Normal and malignant remodelling of epithelial tissues: an integrative IBCell model

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Biocomplexity, October 28, 2009

Integrative Cancer Biology Program
National Cancer Institute

Epithelial tissues

66 CELL BIOLOGY AND HISTOLOGY

TABLE 5-1 *Classification of Epithelia*

Type	Shape of Superficial Cell Layer	Typical Locations
One cell layer		
Simple squamous	Flattened	Endothelium (lining of blood vessels), mesothelium (lining of peritoneum and pleura)
Simple cuboidal	Cuboidal	Lining of distal tubule in kidney and ducts in some glands, surface of ovary
Simple columnar	Columnar	Lining of intestine, stomach, and excretory ducts in some glands
Pseudostratified	All cells rest on basal lamina, but not all reach the lumen; thus the epithelium appears falsely stratified	Lining of trachea, primary bronchi, nasal cavity, and excretory ducts in parotid gland
More than one cell layer		
Stratified squamous (not keratinized)	Flattened (nucleated)	Lining of esophagus, vagina, mouth, and true vocal cords
Stratified squamous (keratinized)	Flattened (without nuclei)	Epidermis of skin
Stratified cuboidal	Cuboidal	Lining of ducts in sweat glands
Stratified columnar	Columnar	Lining of large excretory ducts in some glands and cavernous urethra
Transitional	Dome-shaped (when relaxed), flattened (when stretched)	Lining of urinary passages from renal calyces to the urethra

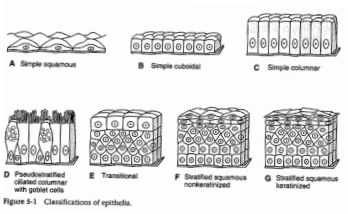
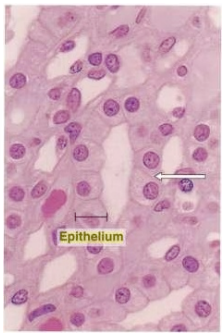
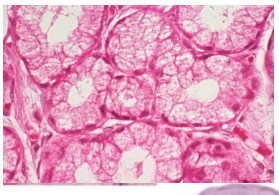
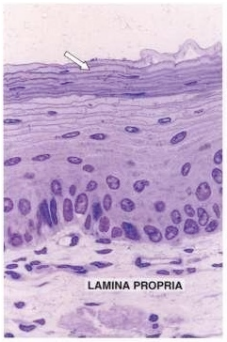
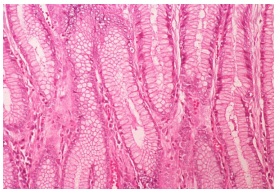


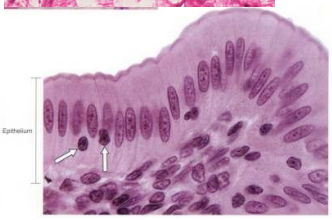
Figure 5-1 Classifications of epithelia.











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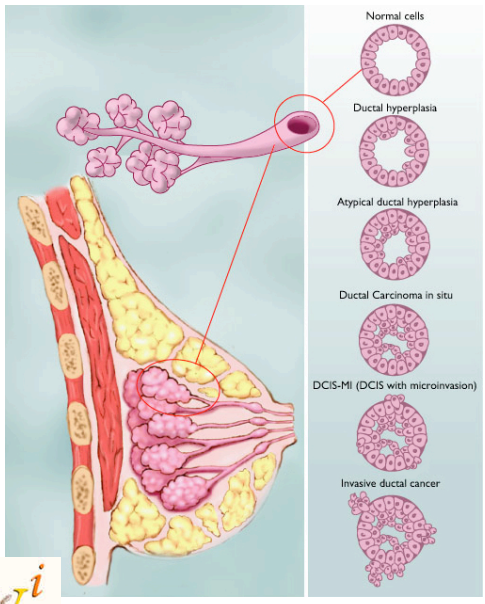
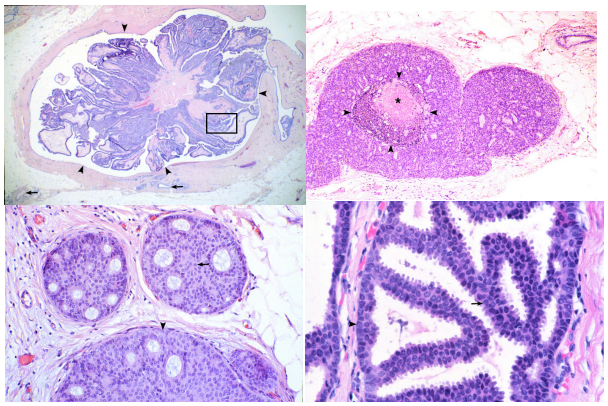
Motivation: how epithelial tumors arise from normal epithelial cells *in vivo*?





Patterns: papilloma, solid, cribriform, roman bridges;
A.Fischer
http://mammary.nih.gov/reviews/tumorigenesis/Fischer001/slides/intropg2.htm

ViCBC

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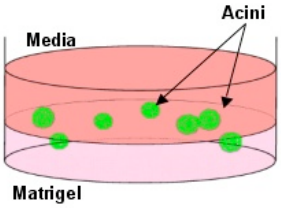
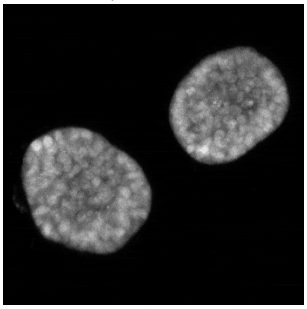
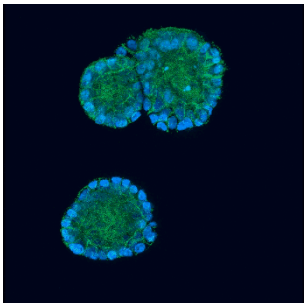
MCF10A mammary acini 3D experimental *in vitro* system



Jerome Jourquin, Quaranta lab, ViCBC

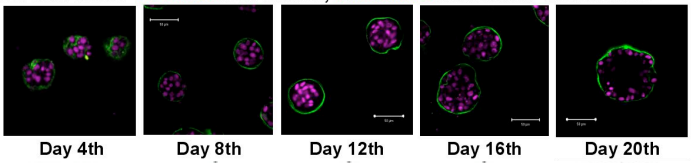


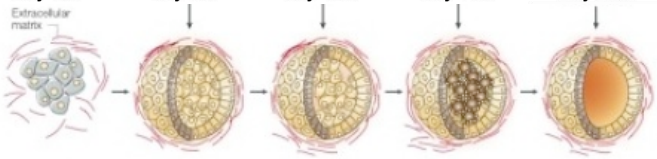
Nicole Bryce, Weaver lab, ViCBC



MCF10A acini: TOPRO3:cell nuclei; Laminin 5:basement membrane

Emily Wang
ViCBC

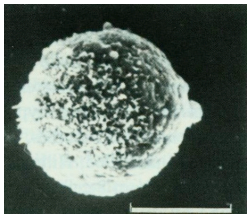
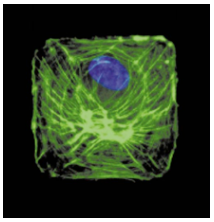
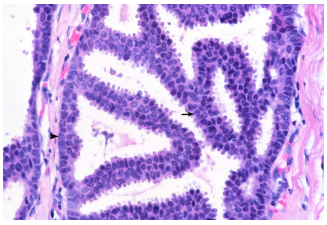
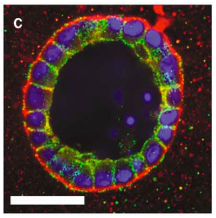




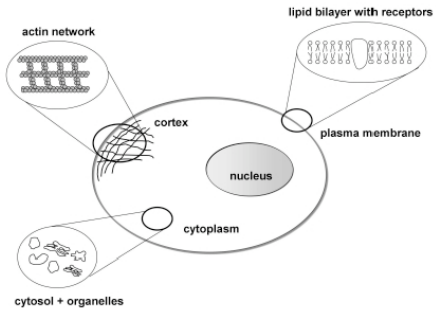
Debnath & Brugge,
Nat Rev Cancer 2005

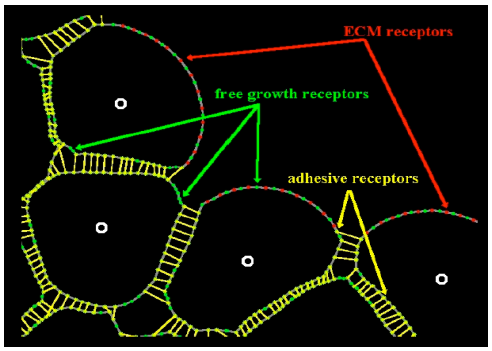
ViCBC

Cell plasticity

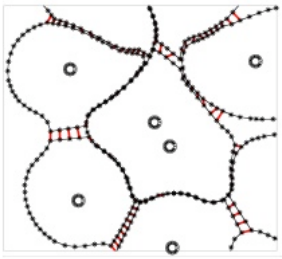





IBCell model

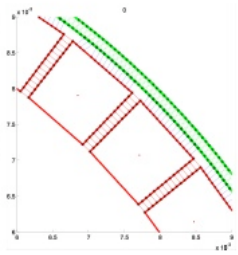


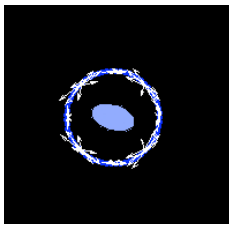


Mechanical forces exerted by the cell on its microenvironment

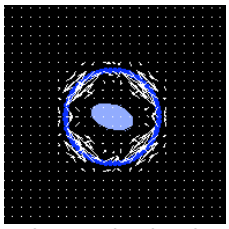


The cell elastic boundary, the cell contractile ring and cell-cell adhesion links are all defined as short linear springs acting on cell boundaries and then transmitted to the surrounding microenvironment.

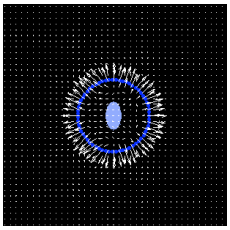




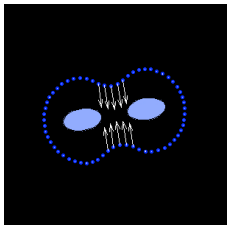
elastic forces acting in a resting cell



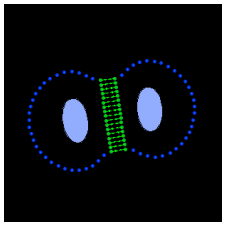
and transmitted to the cell microenvironment



forces exerted by a growing cell



contractile forces in cell division

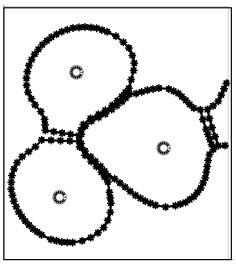


adhesive forces between neighboring cells

Immersed Boundary Method

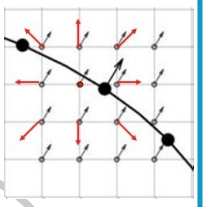
The coupled mechanical system in the immersed boundary method consists of two different structures - a collection of **elastic neutrally buoyant cells** (Lagrangean formulation) and a **viscous, incompressible fluid** of the same constant viscosity and density inside and outside the cells (Eulerian formulation).

[Developed by Ch. Peskin, 1972] [Formulation by Dillon & Othmer 1999]



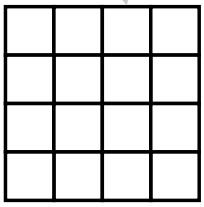
$$f(x,t) = \int_{\Gamma} F(l,t)\delta(x - X(l,t))dl$$

Elastic forces: boundary, adherent, contractile



$$s(x,t) = \sum_{k \in \Xi} S_k(t)\delta(x - Y_k(t))$$

Fluid sources used to model cell growth and death



$$\frac{\partial X}{\partial t} = \int_{\Omega} u(x,t)\delta(x - X(l,t))dx$$

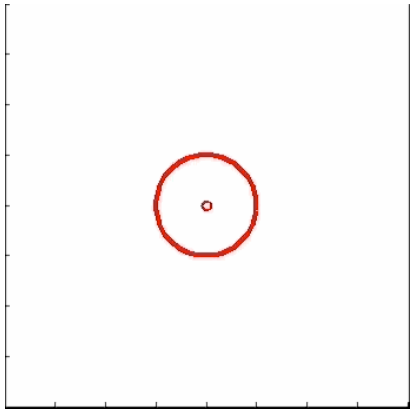
$$\rho\left(\frac{\partial u}{\partial t} + (u \bullet \nabla)\, u\right) = -\nabla p + \mu\, \Delta u + \frac{\mu}{3\rho}\nabla s + f$$

$$\rho\, \nabla \bullet u = s$$

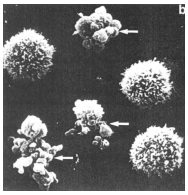
p- pressure, μ- viscosity, ρ-density

$$\rho \int_{\Omega} (\nabla \bullet u)dx = 0$$

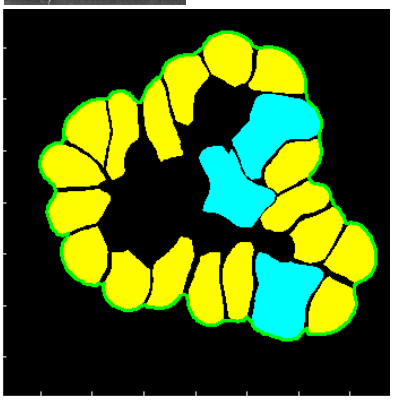
Model of cell growth, division and apoptosis



Transport of the fluid across the cell boundary during cell growth (from outside in) is modeled by placing fluid sources and sinks along the cell membrane intside and outside the cell, respectively



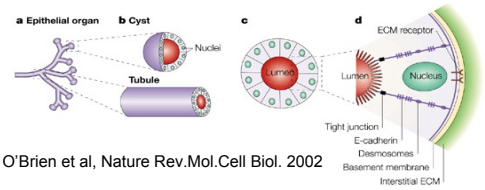
Ardens & Wyllie, Inter.Rev.Exp. Path 1991



Transport of the fluid across the cell boundary during its apoptotic death (from inside out) is modeled by placing fluid sources and sinks along the cell membrane outside and inside the cell, respectively

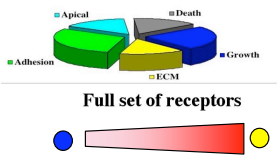
Rejniak JTB 2007, Rejniak & Anderson BMB 2008a,b

Model of cell epithelial polarization and cell receptor dynamics

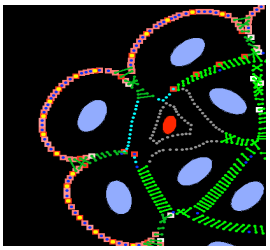


O'Brien et al, Nature Rev.Mol.Cell Biol. 2002

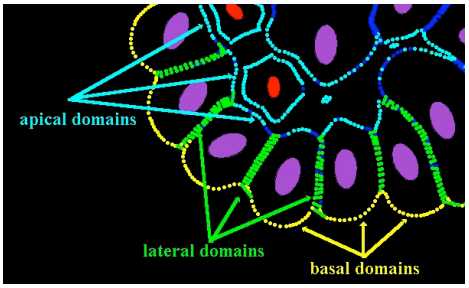
Cell receptors signature



Full set of receptors



Three membrane domains in a polarized cell



- purple—cell nuclei
- blue—cell boundaries
- red—apoptotic cells
- yellow—ECM sensors
- green—lateral sensors
- cyan—apical sensors

Rejniak & Anderson BMB 2008a,b

Receptors:

green: cell-cell – become active if two receptors from distinct cells are close enough

yellow: ECM – become active if the expression of laminin-332 around exceeds the threshold

cyan: apical markers– emerge during the development of cell apical membrane domain

grey: death – emerge as a result of detachment from polarised and other dying cells

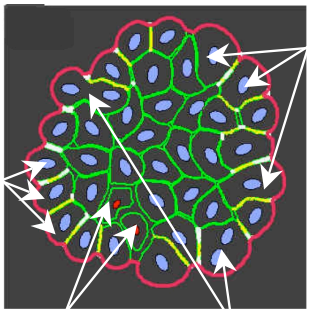
blue: free growth – all remaining receptors are free from any contacts and become growth receptors

In silico model of a single eukaryotic cell (*IBCell* model)

Subcellular elements	Molecular complexes	Cellular processes	Extracellular elements
Nucleus	Contractile ring	Proliferation	ECM/Matrigel
Cytoplasm	Adherens junctions	Division	
Plasma membrane	Membrane receptors	Apoptosis	
(Cytoskeleton)		Polarisation	
		Communication	

Assumptions about cell life processes:

- Only **mechanical aspects** of cell processes are modeled, the underlying biochemical reactions or gene expressions are not included explicitly
- All cell life processes are driven by **membrane sensors** configuration in a host cell
- All **interactions are local** and are driven by cues sensed from other cells and from cell local microenvironment



Cell staining:

- cell nuclei
- apoptotic cells
- ECM sensors
- cell-cell sensors
- cell boundaries

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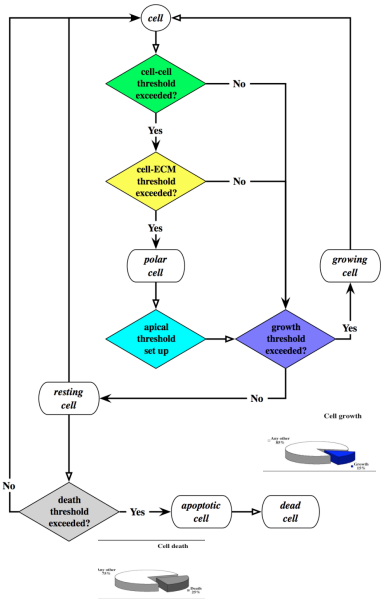
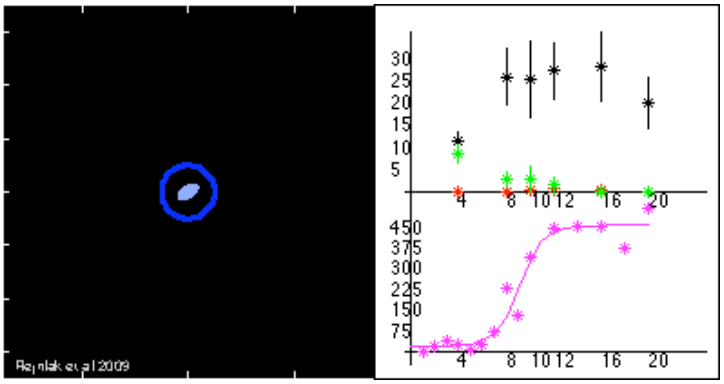
Cell self-organization into an acinar structure





Nucleus:
blue:viable cells;
red:apoptotic cells;

pink/red: LN332 intensity

Receptors:
blue:growth;
yellow: basal (ECM);
green: cell-cell & lateral;
cyan: apical;
grey: death;

CBC

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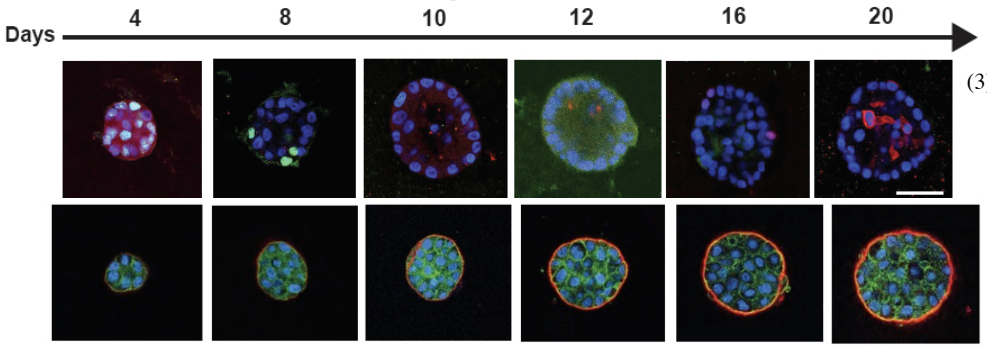
MCF10A experimental data

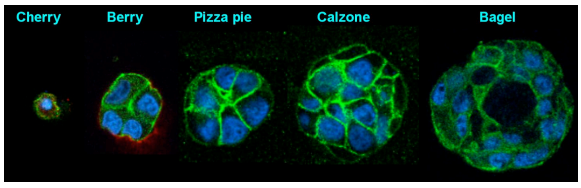


(3)

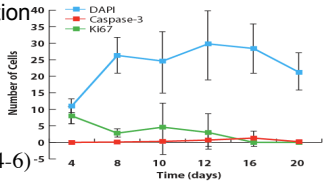
(7)

Experimental data:

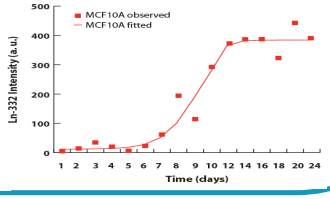
1. Acinar structure
2. Acinar area & perimeter
3. Spatial distributions of cell processes
4. Counts of viable cells
5. Counts of prolifrating cells
6. Counts of apoptotic cells
7. Laminin-332 spatial distribution
8. Laminin-332 intensities



(1)



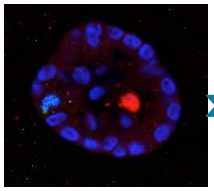
(4-6)



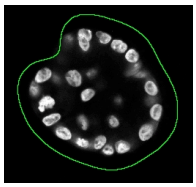
(8)

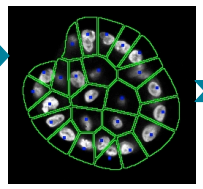
CBC

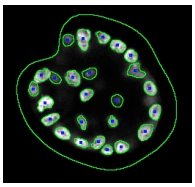
Quantification of experimental data: cell staining and counts using BioSig (LBNL)



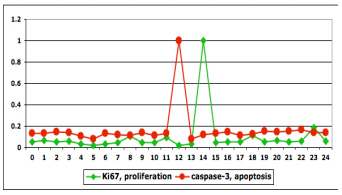
Experimental acini images stained for Ki67(green) and Caspase-3 (red)

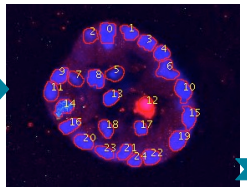


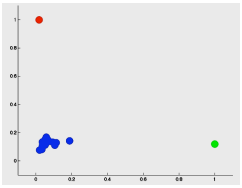




acini segmentation BioSig (LBNL)

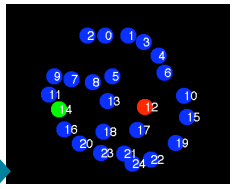


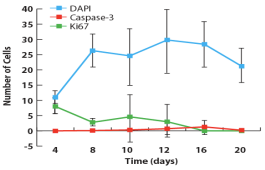




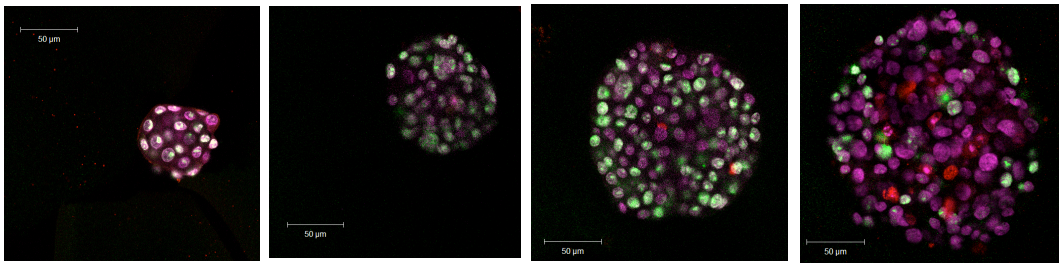
fluorescence intensity profiles, BioSig (LBNL), cluster analysis

Colony reconstruction and average cell counts determination



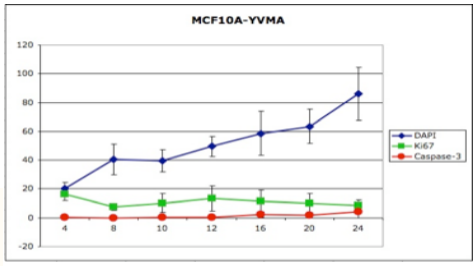


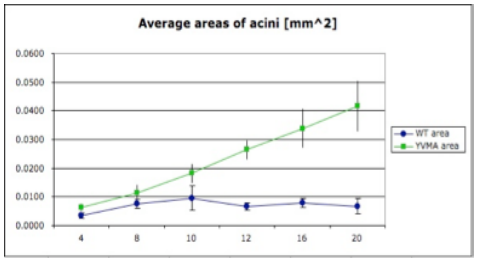
MCF10A/Her2^{YVMA} mutant



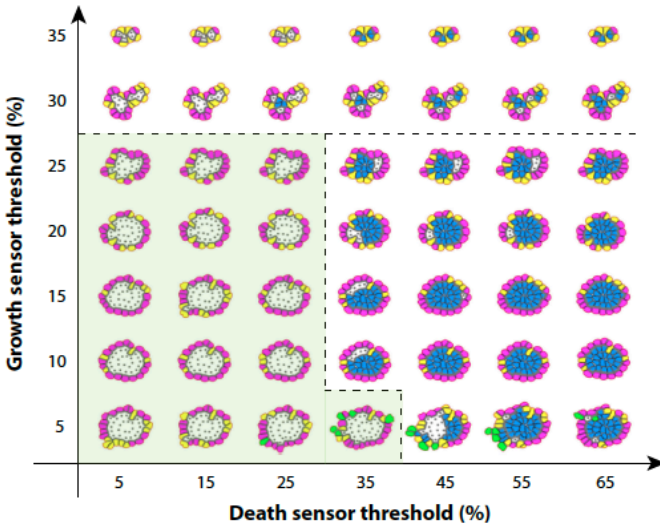
E. Wang, ViCBC

- HER2/Neu (ErbB2)-a member of the ErbB family of transmembrane receptor tyrosine kinases;
- amplification of HER2 gene found in 20% of metastatic breast cancers (poor patient outcome);
- a retroviral vector contining a G776^{YVMA} insertion in exon 20 was stably expressed in MCF10A;
- expression of HER2^{YVMA} mutant is more potent than wild-type HER2;
- its activation upregulates cell proliferation and downregulates cell apoptosis;





IBCell morpho-charts



Morpho-charts:

Show a collection of morphologies at various ranges of sensor thresholds, ie. for cell proliferation (growth sensors) and cell death (death sensors).

Allow for test how changes in model parameters are reflected in the final model outcome

green: growing;
blue: resting inner;
yellow: resting outer;
purple: polarised;

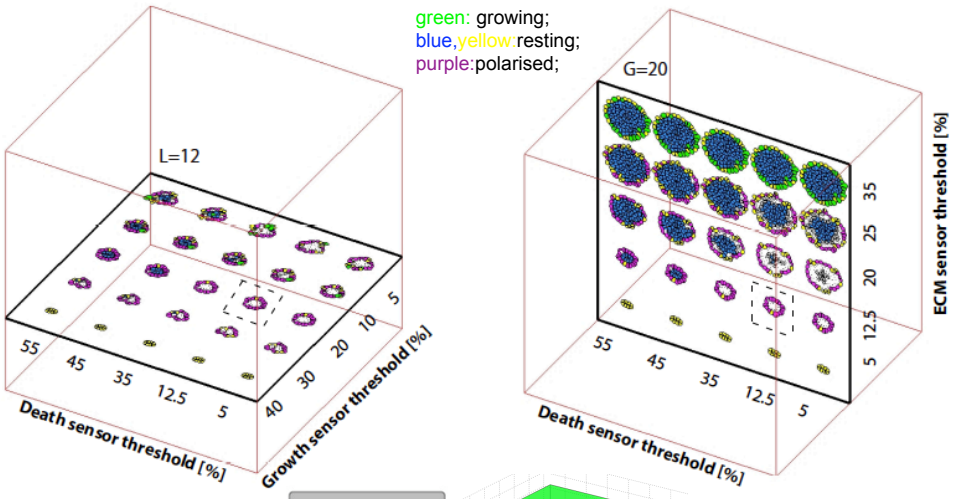
Thresholds:

By **increasing** the death threshold cells need to accumulate **more** death sensors to trigger their death process, so they become **more resistant** to death

By **decreasing** the growth threshold cells need **less** growth sensors to continue the process of cell proliferation, so they become **less sensitive** to contact inhibition

Diagram of acinar morphologies: growth vs. death vs. ECM receptors

green: growing;
blue,yellow: resting;
purple: polarised;



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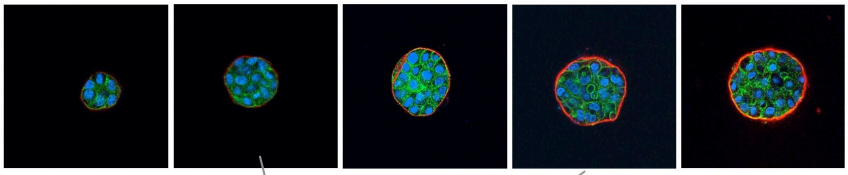
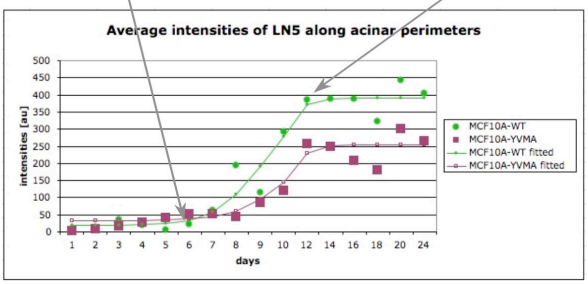
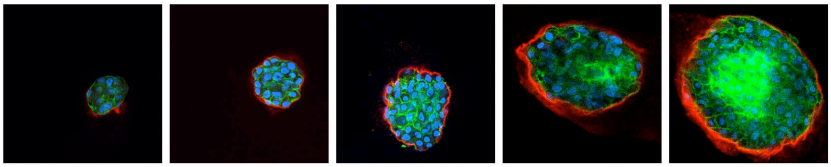
Model driven experiment:

Laminin 332 expression in MCF10A and MCF10A/HER2^{YVMA}





Data collection and staining:
E. Wang (ViCBC)



Intensity of Ln-332 (red staining) accumulated around the acinar cluster estimated using the MetaMorph program; Jerome Jourquin (ViCBC)

ViCBC

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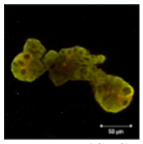
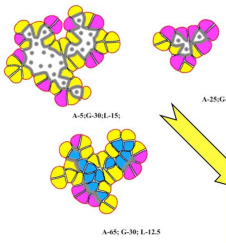
Acinar morphology mapping on the model parameter space

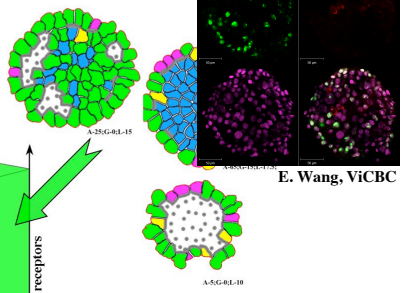
Yellow: stabilised, degenerate



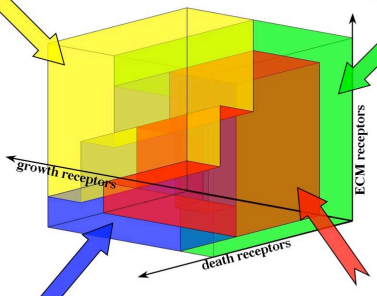
N. Bryce, ViCBC



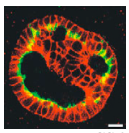
Green: non-stabilised, g



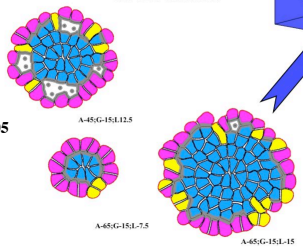
E. Wang, ViCBC



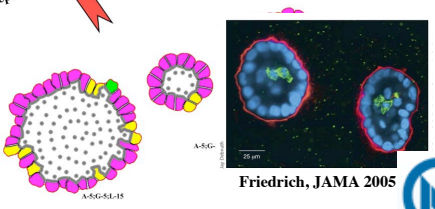
Blue: stabilised with partial or no lumen



Latorre et.al JCS 2005



Red: stabilised hollow acini



Friedrich, JAMA 2005

ViCBC

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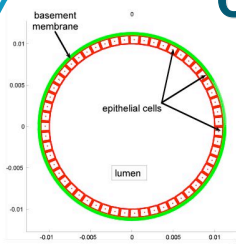
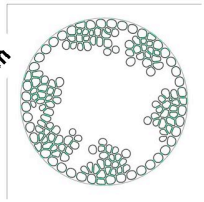
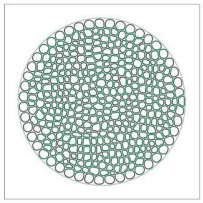
Computer simulations of DCIS

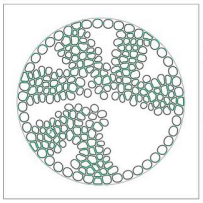
Joint work with R.H. Dillon



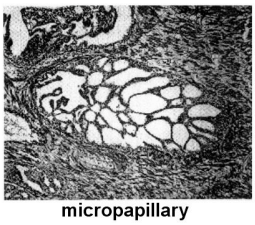
Tumour initiation



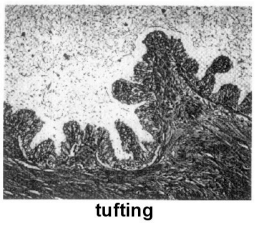




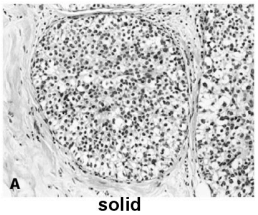
RHD's model



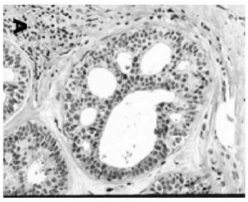
micropapillary



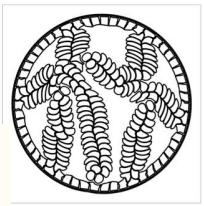
tufting

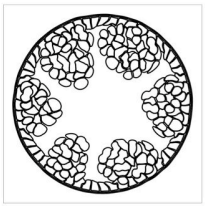


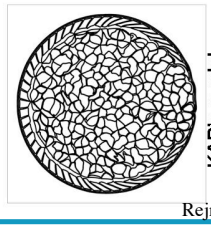
solid



cribriform







KAR's model

Rejniak&Dillon, CMMM 2007

CBC

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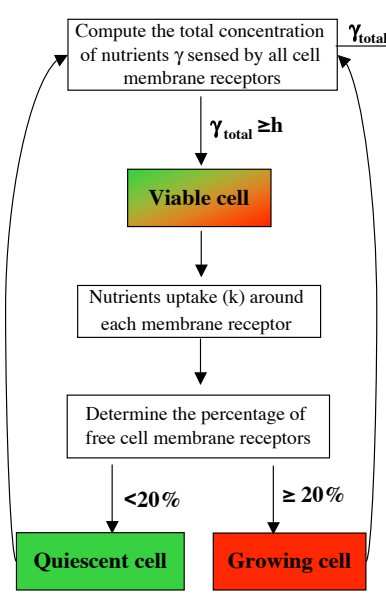
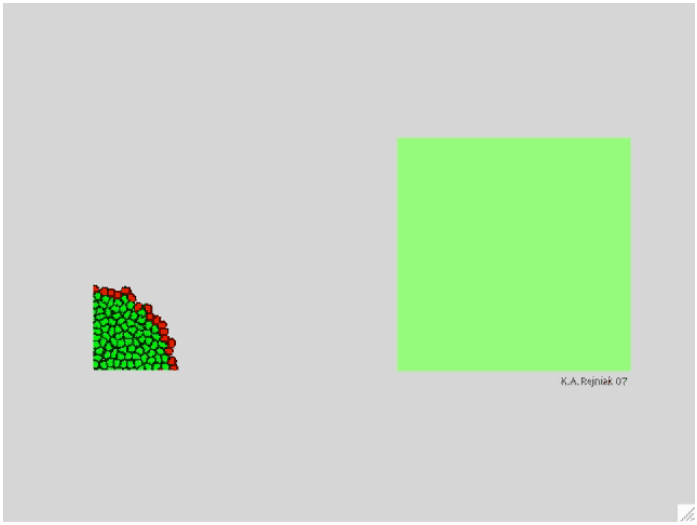
Cell competition for resources - a life cycle



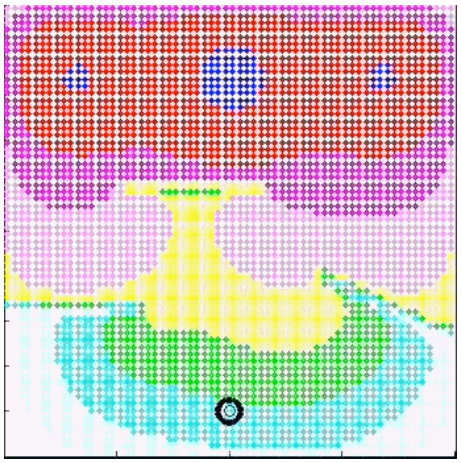


Anderson, Rejniak, Gerlee, Quaranta, JMB, 2009

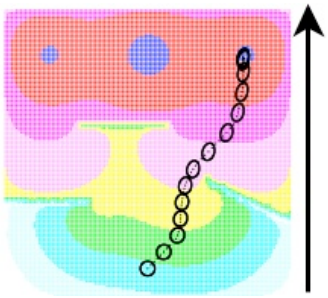
CBC

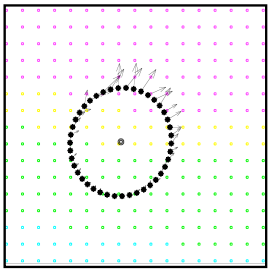
Cell movement driven by hapto-gradient

Directed cell movement of a single cell toward a static gradient of a haptoattractant



Haptotactic movement of the cell is modeled by introducing linear drag motility forces F^{chem} defined at each point on the cell boundary in the direction of increasing gradient of γ:

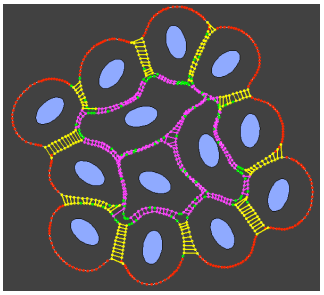




The haptotactic drag forces depend on local gradient of proteinase sensed from the cell microenvironment

Conclusions

- Main modeling object: an eukaryotic cell (epithelial)
- Main cell processes (behaviors): deformable shape, growth, division, death, epithelial polarization, cell-cell adhesion, cell-ECM adhesion, movement
- Cell mechanism of action: cell membrane receptors (sensing the microenvironment, reacting on changes in the microenvironment, modifying the microenvironment)
- Microenvironmental elements: nutrients, hapto- and chemotactic gradients, ECM proteins, intraductal fluid lumen, extracellular gel (matrigel)
- Tissue scale outcome: formation of epithelial acini, acinar mutants, DCIS, invasive tumors



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Conclusions

- *IBCell* bio-mechanical model simulates a **self-organizing** acinar structure (from a single cell to a shell of epithelial cells enclosing a hollow lumen) based **entirely** on local cell-cell interactions
- *IBCell* can be **parameterized** using empirical observations and can **qualitativily** and **quantitativily** match experimental data
- *IBCell* has been used to investigate **intrinsic** (sensitivity to growth/death signals) and **extrinsic** (ECM accumulation) **mechanisms** leading to normal acini formation (*MCF10A*) vs. tumor-like (*MCF10A-HER2^{YVMA}*)
- *IBCell* showed that to stabilize an acinar structure an **inhibition mechanism** is necessary; we hypothesized that ECM produced by cells acts as an inhibitory mechanism on proliferation via a negative feedback loop; the preliminary data show that Ln-332 may play such a role since its accumulation is disregulated in *MCF10A-HER2^{YVMA}* when compared to wild type *MCF10A*
- *IBCell* multidimensional Morpho-Charts can be produced by varying thresholds for different cell processes, and then used to **map** final normal and mutant **morphologies** to the set of **quantitative thresholds** to obtain novel **cues on the molecular basis** underlying the pathogenesis that can be further **investigated experimentaly**.

VICBC

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IMO Integrated Mathematical Oncology

Acknowledgment

Modeling
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Jerome Jourquin, Vanderbilt University
Emily Shizen Wang, Vanderbilt University
Nicole Bryce, Vanderbilt University

Image Processing
Bahram Parvin, LBNL
Hang Chang, LBNL

VICBC