Phenotypic transition maps of 3D breast acini obtained by imaging-guided agent-based modeling

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3D Agent-Based Model of Mammary Acini Morphogenesis

Tang et al., Integr Biol 2011
Our Multiscale challenge

**NSCOR – Mammary gland**

Specialized Center of Research (NYU-LBNL)

**ICBP – 3D co-cultures**

St Elisabeth’s Medical Center (TUFTS-UCB)

**PSOC - Reversion**

Bay Area PSOC (UCB-LBNL)
PSOC Project 3: Modeling reversion

• **Concept:**
  - Integrate 3D image analysis with modeling
  - Create the tools to test *in silico* the key factors influencing reversion
  - Scalable platform

• **STEP1:** Develop 3D model of acinus formation and validate it
  - Use of MCF10A cellular model

  __Previous approaches:__
  - Rejniak et al\(^2\), Grant et al\(^3\): 2D agent-based model of acinus formation
  - This is a 3D problem, needs to be treated as such

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Three axis of homeostasis

- PROLIFERATION
- POLARIZATION
- SURVIVAL
The model

Jonathan Tang
Phenotypic maps – Polarization ON

A

Cell Count

Proliferation Potential

Apoptosis Efficiency (i)

C

% Growth Arrest

Proliferation Potential

Apoptosis Efficiency (ii)

F

Acini Volume

Proliferation Potential

Apoptosis Efficiency (i)

H

Sphericity Index

Proliferation Potential

Apoptosis Efficiency (ii)
Phenotypic maps – Polarization OFF

A: Cell Count
B: % Growth Arrest
C: Apoptosis Efficiency (l)
D: Proliferation Potential
E: (0.3, 0.9), (0.9, 0.9), (0.3, 0.4), (0.9, 0.4)
F: Acini Volume
G: Apoptosis Efficiency (l)
H: Sphericity Index
Findings summary 1

• Apoptosis is required for proper lumen formation
• Percentage of proliferating cells dictates acinar growth arrest
• Polarization is required for spherical structures and reduced acinar proliferation
Imaging workflow

Automatic acini segmentation + Human validation

Imaging Properties for each acinus:
- Volume
- Number of nuclei ($num_nuc$)
- Sphericity
- Percent proliferation (pp)
- Cell density
3D nuclear count

\[ \text{num}_{\text{nuc}} = \frac{\sum_{i,j,k \in \text{nuc\_mask}} I_{\text{nuc}}(i,j,k)}{\sum_{i,j,k \in \text{isolated nucleus}} I_{\text{nuc}}(i,j,k)} \]

Manual count = 19

\[ \text{num\_nuc} = 17.9 \]

Isodata thresholding

\[ y = 0.9704x \quad R^2 = 0.9307 \]

**Other measurements**

<table>
<thead>
<tr>
<th>DAPI</th>
<th>α6</th>
<th>Ki67</th>
<th>DAPI/α6/Ki67</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>

- **num_nuc** = 17.9
- **sphericity** = 1.21
- **%pos** = 9.2%
- **density** = 0.8

**Isodata thresholding**
- ![Image]
- ![Image]

**Manual thresholding**
- ![Image]
- ![Image]

**Binary overlay**
- ![Image]
- ![Image]
Locating normal Phenotype on maps

- Orange: 74.1 < Average Cell Count < 96.2
- Purple: 93.9 < Average Acini Volume < 135.4
- Green: 74.1 < Average Cell Count < 96.2 AND 93.9 < Average Acini Volume < 135.4
- Yellow: 1.20 < Average Sphericity Index < 1.34
- Pink: 0.05 < % Growth Arrest
Modeling normal phenotype
Simulations – 3D rendering

$\theta = 0.9$, $P_p = 0.4$

Polarization OFF

Polarization ON
New imaging metrics for lumen

**A**
- \( \beta = 1 \) epithelial layer
- Density = 88%
- \( \beta = 2 \) epithelial layers

\[
\text{density} = \left( \frac{d}{R} \right)^3
\]

**B**
- Acinar volume (V)
- Density vs. Acinar volume (V)
- Experimental data
- Average simulation, \( \theta = 0.9 \), Pp = 0.4
- Average simulation, \( \theta = 0.9 \), Pp = 0.9
- Average simulation, \( \theta = 0.3 \), Pp = 0.4
- Fit, \( \beta = 1.9 \)
- Fit, \( \beta = 1.45 \)
- Fit, \( \beta = 1.05 \)

**C**
- Acinar volume (V)
- Density vs. Acinar volume (V)
- Experimental data
- Average simulation, \( \theta = 0.9 \), Pp = 0.4

**D**
- Average \( \beta \)
- Pp vs. \( \theta \)
Impact of polarization on lumen formation

Impact of polarization on lumen formation.

Beta (without polarization)

With Polarization

A. Pp = 0.3, μ = 0
Acinar Density = 0.96 ± 0.6

B. Pp = 0.9, μ = 0
Acinar Density = 0.99 ± 0.13

Without Polarization

C. Acinar Density = 1.0 ± 0.0

D. Acinar Density = 1.0 ± 0.0
Findings summary 2

• Apoptosis is required for proper lumen formation
• Percentage of proliferating cells dictates acinar growth arrest
• Polarization is required for spherical structure and reduced acinar proliferation
  • Apoptosis is necessary and sufficient to form a lumen, but polarization enhances lumen formation
• Polarization is necessary to model normal phenotype
Locating DCIS on phenotypic maps

**Graphs and Images:**

- **Graph A:**
  - X-axis: Days (0, 3, 6, 9, 12)
  - Y-axis: # Cells per Acinus

- **Graph B:**
  - X-axis: Days (0, 3, 6, 9, 12)
  - Y-axis: Acinus Volume (Cell Units)

- **Graph C:**
  - X-axis: Days (0, 3, 6, 9, 12)
  - Y-axis: Sphericity Index

- **Images:**
  - Day 1-2
  - Day 3
  - Day 7
  - Day 12

- Scale: 10 μm
Conclusions

- Apoptosis is required for proper lumen formation
- Percentage of proliferating cells dictates acinar growth arrest
- Polarization is required for spherical structure and reduced acinar proliferation
- Apoptosis is necessary and sufficient to form a lumen, but polarization enhances lumen formation
- Polarization is necessary to model normal phenotype
- Model is incomplete. DCIS model suggests the need to add basement membrane/cell interaction
  - Proliferation levels are not mechanistically driven, need to modify code to reflect the impact of basement membrane on proliferation
  - ODE approach to add signaling to the model