF-10A cells, or mouse mammary epithelial HC11 cells (for review see Debnath and Brugge, 2005; O’Brien et al., 2002; Nelson and Bissell, 2005; Xian et al., 2005). In these experimental systems, single cells are seeded within the culture media containing adequate growth factors. The cells proliferate to form small 3-dimensional aggregates of randomly oriented cells consisting of two cell populations: Outer cells having contact with the culture media and inner cells surrounded entirely by other cells. Cells in the outer layer eventually develop an axis of apical-basal polarity and become unresponsive to proliferative signals. Differentiation of outer cells is followed by apoptotic death of inner cells that results in the formation of the hollow lumen and the acinar structure remains hollow thereafter. A schematic of a typical acinus consisting of a monolayer of polarized epithelial cells enclosing the hollow lumen and surrounded by the extracellular matrix is shown in Fig. 1.

These experimental models of normal epithelial acini are often used to reconstruct known phenotypes of epithelial tumors by identifying cellular genes that induce phenotypic alterations similar to those observed in clinical research. Here, we list a few examples of mutants derived from normal epithelial cells modulated by specific oncogenes. Large, distorted structures build up from cells of variable sizes and shapes, and with viable cells partially filling the internal lumen as a result of the activation of Akt receptors in the MCF-10A cells (Debnath and Brugge, 2005; Debnath et al., 2003). Single acinar structures with multiple luminal spaces arise due to a lack of expression of PALS1 receptors in MDCK cells (Debnath and Brugge, 2005; Straight et al., 2004). Multiacinar structures characterized by excessive proliferation and luminal filling are a consequence of activation of the ErbB2 or HER2 receptors in MCF-10A cells (Debnath et al., 2002; Wang et al., 2006). Such experimental models give invaluable insight into the role of intracellular bio-chemical

reaction pathways, that upon their activation or suppression lead to the development of abnormal acini. Activating a particular bio-chemical pathway may affect the course of other cell life processes, and, therefore, the results of such modifications are difficult to quantify in isolation. For example, MCF-10A cells expressing proliferative oncogenes, such as Cyclin D1 or HPV 16E7, do not exhibit the growth suppression characteristic of normal MCF-10A cells. It might then be expected that due to increased proliferation these cells would grow to form large fully filled clusters. It turns out, however, that these proliferative oncogenes do not inhibit luminal apoptosis, and the cells overexpressing Cyclin D1 or HPV 16E7 develop into large, but still hollow acini (Debnath and Brugge, 2005; Debnath et al., 2002).

Our goal is to use mathematical modeling to systematically investigate how changes occurring in individual normal cells or in the interactions between individual cells during the acinar development influence the final multicellular structure. Previously, each author has modeled independently certain aspects of the development of various tumors. Anderson has investigated the growth and evolution of clonal tumors under different selective environmental conditions using a hybrid discrete-continuous individual cell based model and showed that under harsh conditions the formation of finger-like invasive cohorts emerged containing the most aggressive clones in the population (Anderson, 2005; Anderson et al., 2006, 2008; Gerlee and Anderson, 2007). Rejniak has simulated distinct tumor morphologies resulting from cell competition for nutrients and space, and the formation of different patterns of intraductal carcinomas emerging as a result of alteration of certain bio-mechanical properties of proliferating epithelial cells (Anderson et al., 2008; Rejniak, 2005, 2007; Rejniak and Dillon, 2007). In this paper, we focus on the role of direct interactions between individual cells during the formation of normal epithelial layers, in particular, we are interested in characterizing the alterations in normal cell behavior that lead to the growth of different degenerate acini or tumors. Specifically, we will investigate the relationship between a cell's ability to sense free space available for its growth and the final form of the developed cell cluster. We will also explore mechanisms for the initiation of cell apoptosis that result in different patterns of dying cells, as well as various conditions for regrowth of inner cells and repopulation of the hollow lumen. In addition, we will examine the stability of the whole acinar structure in relation to the emergence of polarized cells and their growth suppression. We address all of these points by using the computational bio-mechanical model of the development of normal hollow acini introduced in our previous paper (Rejniak and Anderson, 2008). Using this model, we have previously shown that it is sufficient to consider local interactions between individual cells (such as the development of lateral membrane domains or initiation of apoptosis due to the loss of cell-to-cell contacts) and local interactions between cells and their micro-environment (such as the development of the basal membrane domain and sensing the free space for cell growth) to dynamically develop an acinar structure consisting of one layer of epithelial cells enclosing a hollow lumen. Here, we present several scenarios of the development of abnormal acini, and state hypothetical bio-mechanical conditions that allow for the formation of such mutants. Whenever possible, we draw comparisons with known experimental cases of mutant acini or intraductal tumors and comment on the genetic changes that are responsible for such phenotypic transformations.

The rest of the paper is organized as follows. A summary of our computational model (Rejniak and Anderson, 2008) is presented in Section 2. Computational results including structural changes in developing acini due to alterations in the cell cycle, evolution of cell phenotypes and differentiation of normal epithelial cells are presented in Section 3. Finally, comparison with known experimental and clinical cases of mutant acini together with a final discussion are presented in Section 4.

2. Summary of the model of growing epithelial acini

A computational model of normally growing epithelial acini introduced in Rejniak and Anderson (2008) captures the bio-mechanical processes of individual deformable cells and the collaborative behavior of neighboring cells that together lead to the formation of an acinar structure composed of one layer of polarized cells enclosing the hollow lumen. During this acinar development, each individual cell can undergo certain life processes and certain phenotypic transformations that depend entirely on interactions with other cells and with the cell micro-environment. We have shown in Rejniak and Anderson (2008) that for development of normal hollow acini, it is sufficient to consider five phenotypically different subpopulations of resting cells (outer, inner, dead, partially, and fully polarized) undergoing several cell processes (growth, division, apoptotic death, epithelial polarization). A flowchart summarizing the evolution of cell phenotypes and cell life processes is presented in Fig. 2(a). Morphological changes in phenotypically different cells are summarized in Fig. 2(b).

In general, each cell is modeled as an individual deformable body, with a mesh of elastic springs defining cell shape and a viscous incompressible fluid defining cell mass. All points on the cell boundary play the role of cell membrane receptors and are used by the host cell to sense signals from the surrounding environment and from neighboring cells. In particular, cells can adhere to one another by developing connections between their membrane receptors. Moreover, the cell membrane receptors are used to sense the presence of other cells or extracellular matrix in the cells local vicinity. When the cell senses sufficient free space in its micro-environment (that is, when a certain percent of cell receptors are not connected to the receptors of other cells), it may enter into the process of cell proliferation. The recruitment of fluid from the extracellular matrix during cell growth is modeled by placing couplets of point sources and sinks around the cell boundary to model transport of fluid through the cell membrane (cf. enlarged growing cells in Fig. 2(b)). Once the cell doubles its area, the contractile ring is formed by introducing springs between opposite points on the cell boundary that upon their contraction split the cell into two daughters (cf. changes in the shape of a dividing cell in Fig. 2(b)). All adjacent springs between the neighboring points on the cell boundary, all adhesive connections between separate cells and the contractile rings in dividing cells are modeled as short Hookean springs of constant stiffness. Cell epithelial polarity is acquired by developing three distinct cell membrane domains: one basal domain defined by cell membrane receptors contacting the external media; two lateral domains defined by cell receptors being in contact with other cells; and an apical domain by detaching all of its cell-to-cell connections with inner cells (cf. characteristically wedge-shaped polarized cells in Fig. 2(b)). Cell apoptotic death is modeled by first disassembling all cell adherent connections, and then by placing the sink-source couplets along the membrane of the whole cell to release fluid from the cell interior to the extracellular space (cf. morphological changes in apoptotic cells in Fig. 2(b)).

The acinar structure is formed from a single cell that upon consecutive divisions gives rise to an aggregate of randomly oriented cells consisting of two cell populations: outer cells having contact with the culture media and inner cells surrounded entirely by other cells. Every cell in the cluster can grow if it senses sufficient space, and after doubling its area the cells divide either orthogonally to their longest axis (unpolar division) or orthogonally to its boundary in contact with the external media (polar division). Cells in the outer layer develop an apical-basal polarity and tight junctions with their neighbors that together lead to the formation of a monolayer of epithelial cells. Differentiation of outer cells is followed by apoptotic death of inner cells resulting in the formation of the hollow lumen and the stabilization of the acinar structure. A consecutive sequence of stages in the development of

a typical acinus where each cell follows the phenotypic flowchart presented in Fig. 2(a), is shown in Fig. 3.

The values of the computational parameters defining initiation of different cell transformations and progression of all cell life processes in our basic model are listed in Table 1. In particular, they determine what percentage of cell membrane receptors is required to initiate and continue cell growth; what percentage of cell receptors is needed to initiate cell death and at which point the cell is considered dead; what percentage of cell membrane receptors is necessary to determine the basal and lateral membrane domain of partially polarized cells and when can the lateral cell-to-cell connections be transformed into tight junctions. In the subsequent sections, we will alter these values in order to investigate the final acini structure that develops under these new conditions.

This model is based on the immersed boundary method (Peskin, 2002) and captures interactions between individual elastic cells and a viscous incompressible fluid representing the cytoplasm inside the cells, the extracellular matrix outside the tissue, and the lumen inside the hollow acinar structure. The fluid flow is influenced by couplets of fluid sources and sinks used to model transport of fluid across the membrane of growing and dying cells, as well as forces generated by the elastic cell membranes, while at the same time, the elastic structures move at the local fluid velocity. Equations (1)–(5) describe motion of the fluid and all interactions between cells and the fluid.

$$\rho \left(\frac{\partial \mathbf{u}(\mathbf{x}, t)}{\partial t} + (\mathbf{u}(\mathbf{x}, t) \cdot \nabla) \mathbf{u}(\mathbf{x}, t) \right) = -\nabla p(\mathbf{x}, t) + \mu \Delta \mathbf{u}(\mathbf{x}, t) + \frac{\mu}{3\rho} \nabla s(\mathbf{x}, t) + \mathbf{f}(\mathbf{x}, t), \quad (1)$$

$$\rho \nabla \cdot \mathbf{u}(\mathbf{x}, t) = s(\mathbf{x}, t), \quad (2)$$

$$\mathbf{f}(\mathbf{x}, t) = \int_{\Gamma} \mathbf{F}(l, t) \delta(\mathbf{x} - \mathbf{X}(l, t)) dl, \quad (3)$$

$$s(\mathbf{x}, t) = \sum_{k \in \Xi^+} S_+(\mathbf{Y}_k, t) \delta(\mathbf{x} - \mathbf{Y}_k) + \sum_{m \in \Xi^-} S_-(\mathbf{Z}_m, t) \delta(\mathbf{x} - \mathbf{Z}_m), \quad (4)$$

$$\frac{\partial \mathbf{X}(l, t)}{\partial t} = \mathbf{u}(\mathbf{X}(l, t), t) = \int_{\Omega} \mathbf{u}(\mathbf{x}, t) \delta(\mathbf{x} - \mathbf{X}(l, t)) d\mathbf{x}. \quad (5)$$

In this system, Eq. (1) is the Navier–Stokes equation of a viscous incompressible fluid defined on the Cartesian grid $\mathbf{x} = (x_1, x_2)$, where p is the fluid pressure, μ is the fluid viscosity, ρ is the fluid density, s is the local fluid expansion, and $\mathbf{f}$ is the external force density. Equation (2) is the law of mass balance. Interactions between the fluid and the material points $\mathbf{X}(l, t)$ on cell boundaries and at point sources $\mathbf{Y}_k$ and sinks $\mathbf{Z}_m$ placed in the cell local micro-environment $\mathbf{Y}_k, \mathbf{Z}_m \in \Theta_{\Gamma}^{\varepsilon} = \bigcup_{\mathbf{x} \in \Gamma} \{\mathbf{x}: \|\mathbf{x} - \mathbf{X}\| < \varepsilon\}$ are defined in Eqs. (3)–(5). Here, the force density $\mathbf{F}(l, t)$ defined on cell boundaries, as well as sources $S_+(\mathbf{Y}_k, t)$ and sinks $S_-(\mathbf{Z}_m, t)$ defined in the cell micro-environment are applied to the fluid using the two dimensional Dirac delta function, while all material boundary points $\mathbf{X}(l, t)$ are carried along with the fluid. The boundary forces $\mathbf{F}(l, t)$ arise from elastic properties of cell boundaries, from cell-to-cell adhesion and from contractile forces splitting a cell during its division. The sources $S_+(\mathbf{Y}_k, t)$ and sinks $S_-(\mathbf{Z}_m, t)$ are chosen such that they balance around each cell separately. More details on this mathematical framework, its numerical implementation, and solution can be found in Rejniak and Anderson (2008).

3. Alterations in acinar development

We have shown previously in Rejniak and Anderson (2008) that it is sufficient to consider five phenotypically different sub-populations of resting cells (outer, inner, dead, partially, and fully polarized) undergoing several cell processes (growth, division, apoptotic death, epithelial polarization) to simulate the development of normal hollow acini. To determine conditions that are necessary for capturing all stages of acinar formation, the stability of acinar structure and maintenance of the hollow lumen, we suppress particular pathways from the discussed flowchart and investigate alterations in the final tissue structure. In particular, we are interested in four pathways defining respectively: cell growth abilities (Section 3.1), cell responsiveness to apoptotic signals (Section 3.2), transformation into polarized epithelium that includes a stage of cell intermediate transition denoted as the partially polarized phenotype (Section 3.3) and a stage of cell final differentiation into the fully polarized phenotype (Section 3.4).

3.1. Space limited cell growth

One of the assumptions of our model is that cell growth is space limited. Each undifferentiated cell can initiate the process of proliferation provided that it can sense free space in its vicinity. It is known that most normal mammalian cells grow and divide in culture until a confluent monolayer is formed and then stop proliferating. There are two known mechanisms that may contribute to such growth suppression: cell-to-cell contact interactions known as a *contact inhibition of cell division* and local depletion of the medium of extra-cellular growth factors known as a *density-dependent inhibition of cell division* (Alberts et al., 2002). However, whether the growth inhibition signal comes from the neighboring cells or from the surrounding medium, all cells always compete for free space, and we focus here only on this aspect of cell proliferation.

In our model, there are three different cell sub-populations capable of proliferating, i.e. resting inner, resting outer and partially polarized cells. Three corresponding pathways defining space limited cell growth (one for each proliferative cell phenotype), from the full phenotypic flowchart shown in Fig. 2(a), are presented together in Fig. 4. The availability of free space for cell growth and expansion is determined in each cell subpopulation in the same way, by inspecting all cell membrane receptors for contacts with other cells (contacts with the ECM are not taken into account here since cells are allowed to grow into the surrounding medium). If a certain percentage of cell receptors is not bound to other cells, the host cell starts acquiring the fluid from the surrounding extracellular space. If the percentage of free growth receptors falls below this threshold the host cell is considered to be overcrowded by other cells and enters into a resting state corresponding to the G_0 phase of the cell cycle until the space becomes available or the host cell differentiates and gains a non-proliferative phenotype, such as full polarization or apoptotic death. Note that the axis of cell division is determined differently in resting outer and inner cells (unpolar division—orthogonal to the cell longest axis) and in partially polarized cells (polar division—orthogonal to the cell membrane that is in contact with the external media, see Rejniak and Anderson, 2008 for more detail). We also address these two different orientations of cell division in Sections 3.3 and 3.4.

In our primary model, the host cell is required to have at least 20% of free growth receptors to start acquiring the fluid from the extracellular space. Here, we want to investigate how alterations in cell sensitivity to overcrowding will influence the final stable morphology of the developing acini. To do so, we modify the assumption of space limited cell growth by changing the minimal percentage of free growth receptors needed for the initiation and progression of cell growth when all other parts of the phenotypic flow-chart from Fig. 2(a) are left unchanged. The results showing the total area of the final stable cell cluster as well

as the area of the created lumen together with morphologies of selected acini are presented in Fig. 5. It is not surprising that when a large fraction (60–90%) of free growth receptors is required for cell proliferation then the majority of cells are not able to satisfy these conditions, since the development of multiple adherent connections with the neighboring cells reduces the number of free growth receptors in the host cells. Such cells enter early into a phase of growth-arrest without transforming to the fully polarized phenotype. This leads to the formation of small clusters of viable but non-growing cells, Figs. 5(a–b). For a smaller percentage of free growth receptors (55–60%), the cell clusters grow larger but acquire irregular shapes due to a very selective cell growth that is initiated only if a large part of cell membrane is in contact with the external medium. Other cells remain quiescent, since they do not sense enough space to grow. Some cells in the outer layer become polarized and even small luminal spaces can be created inside the cluster, but the final stable structures remain filled with resting inner cells that only sporadically enter into apoptosis, Figs. 5(b–c). The first fully hollow acinus arises when the required fraction of free growth receptors is equal to 54%, Fig. 5(d). In this case, a sufficient number of outer cells have acquired a fully polarized phenotype that enabled the initiation of apoptotic death of all inner cells. However, the final acinar structure has again a very irregular shape, similar to Fig. 5(c) that requires 55% of free growth receptors. This is again a result of a very restrictive cell growth rule that is satisfied only by some of the outer cells. However, in contrast to the cluster from Fig. 5(c), more partially polarized cells were able to grow and divide that resulted in their transformation into fully polarized cells leading to the formation of the lumen. In all other cases, when a lower percentage of free growth receptors is required for cell growth, the round hollow acini have formed, Figs. 5(e–f). Note that the less sensitive the cells are to overcrowding, i.e. when the smallest percentage of free membrane receptors is needed for the initiation and progression of cell growth; the more regularly shaped the final clusters, since in these cases, all cells including inner cells grow more often.

It may seem surprising, however, that in all cases when the hollow acini have been formed (0–50%), their final areas are very similar (within 10% of each other) and the inner lumen occupies about a half of the area of the whole cluster. This is a result of the suppression of growth of the whole cluster due to polarization of individual cells. Since the polarization pathway has been left unchanged in these simulations, the partially and fully polarized cells emerge normally in fast growing clusters and this in turn leads to initiation of apoptosis of inner cells and to stabilization of the whole cluster. Even in the extreme case when cells grow despite overcrowding (0% of free growth receptors), they still interact with their neighbors leading to cell full polarization and stabilization of the whole cluster. In contrast, when a high percentage of free growth receptors is required, the cells enter into the growth-arrest phase before achieving the polarization phenotype, and, therefore, the shapes and sizes of the final cell clusters vary significantly. Note that even in small cell clusters without dead cells, Fig. 5(b), there is a certain amount of free space between neighboring cells due to cell-to-cell links that preserve a distance between the neighboring cells if they are not transformed into tight junctions. This free space is considered to be the extracellular lumen that is not a result of cell apoptosis, but is created passively due to loosely packed neighboring cells. This extracellular lumen accounts for about 10% of the area of the whole cluster and it decreases when fully polarized cells emerge. Therefore, the area occupied by the lumen presented in Fig. 5 includes the total amount of the lumen located between neighboring cells and the lumen created due to cell apoptosis.

To summarize, the transition between the formation of hollow acini and the fully filled clusters takes place when the growing cells are required to maintain about 54–55% of their membrane receptors free from contacts with other cells. In this transition phase, the cell clusters acquire very irregular shapes. When the requirement of free growth receptors is increased, the cells form small clusters of viable non-polarized cells that are in growth-arrest

phase because too many of their membrane receptors are engaged in cell-to-cell adhesion. However, it would seem plausible that such cases (60% or more of free growth receptors) may not exist biologically. When the requirement of free growth receptors is decreased below 50%, the regular hollow acini are formed, and their shapes, the area of the whole cluster, and of the inner lumen are very similar in all these cases. This is due to unchanged pathway of cell polarization which leads to stabilization of the clusters over similar periods of time.

3.2. Adhesion regulated cell apoptotic death

It has been experimentally observed that the lumen of acinar structures emerges as a result of cell apoptosis after the layer of outer polarized cells has been formed (Debnath et al., 2002, 2003). It is also known that this form of cell death can be induced by specific signals from neighboring cells or from the extracellular matrix (Ardens and Wyllie, 1991). In particular, cells may undergo detachment-induced apoptosis (anoikis) when they loose their cell-to-matrix anchorage (Frisch and Screaton, 2001). However, this process may be prevented when cells maintain cell-to-cell contacts within the cell aggregate (Grossmann, 2002; Hofmann et al., 2007). We assume, therefore, that cell apoptotic death is initiated by the adhesion imbalance due to the loss of cell-to-cell contacts and insufficient cell-to-matrix support.

We model the process of lumen formation under the following assumptions. (i) The development of free apical membrane domains in the polarized cells causes disassembly of all their cell-to-cell connections with neighboring inner cells. (ii) All these previously connected membrane receptors of the inner cells are now considered to be death receptors and are blocked from any further cell-to-cell contacts and from sensing free space for cell growth. (iii) If a certain percentage of cell receptors become death receptors, the host cell loses the support from both the extracellular matrix and the neighboring cells and starts the apoptotic process. (iv) Since all dying cells disconnect themselves from their neighbors, this subsequently results in the emergence of new death receptors in the inner cells located more centrally, and if these cells reach the adhesion imbalance level, they can also die. (v) We assume that an apoptotic cell is dead when its diminishing area reaches 15% of its initial value.

We based our first assumption on biological evidence that the first apoptotic events take place near the layer of outer polarized cells and that the expression of certain proteins (such as CEACAM1 or PATJ) at the apical surfaces of epithelial cells is required for lumen formation (Debnath and Brugge, 2005; Debnath et al., 2003; Shin et al., 2005). We address assumptions (ii) and (iv) below by showing that if one of them is omitted in the model, then the developing acini will not form a hollow lumen. We also discuss assumption (iii) below by showing how the final structure of the developing acinus will change if we modify the requirement for apoptotic death initiation (in terms of the minimal percentage of death receptors). Modifying assumption (v), i.e. changing the parameter describing when the cell phenotype transforms from apoptotic to dead, will result in alterations of the length of the period of cell apoptotic death only, but will not introduce any significant changes in the overall acinar morphology. We have shown in our previous paper (Rejniak and Anderson, 2008) that for the chosen parameter, the average time of cell apoptotic death is about 9 hours which is biologically plausible.

When assumption (ii) is neglected, i.e. when the previously connected receptors in the inner cells are not blocked, then they may engage in new adhesion with neighboring cells and may also be used by the host cell as free growth receptors to determine availability of free space and to acquire fluid during cell growth. This subsequently leads to repopulation of the central luminal space, as it is shown in a series of six equally timed snapshots in the top row

of Fig. 6. Note that during the whole process of acinar development, three different cell phenotypes of inner cells are observable: growing (green), dying (grey), and resting (blue) cells. The evolution of these cell sub-populations is very dynamic. First, apoptotic cells arise as a result of the development of apical membrane domains in fully polarized outer cells; compare three dying cells in the snapshot taken after the 8th cell cycle. Since dying cells do not block receptors of their neighbors, the created free space can be sensed by other inner cells that may then start growing; compare the snapshot taken after the 10th cell cycle. The newly formed daughter cells can in turn assemble cell-to-cell connections with their neighbors, including the polarized cells, but since all polarized cells maintain their apical membrane domains and disconnect any new cell-to-cell contacts, these cells are triggered to start the process of apoptotic death and again create free space in their vicinity; compare growing and dying cells in snapshots taken after the 12th and 14th cell cycle, respectively. The last snapshot shows again several cells growing in a space created previously by dying cells. The final acinar structure, in fact, did not stabilize before the end of the presented simulation.

A series of six equally timed snapshots in the bottom row of Fig. 6 show the development of the acinar structure when assumption (iv) is neglected. The first apoptotic cells arise here, as in all previous cases, as a result of the formation of apical membrane domains in the fully polarized cells. All apoptotic cells disassemble their cell-to-cell contacts with the neighboring inner cells, however, since these previously connected membrane receptors are not transformed into death receptors, the inner cells that are not located in the vicinity of fully polarized cells will never reach the required fraction of death receptors and, therefore, will remain viable and resting inside the cell cluster. This prevents the formation of the central hollow lumen but allows for the formation of small multiple lumens near the boundary of the acinar cluster. Note that the subpopulation of inner resting cells (blue) is almost intact during the whole simulation—some centrally located inner cells shown in the snapshots taken after 8th cell cycle remain in the same location even in the snapshot taken after the 16th cell cycle. These cells cannot proliferate since their membrane receptors are blocked from sensing free space in their vicinity. Moreover, the cells cannot die since their membrane receptors are not transformed into death receptors.

To address assumption (iii), we investigate how alterations in cell sensitivity to the environmental cues for adhesion imbalance, that either keep the cell viable or initiate its apoptotic death, may influence the development of the whole acinar structure. A specific part of the phenotypic flowchart from Fig. 2(a) that defines adhesion regulated cell apoptotic death is shown in Fig. 7.

In our primary model, the host cell requires at least 25% of its membrane receptors to become death receptors in order to initiate the process of apoptotic death, and the cell is considered dead when its diminishing area reaches less than 15% of the initial size. Moreover, all cell death receptors are blocked in the sense that they cannot be engaged neither in the formation of new cell-to-cell contacts, nor in sensing the space available for cell growth. Here, we investigate morphological changes in the developing acini when the cells sensitivity to the environmental cues for adhesion imbalance, defined in terms of the minimal percentage of death receptors required to initiate cell apoptosis is altered. All other parts of the phenotypic flowchart from Fig. 2(a) are left unchanged. The results showing the total area of the final stable cell cluster as well as the area of the created lumen, together with morphologies of selected acini are presented in Fig. 8.

We expect that when a small fraction of death membrane receptors is sufficient to initiate cell apoptotic death, the normal hollow acini will form, that is confirmed in Figs. 8(a–b). Note that the change in the minimal percentage of death receptors from 0% to 39% results

only in small variations in the total acinar area of final stable configurations. Similarly, the area occupied by the lumen and the final acinar morphology does not vary significantly. However, the time to emergence of the first apoptotic cells and the overall time taken for lumen development are shorter when the required percentage of death receptors is lower, i.e. in a case of 10% of death receptors, the stable structure is created after the 8th cell cycle, whereas in the case of 35% death receptors, the stable structure is reached after the 10th cell cycle. In contrast, when the minimal percentage of cell death receptors is so large (75% or more) that no inner cell can meet the requirements for the initiation of apoptotic death the structure remains filled, Figs. 8(e–f). Note, however, that even in these cases the whole structure is stable and all outer cells are fully polarized, i.e. they have developed apical membrane domains and have disassembled all cell-to-cell connections with neighboring inner cells. Thus the inner cells possess some death receptors but are insufficient to initiate the apoptotic process. In the transition phase (40–75% of death receptors), all acini show stable formation of multiple lumens located near the outer layer of fully polarized cells with numerous inner cells forming a compact inner cell cluster, Figs. 8(c–e). Note again, that in all hollow acini about 50% of their area is occupied by the lumen, and the fully filled cell clusters contain about 10% of extracellular space between inner resting cells.

To summarize, the transition between the formation of the completely hollow acini and clusters with multiple small lumens takes place when the inner resting cells are required to accumulate about 39–40% of death membrane receptors before the process of their apoptotic death is initiated. Interestingly, the areas of both clusters are similar, but in a partially filled acinus, the created multiple lumens account together for only 25% of the total area of the cluster. Since it is more difficult for inner cells to accumulate higher fractions of death membrane receptors, the created lumens are smaller in these cases, but they are still observable even for a very high requirement of 75% of death membrane receptors. A proper fraction of death membrane receptors is necessary, but not sufficient for the development of a completely hollow lumen as a result of cell apoptotic death. Whereas the emergence of the first apoptotic cells near the layer of polarized cells can be due to the activity of polarized cells, such as the development of apical membrane domains, then the death of inner cells that have no contacts with polarized cells requires some other form of signaling that can be spread or shared among neighboring inner cells and can trigger their apoptotic death. We supported this hypothesis by the introduction of death membrane receptors that are transformed from adherent connections, during the process of disassembling all cell-to-cell contacts for the cell entering into the apoptotic process. Such death membrane receptors are blocked from creating new cell-to-cell contacts and from sensing free space in the cells vicinity, and upon their accumulation will trigger the death of a host cell. We showed that if there is no such signal that blocks cell growth and allows for accumulation of cues for apoptotic death, then either the luminal space will repopulate with viable growing cells, even if the apoptotic events are also present; or some cells will not die and remain viable leaving only small multiple lumens near the boundary of the acinar cluster. For simplicity, we have introduced a direct signal between cell receptors, but such a cell-to-cell communication may occur via some distributed signal sensed by the cells from the luminal space. We argue here that insufficient support from other cells and the ECM (in the form of the accumulation of blocked membrane receptors) may play a role in the initiation of cell apoptotic death.

3.3. An intermediate partially polar transformation

During the process of epithelial polarization, the host cell must develop three different membrane compartments: a basal membrane domain that adheres to the extracellular matrix or to the basal lamina (separating the tissue from the extracellular matrix); a lateral membrane domain that is in contact with neighboring cells; and an apical membrane domain facing the hollow lumen. This is achieved as a result of the redistribution and sorting of

particular membrane proteins on specific sides of the cell plasma membrane. Experiments on 3-dimensional cultured acini derived from MDCK cells reveal that extensive cell-to-cell contact is sufficient for the establishment of segregated domains of apical and baso-lateral membrane proteins. However, the formation of a basal lamina in addition to cell-to-cell contacts is required to establish the epithelial baso-apical axis of cell polarity which in turn is a requirement for the development of a central lumen (Wang et al., 1990; Ojakian et al., 1997). Therefore, the process of cell epithelial polarization is divided in our model into two stages—partial polarization (described in more detail below) established when the cell basal and lateral membrane domains are created, and full polarization (discussed in the next section) initiated after the tight junctions and an apical membrane domain forms. The relevant part of the flowchart describing the cell transition from outer to partially polarized is shown in Fig. 9.

A partially polarized cell is an intermediate state between an outer normally proliferating cell and a fully polarized cell for which the cell growth is suppressed. We call this state partially polarized, since the cells possess some features that are characteristic of polarized cells (such as two well-established membrane domains: basal and lateral with a potential to create an apical side), and some features characteristic of normal viable cells (such as an ability to proliferate, but with a specifically oriented axis of cell division; this is discussed below). The moment at which this phenotypic transition takes place depends on the emergence of the baso-lateral membrane domain. However, it is not known what fraction of the cell membrane receptors should belong to each membrane compartment. It has been shown in Vega-Salas et al. (1987) that depending on the culture conditions, the MDCK cells forming a confluent monolayer can acquire different shapes in which the basal and lateral surface areas can vary significantly. For example, the basal/lateral ratio can be $1.6 (26 \pm 5\%)$ and $16 \pm 21\%$ of the total cell surface area for a basal and lateral membrane domain, respectively) in a low calcium medium, or $0.25 (15 \pm 7.5\%)$ and $58 \pm 23\%$, respectively) in a normal medium. Similarly, the cells that form epithelial tissues in vivo can acquire very different shapes and different ratios of their basal/lateral surface areas depending on their type. For instance, simple columnar cells, such as those lining the intestines, gall bladder, or stomach, have much larger lateral than basal surfaces; the lateral/basal surface ratio is much smaller in simple cuboidal cells of thyroid follicles, kidney tubules or pancreas; whereas the squamous cells in lungs alveoli or capillary endothelium have flattened shapes characterized by small lateral but large basal surfaces (Gartner et al., 2007).

In our primary model (compare Fig. 3), the host cell becomes partially polarized provided that (i) at least 25% of its membrane receptors are in contact with the extracellular matrix (the basal membrane domain); (ii) the host cell adheres to two other outer cells using at least 10% of its membrane receptors separately for each neighbor (the lateral membrane domain); (iii) it has a potential to develop an apical membrane domain, i.e. at least 10% of its membrane receptors are in contact with one or more inner cells, and thus can be transformed into an apical side, or are already blocked due to apoptotic death of previously connected inner cells; (iv) the basal, lateral, and apical membrane receptors form mutually disjoint sets. These rules define a transition from an outer to a partially polarized cell, but subsequently all partially polarized cells develop tight junctions with their neighbors before their phenotype can be transformed into fully polarized. Nevertheless, the partially polar transformation is the first step that allows for the development of the hollow acinar structure. Figure 10 shows results of several simulations in which the minimal fraction of basal receptors was varied from 10% to 50%, and the minimal fraction of lateral receptors from 5% to 25% for each lateral side separately. It is intriguing that in each case the final configuration forms a stable hollow acinar structure composed of cells of three phenotypes: fully polarized, partially polarized, and resting viable cells. The creation of the hollow lumen is a result of the emergence of some fully polarized cells that consequently trigger the

apoptotic death of inner cells. However, in some cases, only a few cells have acquired the fully polarized phenotype. Nevertheless, due to the apoptotic death of all inner cells, all cells in the outer layer (whether polarized or not) possess blocked membrane receptors that prevent them from initiating further growth. Therefore, all cells are in the growth-suppressed state, but not all of them satisfy the polar transformation conditions, and thus some of them remain viable, but non-proliferative. Depending on the chosen fractions of basal and lateral membrane receptors, the final configurations contain different counts of polarized and non-polarized cells. The graph in Fig. 10 shows the percentage of cells forming the outer layer that have been transformed into a fully or partially polarized phenotype together with three characteristic acinar morphologies representing growth-arrested but non-polarized acini (a small percentage of polarized cells), half-polarized acini (40–60% of polarized cells) and almost fully polarized acini.

It is not surprising that for lower fractions of both, basal and lateral membrane receptors, the majority of cells forming the outer epithelial layer have reached the full polarized phenotype and have acquired similar rectangular shapes. All of them have longer lateral sides which one may expect to follow from the transition requirements, but this is due to the dynamical development of tight junctions in already partially polarized cells, naturally leading to an increased area of cell-to-cell contacts, Fig. 10(b). When larger fractions of either basal or lateral membrane receptors are required for a polar transformation, then the final structures grow larger before they reach stabilization. Moreover, such growth suppression is rather due to the blocked receptors that emerge as a result of apoptosis of the neighboring inner cells (preventing the cells from entering into a new proliferation process) than to the polar transformation of such cells. The outer cells that are not able to satisfy the transition requirements remain in a resting state never achieving the partially polarized phenotype. The viable resting cells acquire circular or oval shapes, however, cells that do become polarized acquire more cubical shapes, again a result of the development of tight junctions between neighboring cells, Fig. 10(c). When very high fractions of basal and lateral membrane receptors are required for a polar transformation, then the majority of cells in the outer layer remain in a growth-arrest state without the transition to the partially polarized phenotype, Fig. 10(a).

The main difference between normal proliferating cells and those that are partially polarized is in determining the axis of cell division. Normal cells in our model divide orthogonally to the cell longest axis, that due to their inhomogeneous and irregular shapes gives an impression of randomly oriented cell division. The partially polarized cells, in contrast, divide orthogonally to the cell basal membrane which results in the production of two daughter cells that both acquire baso-apical orientation. We investigated the outcome of altering the polar orientation of cell division in two simulations presented in Fig. 11. Five snapshots in the top row show morphological changes in the developing acinus in which all outer cells (whether they are polarized or not) divide orthogonally to the cell basal membrane domain, i.e. in a polar manner. Five snapshots in the bottom row show the developing acinus in which no polar division is present, and all outer cells (whether they are polarized or not) divide orthogonally to their longest axis. Note that both clusters are identical at the 6th cell cycle, and are identical to our base simulation shown in Fig. 3. The first differences in the acquired axis of cell division and in the shapes of new daughter cells are observable in snapshots taken at the 7th cell cycle. Three such cells are indicated by arrows in both simulations: the cell located in the top-left corner divides orthogonally to its basal membrane compartment (top row) and orthogonally to the cell longest axis (bottom row). The two new daughter cells located at the bottom of the cluster show distinct shapes and differences in relative positions. Note that in our base simulation in Fig. 3, where the orientation of cell division depends on cell phenotype, two discussed daughter cells comply with the simulation presented in the top row in Fig. 11, and the highlighted single cell

behaves identically to that shown in the bottom row in Fig. 11. As a result of the differently imposed orientations of cell division, the final acini differ in their size, shape, and the structure of the outer layer of epithelial cells. Due to polar cell division (top row), the final structure is composed of a layer of tightly packed elongated polarized cells. It is, however, quite irregular since cell full polarization occurs much earlier (compare both snapshots taken at the 8th cell cycle).

When all outer cells divide orthogonally to their longest axis (bottom row), a larger and more circular acinus is formed with outer cells of ovoid or cubical shape that are loosely connected to their neighbors. Note that in our base simulation in Fig. 3 the final acinar structure possess characteristics of both cases discussed here. It is composed of rectangular tightly packed epithelial cells, but its overall shape is round.

To summarize, the introduction of a partially polarized cell phenotype allows us to control the orientation of the axis of cell division as it is observable in epithelial tissues. It has been reported (Ferguson and Anderson, 1981; Ferguson, 1985, 1988) that normal epithelial breast cells can acquire only two specific orientations of the nuclear spindle, and thus two planes of cytokinesis. Cell division perpendicular to the lumen (or to the basal membrane) results in two lumenally positioned daughter cells and leads to the duct outgrowth. Cell division parallel to the lumen (or to the basal membrane), on the other hand, gives rise to one lumenally and one basally positioned daughter cell, and culminates in basal cell differentiation or its death by apoptosis. This allows the organism to control the orientation of cell mitosis in proliferating epithelial cells in such a manner as to maintain the normal tissue architecture and structural integrity. We introduced in our model one controlled orientation of cell cytokinesis that corresponds to cell division producing two lumenally positioned daughter cells. We showed that if such a controlled cell division is introduced too early (in every outer cell), then the developing acini acquire irregular shapes, however, all epithelial cells form a continuous layer built from tightly packed rectangular cells. In contrast, if there is no polar division, then the final acinar structures grow larger, with a majority of outer cells loosely connected to one another displaying more circular or oval shapes. Therefore, we have proposed an additional cell phenotype—a partially polarized cell—and assumed that partially polarized cells divide orthogonally to their basal membrane compartment, whereas all other cells divide orthogonally to their longest axis; this results in the formation of circular acini with a layer of tightly packed columnar cells. However, the acinus shown in the bottom row of Fig. 11 may still represent an epithelium, nonetheless, squamous rather than columnar. Similarly, different kinds of epithelial structures—squamous, cuboidal, columnar—may be obtained from our model by imposing different ratios of basal and lateral membrane receptors in cells that develop their partially polarized phenotype. We have identified a range of parameters defining the basal and lateral membrane domains that lead to fully polarized stable acini and those that produce acinar-like structures that are in a growth-arrest state, however, without a complete polarization of outer cells.

3.4. A final fully polar transformation

The partially polarized cell becomes fully polarized by developing tight junctions with neighboring outer cells to which it adheres, and by establishing a free apical membrane domain by disassembling all adherent connections with inner cells. A sub-section of the flowchart describing the transition from a partially to a fully polarized phenotype is shown in Fig. 12. We are interested in determining changes in the final acinar morphology when some requirements for the fully polarized phenotype are altered in our model, but all other parts of the flowchart are left unchanged.

In epithelial tissues, tight junctions are located on the border between the apical and basolateral membrane surfaces and are formed from transmembrane proteins embedded in the plasma membranes of both adherent cells. They not only hold cells together, but also form a barrier that prevents the passage of molecules and ions through the space between the cells. In our model, tight junctions arise upon dynamical contraction of lateral connections assembled between neighboring outer cells. These lateral connections are modeled as short springs with a resting length h_l that corresponds to the desired separation distance between membranes of neighboring epithelial cells. However, the lateral spring can only be introduced when the distance between adherent membrane receptors is at most four times larger than the spring resting length. This results in the contraction of lateral springs that diminishes the actual separation between boundaries of adherent cells (see Rejniak and Anderson, 2008 for more detail). In our primary model, we assume that such lateral connections are transformed into tight junctions given that contraction of lateral springs results in a separation between lateral boundary points smaller than $1.5h_l$. Moreover, we assume that a partially polarized cell becomes a fully polarized cell if at least 75% of their lateral connections have been transformed into tight junctions. It can be seen in Fig. 10(b) that some fully polarized cells have developed much longer lateral membrane domains than would be expected from the requirements of a partially polar transformation, but this is a result of the dynamic contraction of lateral adhesive connections and their transformation into tight junctions, that takes place after the host cell has acquired the partially polarized phenotype.

Here, we want to examine how the change in conditions defining the establishment of tight junctions will influence the shape of the fully polarized cells and the final structure of the whole acinus. Figure 13 shows results of several simulations in which the fraction of lateral cell-to-cell connections that need to be turned into tight junctions, was varied from 25% to 95%, and the distance between adherent boundary points that need to be reached in order to define a tight junction was varied from h_l to $2h_l$. Generally, the more restrictive the requirements for the assembly of tight junctions, the less likely partially polarized cells will satisfy them. Therefore, the cells remain in a partially polarized state and can enter into new proliferation processes that results in the formation of larger clusters before the cells get more tightly packed and finally can establish tight junctions. For example, for all three simulations presented in the last column of Fig. 13, where as much as 95% of lateral connections are required to turn into tight junctions, only every third cell that has acquired the partially polarized phenotype was transformed directly into a fully polarized state, and more than a half of them were forced to start proliferating. In contrast, almost every partially polarized cell turned instantly into a fully polarized phenotype in all six cases when the required lateral point separation was quite large: $1.5-2h_l$ and the required tight junctions fraction was between 25% and 75%. For the lateral point separation distance equal to the resting length of lateral contractile springs h_l , the size of the final acini, and the number of partially polarized cells that initiated the proliferation process, increases with the increased requirements of the fraction of tight junctions. Nevertheless, in all cases presented in Fig. 13, round hollow acini were formed, showing that our model is quite robust to changes in the conditions defining the establishment of tight junctions. Once the required fraction of tight junctions has been established, an apical membrane domain is formed automatically by disassembling all adhesive connections with the neighboring inner cells, along the part of the cell membrane that is disjoint from the lateral and basal domains. Moreover, all apical membrane receptors are blocked from any further cell-to-cell contacts. It has been shown in Section 3.2 that if these receptors are not blocked, then they may be used as growth receptors and in turn lead to cell regrowth and repopulation of the hollow lumen.

We assume in our model that fully polarized cells are unable to proliferate resulting in growth suppression of the whole acinar structure. This is consistent with in vitro

experiments on 3-dimensional acini that exhibit stability with respect to both acinar size and cell number when fully developed (Debnath et al., 2002, 2003). However, the *in vivo* epithelial ducts are exposed to certain changes in their size and cell number during normal tissue maintenance and turnover. For example, the mammary glands in breast tissues can expand significantly during the pregnancy and lactation periods. Therefore, we now investigate how sensitive our model is to the initiation of cell proliferation in an already stable acinar structure, and how this structure will change when either polar or un-polar orientation of cell division is imposed on the proliferating cells. Figure 14 shows several snapshots taken at the same time points during two simulations that differ only in the manner the orientation of cell division is determined, whereas, all other cell life processes are executed identically in both cases. Both simulations start with the same stable acinar structure and all new proliferation events are initiated in the same cells.

In the simulation shown in the top row of Fig. 14, all cells divide orthogonally to their basal membrane compartments (polar division); whereas in the simulation shown in the bottom row of Fig. 14 all cells divide orthogonally to their longest axis (un-polar division). Note that both acini grow identically during the first cell cycle until the first division is executed, but further divisions, consecutively either polar or un-polar, result in completely different final acinar structures. The consecutive un-polar divisions give rise to new inner cells that are triggered to die by apoptosis (several grey cells in all snapshots taken after the first cell cycle in the bottom row of Fig. 14), whereas cell apoptosis occurs very sporadically when all cells divide in a polar manner (single grey cells in snapshots taken after the second and half cell cycle in the top row of Fig. 14). In the later case, the dying cells are located always between the actively proliferating and fully polarized cells and their apoptotic death is a consequence of the loss of contacts with the extracellular matrix due to the expansion of neighboring growing cells that push loosely attached outer cells into the lumen. These new inner cells are subsequently triggered by fully polarized cells to commit apoptotic death. The majority of daughter cells, however, remains in the outer layer after each polar division leading to the outgrowth of the epithelial layer and an increase in the number of epithelial cells—the epithelial layer contains 26 cells after the first cell cycle, but 41 after the 4th cell cycle. However, such an increase in the number of epithelial cells is not accompanied by an enlargement in the luminal space (since cell apoptosis is infrequent) and, therefore, results in the elongation and folding of the epithelial layer, and a very tight packing of the outer layer of epithelial cells, and in the degeneration of the whole acinar structure. In contrast, cell un-polar division allows for continuous emergence of new inner cells that subsequently die by apoptosis increasing the area of the inner lumen. Moreover, the overall acinar shape is round, cell shapes remain oval and the number of outer epithelial cells increases much slower—from 25 cells after the first cell cycle to 28 after the 4th cell cycle. Therefore, in order to maintain the acinar structure of the growing duct, the outgrowth of the epithelial layer must be accompanied by an enlargement of the luminal space, and one way of achieving this is to execute cell proliferation in which one of the daughter cells remains in the outer layer, but the other is forced to die and create the lumen.

To summarize, we have shown that our model is robust with respect to changing the conditions under which the partially polarized cells can transform into the fully polarized phenotype. We have identified a wide range of parameters defining the transition of lateral adhesive connections into tight junctions that result in the formation of hollow acini, however, of different sizes and shapes. Since cell epithelial polarization is more difficult to tackle experimentally than cell proliferation or apoptosis, it is not known how long it may take for a cell to establish different membrane domains and to re-distribute specific proteins to the cell sides. In our model, most of the successful transformations from the partially to the fully polarized cell phenotype took place instantly in one iteration step. From those that took longer, the majority were completed within one cell cycle. However, a few cells needed

2–3 cell cycles to acquire full polarization when the requirements were very restrictive (e.g. tight junctions fraction of 95%, or points separation no larger than h_I). This wide spectrum of conditions for tight junction establishment allows us to adjust the parameters in our model in order to represent acini derived from different cell lines. We have also shown that the maintenance of acinar structure during the growth of already established ducts requires de-differentiation of fully and partially polarized epithelial cells and the introduction of a specific orientation of cell division that will contribute to the formation of the luminal space. We have shown that polar cell division was necessary for assembling the epithelial structure and is characteristic for resting epithelia, but is not sufficient for maintaining the epithelial shape and function.

4. Conclusions and discussion

Normal experimental acini are stable spherical or ovoid 3-dimensional structures composed of one layer of tightly packed epithelial cells enclosing a hollow lumen. They resemble the structure and function of real cysts or glands and are used to experimentally reconstruct known types of acinar mutants and epithelial tumors. To systematically investigate how changes occurring in individual cells or in the interactions between individual cells during the acinar development process influence the final structure, we used a computational single-cell-based bio-mechanical model of a two-dimensional cross section through the developing acinus that was introduced in our previous paper (Rejniak and Anderson, 2008). In this paper, we focused on determining which factors are necessary for the maintenance and stability of the epithelial monolayer and the hollow lumen. We examined how alterations in several key parameters of our model can influence the behavior and interactions of individual cells that finally lead to the formation of either normal or mutated acini.

Normal experimental acini grow in culture as multicellular structures with spherical or ellipsoidal shapes. Depending on the cell line and culture conditions, the time of their development may vary and the final acini may reach different sizes, but their overall form is usually quite regular (Debnath and Brugge, 2005; O'Brien et al., 2002; Nelson and Bissell, 2005; Xian et al., 2005; Debnath et al., 2003; Mills Shaw et al., 2004). In contrast, the multicellular structures derived from transformed or mutated cell lines acquire highly irregular shapes. For instance, MCF-10A cells expressing chimaeric ErbB2 receptors overcome growth inhibition and elicit multi-acinar structures that look like aggregates of several connected acini. The cells, however, still retain epithelial properties and do not invade the basement membrane or display anchorage independence (Debnath and Brugge, 2005; Debnath et al., 2002; Muthuswamy et al., 2001). Other cell lines, such as human breast carcinoma cells MCF-7 or tumorigenic cells T4-2 form large loosely disorganized invasive colonies that recapitulate malignant breast tissues in vivo (Muschler et al., 2002; Mills Shaw et al., 2004; Petersen et al., 1992; Weaver et al., 1997).

Our simulations reveal that normal regularly shaped acini arise for a broad range of parameters, and in all such cases, their final hollow structures become round and develop both similar geometric size and luminal space (cf. Figs. 5, 8, 10, and 13), even in the case of unrestricted cell growth, Fig. 5(f). The ability of the model to consistently produce acini with regular morphologies and similar overall sizes shows that the process of epithelial stabilization due to cell full polarity is quite robust. Moreover, for the majority of cases presented in Fig. 8, the stable acini are also round, regardless of whether they are hollow or filled with inner cells, which shows that the time of emergence of the hollow lumen and the progress in cell apoptotic death are not the key factors in acquiring the final regular shape. Only when the ratio of free growth membrane receptors required for cell growth or the ratio of death membrane receptors needed for the initiation of cell apoptosis are very high (at least

50% in Figs. 5(c–d) or 70% in Figs. 8(e–f), respectively), do the final structures show elongated irregular protrusions. These high requirements for cell membrane receptors are rather biologically implausible. However, these simulations indicate some cellular features (such as the imbalance in cell growth and polarization; or the continuing proliferation of cells that are unable to create apical sides) that may lead to the emergence of particular invasive mutants. Certainly, the initiation of invasive tumors will be a subject of our further investigation. In this paper, we also observed irregularly shaped acinar structures when the polar orientation of cell division was imposed on all proliferation events in the developing acinus (presented in the top row of Fig. 11) and in the expanding acinus presented in the top row of Fig. 14. In contrast, when un-polar divisions were combined with cell apoptosis, the whole cluster grew in a more regular manner (bottom rows in both figures). This would suggest that for the proper rearrangement and maintenance of the acinar structure a balance in the polarization-depolarization of outer epithelial cells is needed.

Another characteristic feature of normal acini is a completely hollow centrally located lumen that arises as a result of apoptotic death of all inner cells (Debnath and Brugge, 2005; O'Brien et al., 2002; Nelson and Bissell, 2005; Xian et al., 2005; Debnath et al., 2002). In contrast, many acinar mutants or intraductal tumors are characterized by multiple luminal spaces surrounded by viable cells forming intraductal bridges, or even by cells completely filling the inner space. For instance, the activation of iFGFR1 receptors in the growth-arrested and polarized acini derived from HC11 mouse epithelial cells leads to repopulation of the lumen and to the formation of multiple luminal spaces (Xian et al., 2005). Similar multiple mini-lumens develop in the cysts derived from a MDCK cell line stably expressing the tight-junction associated proteins: either PALS1 (Straight et al., 2004) or E4-RF1 (Latorre et al., 2005). Other cell lines, such as MCF-10A expressing ErbB2 receptors develop multi-acinar structures that are fully filled with viable cells (Debnath and Brugge, 2005; Debnath et al., 2002; Muthuswamy et al., 2001).

Our simulations reveal that the fully hollow lumen emerges in all cases when the required fraction of death receptors necessary for the initiation of cell apoptotic death does not exceeds 40% of all cell membrane receptors. It is known that apoptosis can be initiated by either extracellular or intracellular death signals which cause intracellular adaptor molecules to aggregate and activate a proteolytic caspase cascade that mediates the apoptotic process (Alberts et al., 2002). By varying the minimal percentage of death receptors necessary for activation of the apoptotic process, we showed that completely hollow lumens are created even if the required amplification of the death signal is as much as 39% of cell membrane receptors, (cf. Fig. 8). The formation of multiple mini-lumens has been observed in our simulations in two circumstances. First, when the required amplification of death signals was moderate (25% of all cell membrane receptors were death receptors), but the signal was passed on only from the polarized to nearby inner cells and was not transmitted further to more centrally located inner cells, (cf. bottom row of Fig. 6). In this case, the inner cells that have no contact with polarized cells were not “instructed” to die and, therefore, they remain viable. In the second case, the death signal was transmitted subsequently from all dying cells to their neighbors, but the requirements for the initiation of cell apoptosis were so elevated (40–75%) that most of the inner cells were not able to satisfy them and they remained in a resting viable state, (cf. Figs. 8(c–e)). In these cases, only small mini-lumens were formed near the rim of polarized cells and their number and areas became smaller with increased requirements for the initiation of cell death. Therefore, the emergence of the first apoptotic cells is due to the development of apical sides in polarized cells, whereas the death of inner cells that have no contact with polarized epithelium requires some other form of signaling between neighboring inner cells. For simplicity, we have introduced here a direct signal between cell receptors, but such cell-to-cell communication may occur via some distributed signal sensed by the cells from the luminal space. We argued here that insufficient support

from other cells and the ECM, in the form of the accumulation of death (blocked) membrane receptors, may play a role in the initiation of cell apoptotic death. When the prerequisites for cell death reached more than 75% of death receptors, the lumens remained completely filled with viable resting cells, (cf. Figs. 8(e–f)). Other instances where no lumen formed were either a consequence of the lack of fully polarized cells that can initiate the apoptotic process, (cf. Figs. 5(a–c)), or were an effect of repopulation of the lumen when the growth of inner cells was not inhibited, (cf. top row of Fig. 6).

Cell polarity is broadly defined as asymmetry in cell shape, protein distribution, and cell function (Nelson, 2003). In epithelial cells, this means a redistribution of surface-specific transmembrane proteins to three different cell surfaces: apical, basal and lateral, and relocation of certain cell organelles, i.e. the Golgi apparatus should be located near the cell apical surface and the nucleus near the cell basal surface (Debnath et al., 2002, 2003). Due to its complex and multi-component nature, it may be necessary to simultaneously apply several biological markers in order to determine whether the cell is polarized or not. Moreover, since cell epithelial polarization is difficult to tackle experimentally, many questions remain unanswered. It is not known how long it takes to establish different membrane domains and redistribute specific proteins adequately to the cell sides, what are the relative ratios of membrane receptors expressed in each of the cell surfaces, and how the cell behavior changes when membrane protein distribution is varied. Because of such uncertainty and complexity of polarization, we have divided this process into two stages: a transition phase called cell partial polarity and a terminal phase called cell full polarity. Whether or not such different stages of cell polarization can be distinguished experimentally remains to be evaluated.

We have shown in our simulations that stable polarized acini are formed for a wide range of parameters defining redistribution of basal (10–30%) and lateral (5–15% for each side) membrane receptors in partially polar transformation, and for a wide range of parameters defining lateral point separation ($h_l - 2h_l$) and the fraction of lateral connections that become tight junctions (25–75%) in the fully polar phenotype. When parameters in a partially polar transformation were chosen outside this range, the acinar-like structures that formed consisted of one layer of growth-arrested non-polarized cells that acquired square or round shapes and were loosely adherent to their neighbors, (cf. Fig. 10). This suggests that different kinds of epithelial structures (such as squamous, cuboidal, columnar epithelia) may be obtained from our model by imposing different ratios of basal and lateral membrane receptors in cells that develop their partially polarized phenotype. The wide spectrum of parameters that allow for the establishment of tight junctions shows that our model is robust with respect to transformations of partially polarized cells into fully polarized, (cf. Fig. 13). This could suggest that the partially polarized phenotype may be omitted in our simulations, but the main reason for using it was to introduce in a controlled way a polar orientation of cell division that corresponds to cell division producing two lumenally positioned daughter cells. This allows us to handle the orientation of cell mitosis in proliferating partially polarized cells in such a manner as to maintain the epithelial architecture in the developing acinus. We showed that if such a controlled cell division is introduced too early (in every outer cell), then the developing acini acquire irregular shapes, however, all epithelial cells form a continuous layer built from tightly packed rectangular cells. In contrast, if there is no polar division, then the final acinar structures grow larger, but a majority of the outer cells are loosely connected to one another and acquire more circular or oval shapes, (cf. Fig. 11). On the other hand, re-initiation of cell growth in an already stable acinar structure leads to tissue folding and acinar degeneration if only polar divisions are executed, since this does not lead to the creation of any new luminal space (cf. Fig. 14). We have also shown that the maintenance of acinar structure during the growth of an already established duct requires de-differentiation of fully and partially polarized epithelial cells and the introduction of

orientated cell division that also contributes to the formation of the luminal space. We have shown that polar cell division is necessary for assemble of the epithelial structure and is characteristic for resting epithelia, but is not sufficient for maintaining the epithelial shape and function.

The model presented in Rejniak and Anderson (2008) and examined in detail here can be improved and extended in several ways in the future. We plan to incorporate known biochemical pathways that govern the behavior of particular cell membrane receptors and cell life processes. The model is general enough that it can be applied to other cell types and other multicellular systems in embryology, vasculogenesis, or tubulogenesis. Moreover, in this paper, we have not investigated the effects of changing multiple model parameters simultaneously since this leads to an extensive search in the multidimensional parameters space. In our opinion, it would be more effective to first tune the model to a particular experimental cell line, i.e. by adjusting cell growth and death rates and then examine how alterations in other cell processes such as cell polarization or cell-cell interactions influence subsequent acinar development, and this is a subject of our ongoing research.

We cannot underestimate the importance of understanding the critical conditions that cause stable epithelial acini structures to break down. The developmental processes that lead to these structures must ultimately be the same processes that drive epithelial cancer. We have investigated a wide range of parameters and assumptions in our model that lead to degenerate epithelial structures. In particular, we have highlighted the important roles that both cell polarization and apoptosis play in forming stable acini structures. Whilst degenerate acini do not represent cancer, they can give crucial insight into the initial properties a malignant phenotype will need to acquire in order to become a full blown cancer. The deeper computational understanding of these properties must be complimented by experimental testing and validation, however, this will certainly be difficult in relation to epithelial polarization. Mathematics driven experimentation is still in its infancy, but we believe this integrated approach holds great promise.

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Fig. 1.
A schematic representation of a typical acinus consisting of a monolayer of polarized epithelial cells enclosing the hollow lumen and surrounded by the extracellular matrix.

(a)

Fig. 2.

(a) Flowchart describing the evolution of phenotypically different sub-populations of resting cells—outer, inner, partially and fully polarized, dead; and the associated cell life processes—growth, polar and unpolar division, establishment of different membrane domains and tight junctions (TJ) leading to cell epithelial polarization, apoptotic death. (b) Morphological alterations in phenotypically different cells—enlargement of growing cells, formation of the contractile furrow between a pair of daughter nuclei in the dividing cell, shrinkage of the area of apoptotic cells, and development of distinct membrane domains in polarized cells. More details can be found in Rejniak and Anderson (2008).

Fig. 3.

A consecutive sequence of stages in the development of a typical acinus where each cell follows the phenotypic flowchart in Fig. 2(a). The snapshots are taken after each full cell cycle from 0 to 9. An accompanying simulation movie is available at: <http://www.maths.dundee.ac.uk/~rejniak/BMBsimulations.html>.

Fig. 4.

Flowchart of space limited cell growth. An undifferentiated cell (outer, inner, or partially polarized) can enter the proliferation process if there is free space available for its growth. After doubling its area, the cell divides either in an unpolar or in a polar manner (see text for more detail) giving rise to two daughter cells that can again start growing if free space is available.

Fig. 5. Morphological changes in developing acini under different conditions of growth initiation ranging from 0% to 90% of free growth receptors. Total areas of the final stable cell clusters (blue), areas of the created lumen (red), and the fraction of the total area occupied by the lumen (green) are reported for all cases. The selected cluster morphologies represent: (a–b) growth-arrested clusters of viable cells; (c–d) transition phase from growth-arrested clusters to hollow acini; (e–f) regular round hollow acini. For explanation of color-coded cell phenotypes, see Fig. 3.

Fig. 6.

A sequence of consecutive stages in acinar development when the function of death receptors in inner cells is altered: (top) the receptors are not blocked from sensing the space for cell growth, assumption (ii); (bottom) the death receptors arise only from apical contacts with polarized cells, assumption (iv). For explanation of color-coded cell phenotypes, see Fig. 3. An accompanying simulation movie is available at: <http://www.maths.dundee.ac.uk/~rejniak/BMBsimulations.html>.

Fig. 7.

Flowchart of adhesion regulated cell apoptotic death. An inner cell becomes apoptotic due to adhesion imbalance resulting from the breakage of cell-to-cell connections either by polarized cells developing free apical membrane domains, or by dying neighboring cells that disassemble all their adherent connections.

Fig. 8. Morphological changes in developing acini under different conditions of apoptosis initiation ranging from 0% to 90% of death membrane receptors. Total areas of the final stable cell clusters (blue), areas of the created lumen (red), and the fraction of the total area occupied by the lumen (green) are reported for all cases. The selected cluster morphologies represent: (a–b) regular round hollow acini; (c–e) transition phase showing stable multiple lumens; (e) fully filled cell clusters. For explanation of color-coded cell phenotypes, see Fig. 3.

Fig. 9.

Flowchart of the emergence of the partially polarized cell phenotype. An outer cell becomes partially polarized if it develops a baso-lateral membrane domain that adheres to the extracellular matrix (basal compartment) and to the neighboring cells (lateral compartment).

Fig. 10.

Emergence of polarized cells in the final acinar structure under different ratios of basal and lateral membrane receptors required for the transition from an outer to a partially polarized cell. The graph shows the percentage of fully and partially polarized cells in the outer layer of the final acinar structure together with selected characteristic acinar morphologies representing growth-arrested but (a) non-polarized, (c) half-polarized and (b) almost fully polarized acini. For explanation of color-coded cell phenotypes, see Fig. 3.

Fig. 11.

Morphological changes in developing acini under different orientations of cell division, Top row: all outer cells divide orthogonally to the cell basal membrane domain (all cells divide along the polar orientation); Bottom row: all outer cells divide orthogonally to their longest axis (no polar orientation of cell division present). Note the difference in division of the cell located in the left-top part of the cluster and the difference in two cell phenotypes at the bottom of cell cluster at the 7th cell cycle (arrows). For explanation of color-coded cell phenotypes, refer to Fig. 3. An accompanying simulation movie is available at: <http://www.maths.dundee.ac.uk/~rejniak/BMBsimulations.html>.

Fig. 12.

Flowchart of the emergence of the fully polarized cell phenotype. All partially polarized cells must develop tight junctions (TJ) with neighboring outer cells and a free apical membrane domain in order to become fully polarized.

Fig. 13.

Morphological changes in developing acini under different rules of tight junction assembly: the fraction of the lateral membrane receptors that need to be turned into tight junctions, and the distance between adherent boundary points that need to be reached in order to define tight junctions; h_l is the resting length of the lateral contractile springs. All acini are shown at the same time after initiation of growth, but only the structures in the last column have not stabilized. For explanation of color-coded cell phenotypes, see Fig. 3.

Fig. 14.

Morphological changes in expanding acini with polar vs. un-polar orientation of cell division shown after the indicated number of cell cycles. Both simulations start from the same stable acinar structure (first column) and new proliferation events are initiated in the same cells. Top row: all cells divide orthogonally to the cell basal membrane domain (polar division); Bottom row: all cells divide orthogonally to the cell longest axis (un-polar division). For explanation of color-coded cell phenotypes, see Fig. 3. An accompanying simulation movie is available at: <http://www.maths.dundee.ac.uk/~rejniak/BMBsimulations.html>.

Table 1

Numerical values of computational parameters in the basic model (Rejniak and Anderson, 2008)

Computational parameter	Basic value	Alteration discussed in
min % of free receptors for cell growth	20%	Section 3.1
min % of death receptors for cell apoptosis	25%	Section 3.2
min % of cell area for cell death	15%	Section 3.2
min % of basal receptors for cell partial polarity	25%	Section 3.3
min % of lateral receptors (for each side) for cell partial polarity	10%	Section 3.3
min % of tight junctions for cell full polarity	75%	Section 3.4
max distance for tight junctions assembly (h_l -length of a lateral link)	$1.5h_l$	Section 3.4