

Signaling Hierarchy

Mammary Epithelial Cells

Part I & II

Mammary Epithelial Cell

Signaling Hierarchy

- Stages operational during pregnancy (just before the onset of lactation to completion of lactation)
- Epithelial cells - ECM are the tissue level players
- Construction and destruction of steps with various 'go' checkpoints to the next step

Signaling Hierarchy

- Flow of information between cells and tissues are integrated into a signaling hierarchy that is :
a) constructed and then b) dismantled in a cyclical manner
- First tier of hierarchy involves mechanical signals : cell rounding that trigger lactoferrin gene expression
- Rounded cells deposit ECM and initiate a laminin mediated hierarchy leading to biochemical signal transduction and activation of a wide range of genes

Signaling Hierarchy

- The third tier of hierarchy signaling relies on the ECM morphogenesis, wherein presence of ECM directs cell polarity, formation of central lumen and expression of WAP.
- WAP is expressed late in pregnancy and just before the onset of lactation.
- Dismantling of this hierarchy begins at weaning is mediated by ECM-degrading enzymes, which act in a development stage manner to induce programmed cell death.

Architecture

- Composition of ECM is important: e.g. myoblast proliferate (Fn) or form tubes (ln)
- Decreased adhesion to rigid substratum: *mechanical in nature*
- Increased cell rounding
- Reorganization of cytoskeleton (markers)

Laminin Signaling

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Please see:

Figure 6 in Gabriela Bezakova, Markus A. Ruegg.
"New insights into the roles of agrin."
Nature Reviews Molecular Cell Biology 4,
295 - 309 (01 Apr 2003).

- Integrin link very important signaling: inside-out and outside-in
- Laminin-specific integrin clustering and activation - laminin based cytoskeleton reorganization

Integrin Family & Signaling

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Please see:

Figures 1 and 2 in Richard O. Hynes.

"Integrins: Bidirectional, Allosteric Signaling Machines."

Cell 2002 110: 673-687.

Integrins

Mammary Gland Development

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Integrin chains

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Integrin Signaling I

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Please see:

Figures 4, 5a, and 7 in Richard O. Hynes.

"Integrins: Bidirectional, Allosteric Signaling Machines."

Cell 2002 110: 673-687.

BE 440. Analysis of Biological Networks

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Integrin Signaling

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Please see:
Figure 1a in Cindy K. Miranti, Joan S. Brugge.
"Sensing the environment: a historical perspective
on integrin signal transduction."
Nature Cell Biology 4, E83 - E90 (01 Apr 2002).

Vinculin

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Adhesion Complex

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Actin/Vinculin Complex

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Cytoskeleton

- Microfilaments [actin monomers]
- Microtubules [α and β - tubulin]
- Intermediate filaments [various different types of monomers]

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Properties of the actin subunit

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Actin Polymerization

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Actin/Vinculin Complex

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Cytoskeleton & Integrin signaling

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Please see:

Figure 1b in Cindy K. Miranti, Joan S. Brugge.

"Sensing the environment: a historical perspective on integrin signal transduction." *Nature Cell Biology* 4, E83 - E90 (01 Apr 2002).

Cell-ECM contact

- Cell shape is a set point for proliferation *versus* differentiation
- Integrin signaling - Cross talk to make sure that differentiation signaling is different from proliferation
- Cessation of proliferation - exit cell cycle
- Decreased AP1 transcription factor activity

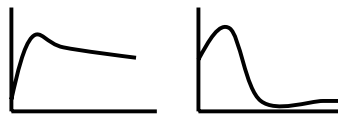
Integrin Signaling Diversity

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Please see:

Figure 3 in Cindy K. Miranti, Joan S. Brugge.
"Sensing the environment: a historical perspective
on integrin signal transduction." *Nature Cell Biology*
4, E83 - E90 (01 Apr 2002).

Integrin Signaling II

- Cross talk to make sure that differentiation signaling is different from proliferation
- Modulation of insulin signal transduction pathway MAP kinase pathway
- coupled with growth factor signaling: kinetic activation of transcription factors is modulated



Integrin Coupled with growth factor Signaling: Cell Cycle *in or out*

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Please see:

Figure 2 in Cindy K. Miranti, Joan S. Brugge. "Sensing the environment: a historical perspective on integrin signal transduction." *Nature Cell Biology* 4, E83 - E90 (01 Apr 2002).

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Receptor Mediated Signaling

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Growth Factor Signaling

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Substrate Mediated Signaling

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IGF *signaling*

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IGF

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IGF

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Growth Factor Signaling

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Hormone Binding

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Hormone Binding

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Differentiation Specific Effects

- STAT-5 interacts with activated prolactin receptor: gets P, and becomes a transcription factor
- BCE-1 contains STAT-5 binding sites
- beta-casein expression is on

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STAT pathway

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Please see:

Figure 1 and Box 1 in David E. Levy, J. E. Darnell.
"STATs: transcriptional control and biological impact."
Nature Reviews Molecular Cell Biology 3,
651 - 662 (01 Sep 2002).

STATs

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Please see:

Figure 3 in David E. Levy, J. E. Darnell.
"STATs: transcriptional control and biological impact."
Nature Reviews Molecular Cell Biology 3,
651 - 662 (01 Sep 2002).

Laminin Signaling

- Differentiation specific elements are activated (*BEC-1 which contains C/EBP binding: ECM responsive elements*)
- Right ECM for the proper loading of the transcription factors: appropriate histone organization
- BEC-1 leads to prolactin based activation of STAT-5 leading to beta-casein expression

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Diverse signaling pathways activated by growth factor receptors induce broadly overlapping, rather than independent, sets of genes.

Micro array

*Prolactin putative target
genes*

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Ready for Third tier

- Reciprocal cell-mediated changes in ECM composition
- *rigid substratum results in flattening, de-differentiation and beta-casein production off*
- The next signal is for the cells to migrate and this requires a change in the FAK based signaling as well as ECM-integrin-cell interactions
- Cell spreading on ECM - actin stress fibers

Migration

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Steps in migration *polarization*

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G-protein coupled receptor activation

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GPCRs

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GPCR -oligomerization

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Integrins

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Steps in migration *Protrusion and Adhesion Formation*

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Steps in migration *Rear Retraction*

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Steps in migration

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Migrating cells

- Entire complex
- FA and migration
- Migration

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Please see:

Figure 1 in J. Victor Small, Benjamin Geiger, Irina Kaverina, Alexander Bershadsky.
"How do microtubules guide migrating cells?"
Nature Reviews Molecular Cell Biology 3,
957 - 964 (01 Dec 2002).

alpha5 integrin clustering, diffusion and interactions in the cell

- Image Correlation Microscopy (ICM) allows the detection of submicroscopic alpha5 integrin clusters with 3-4 proteins.
- Two color ICM shows that alpha5 integrin and alpha-actinin localize and move together even in regions of the cell with no discernable adhesions.
- A temporal ICM analysis reveals heterogeneity in both alpha5 integrin and alpha-actinin dynamics across the cell with the proteins being more dynamic in regions of the cell that are ruffling and protruding.
- When adhesions disassemble three proteins have very different fates:
 - alpha5 integrin diffuses away slowly,
 - alpha-actinin moves away with a directed motion and
 - paxillin diffuses away rapidly into the cytosol.

Migration, proliferation & differentiation

- Growth factors in the ECM become key for sending proliferative signals: FGF
- Polarization of the cells leads to self-assembly and the formation of alveoli like structures: morphogenesis (HGF)
- Production and deposition of new ECM
- Down regulation of TGF- β

Glycosaminoglycans

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Core: ECM CS

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Core: Cell Surface HSGAG

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ECM and Growth Factor

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FGF-FGFR complex

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Conformation of H

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FGF-FGFR-H mediated signaling

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Tube formation

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HGF

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TGF-beta

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Please see:

Figures 1 and 4 in Joan Massague.

"How cells read TGF-beta signals."

Nature Reviews Molecular Cell Biology 1,

169 - 178 (01 Dec 2000).

Morphogenesis

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Heparanase over expression

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Destruction & Involution

- Inhibition of milk protein expression
- Increased Matrix Metalloprotease production
- Decreased production of MM inhibitors
- Basement membrane destruction and Enactin fragmentation and increase tenascin production
- Loss of cell function
- ICE dependent apoptosis

MMPs

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MMP gene regulation

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Please see:

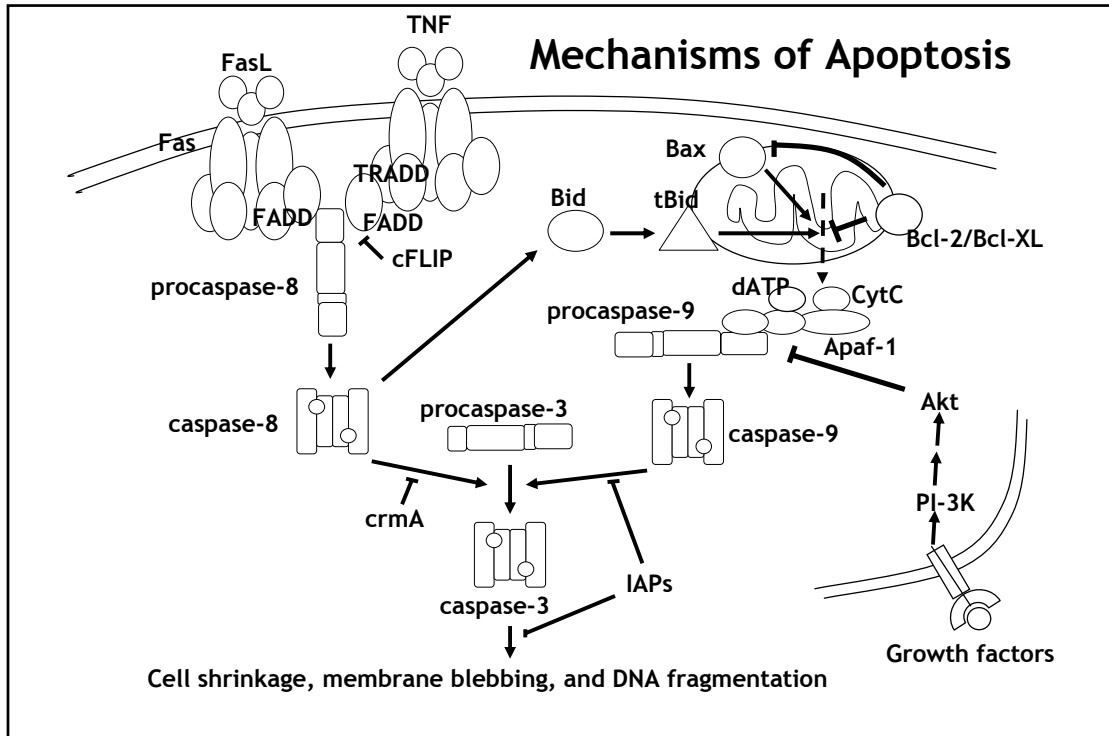
Figure 3 in Christopher Mark Overall,
Carlos Lopez-Otin. "Strategies for mmp inhibition
in cancer: innovations for the post-trial era."
Nature Reviews Cancer 2, 657 - 672 (01 Sep 2002).

MMP regulation

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Please see:

Figure 4 in Christopher Mark Overall, Carlos Lopez-Otin.
"Strategies for mmp inhibition in cancer: innovations
for the post-trial era." *Nature Reviews Cancer* 2,
657 - 672 (01 Sep 2002).

BE 440. Analysis of Biological Networks



Key Points: I

- Flow of information between cells and tissues are integrated into a signaling hierarchy that is :
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- First tier of hierarchy involves mechanical signals :
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Key Points: II

- The third tier of hierarchy signaling relies on the ECM morphogenesis, wherein presence of ECM directs cell polarity, formation of central lumen and expression of WAP.

WAP is expressed late in pregnancy and just before the onset of lactation.

- Fourth tier: Dismantling of this hierarchy begins at weaning is mediated by ECM-degrading enzymes, which act in a development stage manner to induce programmed cell death.

Summary

- Signaling hierarchy emerges as a universal integrator of function for a given physiology
- Fundamental cellular processes modulated by biochemical signals- cycles of growth, differentiation, morphogenesis and apoptosis
- Molecular (biochemical, mechanical, physical interactions) - cellular - tissue - organ system