Biology of Precancer

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• no relevant financial relationships with commercial interests to disclose
Hundred of Abnormalities in the Human Breast

Wellings-Jensen Model (JNCI 55:231, 1975)

Evidence Supporting Model:
- **Histological continuity**
- **More common in breasts with IBC**
- **Risk factors for developing IBC**
- **Shared genetic alterations with IBC**
- **Similarities with in vivo models.**
Evidence Supporting Wellings-Jensen Model

Histological Continuity

TDLU  CCH  ADH  DCIS  DCIS+IBC

TDLU  ALH  LCIS  LCIS+ILC

Time
Evidence Supporting Wellings-Jensen Model

More common in cancerous breasts.

<table>
<thead>
<tr>
<th>Incidence in Breasts Without Invasive Cancer</th>
<th>Incidence in Breasts With Invasive Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH and ALH:</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>DCIS and LCIS:</td>
<td>5-10%</td>
</tr>
<tr>
<td></td>
<td>&gt;50%</td>
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<tr>
<td></td>
<td>&gt;90%</td>
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</table>

From 24 autopsy/mastectomy studies involving 5,300 patients since 1930.
Evidence Supporting Wellings-Jensen Model

Escalating risk factors for developing IBC.

<table>
<thead>
<tr>
<th>RR of IBC after Bx Alone</th>
<th>CCH</th>
<th>ADH</th>
<th>DCIS</th>
<th>ALH</th>
<th>LCIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;2X</td>
<td>5X</td>
<td>10X</td>
<td>6X</td>
<td>12X</td>
</tr>
</tbody>
</table>

From 30 studies involving > 120,000 patients since 1960.
Assigned risk to specific lesions.
Defined histological criteria for diagnosing them.

Enigma: ADH, ALH, LCIS ⇒ Bilateral Risk.

Risk factors and precursors if distribution is multifocal and bilateral...and it is.

Only Risk Factors?
The true *in situ* risk of IBC associated with ADH/DCIS and ALH/LCIS is probably higher than we think...

...nearly all pathological/epidemiological studies establishing risk were based on excised lesions ⇒ many patients cured.
# Evidence Supporting Wellings-Jensen Model

## Shared Genetic Defects with Invasive Breast Cancer

<table>
<thead>
<tr>
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<th>With $\geq 1$ Mutation</th>
<th>Share $\geq 1$ Mutation with IBC same breast</th>
</tr>
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<tbody>
<tr>
<td>ADH/ALH:</td>
<td>40-50%</td>
<td>&gt;60%</td>
</tr>
<tr>
<td>DCIS/LCIS:</td>
<td>80-90%</td>
<td>&gt;90%</td>
</tr>
</tbody>
</table>

From over 30 studies assessing allelic imbalance (LOH and CGH) at more than 100 loci on 17 chromosomes.
Columnar Cell Hyperplasia (CCH)

*Increased Growth*

- ↑ Estrogen receptor
- ↑ Proliferation and ↓ Apoptosis
- Suppression adult differentiation (e.g. ↓ EGF)
- Re-activation embryonic pathways (e.g. ↑ AREG)
- Few genetic mutations
Practical Issue: The Clinical Significance of “Atypia” in CCH

Worrisome:
Some studies show up to 20% CCHA on CNBX associated with cancer on FU excision...significantly higher than CCLs without A.

Modern Pathol 20:30A,
Human Pathol 38:35, 2007
Histopathology 52:11, 2008

Confusing:
Some studies do not...
and some show the opposite (i.e. cancer rates CCLs > CCHA).

Sem Diag Pathol 11:223, 1994
J Clin Oncol 19:2263, 2001
Am J Pathol 160:597, 2002
Semin Diag Pathol
Practical Issue:  
The Clinical Significance of “Atypia” in CCH

Cytological “atypia” is difficult to define:

Moderate Atypia

Typical CCH in non cancerous breast.

Minimal Atypia

There is poor reproducibility between pathologists.

My Opinion: Some CCH probably represent relatively high risk lesions, but histological features alone may not be sufficient to identify them.
CCHs → ADH: 
*Increased Growth + Alterations in Adhesion/Polarity*

↑ Estrogen receptor
↑ Proliferation and ↓ Apoptosis
Suppression adult differentiation (e.g. ↓ EGF)
Re-activation embryonic pathways (e.g. ↑ AREG)
Few genetic mutations
20% ADH on CNBX = DCIS and/or IBC on FU EXB

Treatments:
- Nothing
- Local excision (clear margins)
- Mastectomy (only cure except good luck)
- Hormonal therapy (↓ risk of IBC by 80-90%)
ADH and DCIS are “Same” Disease

- **Features of ADH**: <2 mm and/or <2 spaces
- **Distinction**: artificial and controversial... based on extent and amount.
- **DCIS**: Excision with Clear Margins + Adjuvant Radiation

A little bit..........................................................................................................................more.
ALH

Increased Growth + Changes Adhesion/Polarity

↑ Estrogen and progesterone receptors
Loss of e-Cadherin expression in 90%
LOH/mutation/methylation at 16q24 (e-Cadherin) in 50-70%
10-15% ALH/LCIS on CNBX = DCIS or IBC FU EXB

Treatments:
- Nothing
- Local excision
- Mastectomy (sometimes bilateral)
- Hormonal therapy (↓risk IBC 50-60%)
ALH and LCIS are “Same” Disease

ALH
- Solid Growth Pattern
- Uniform “Fried-Egg” Cytology
- Partial involvement TLDUs
- Extension into Proximal Ducts

LCIS
- Solid Growth Pattern
- Uniform “Fried-Egg” Cytology
- Complete involvement TLDUs
- Extension into Proximal Ducts

Distinction artificial and controversial... based on extent and amount.

A little bit...............................................................more.

**Brief Aside:**

**Crown-Like Structures (CLS) New Type of Premalignant Breast Lesion?**

ADH $\rightarrow$ DCIS:

*Increased Growth + Alterations Adhesion/Polarity + \(^\uparrow\)Diversity*
Importance of DCIS: Immediate Precursor of IBC

DCIS
No Invasion
Non-Lethal

IBC
Invasion
Potentially Lethal

No Invasion

Point of Invasion
Classification of DCIS

Subtypes of DCIS based on gross appearance and predominant microscopic growth pattern

Comedo  Cribriform  Solid

Micropapillary  Papillary
Classification of DCIS

“Differentiation” = degree tumor cells resemble normal cells (histological = tumor grade)

Histological Scoring and Grading of DCIS

<table>
<thead>
<tr>
<th>Microscopic Feature</th>
<th>Score</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>A. Glands/papillae</td>
<td>&gt; 75%</td>
</tr>
<tr>
<td>B. Nuclear grade</td>
<td>low</td>
</tr>
<tr>
<td>C. Mitotic rate</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>D. Central necrosis</td>
<td>&lt; 10%</td>
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</tbody>
</table>

Σ = total score (range 4-12)
Grade 1 = 4 - 7 points (well differentiated)
Grade 2 = 8-9 points (intermediate differentiated)
Grade 3 = 10-12 points (poorly differentiated)

Clin Cancer Res 14:339, 2008 (based on SBR method of grading IBC)
DCIS is a Histological and Biological Continuum

Histological Score (Modified SBR)

Well Differentiated

Poorly Differentiated

DCIS (n=400)

% ER+
% PgR+
% HER2+
% p53+
avg %Ki67+
% Cases
DCIS = IBC (and its DCIS Component)
Conventional Histological Score/Grade
and Standard Prognostic Biomarkers
Intrinsic Molecular Subtypes
Specific Mutations
More…

Diversity for these features arises first in DCIS and is later propagated to IBC independent of invasion.
Histological (Biological) Differentiation in DCIS Related to Rate but not Fate of Progressing to IBC.

ERα in DCIS
NSABP B24: Tam vs. Placebo after Lump/Rad
Allred et al, J Clin Oncology, 2012

Data as of December 31, 2006

- ER neg/Placebo: 94 patients, 25 events
- ER neg/Tamoxifen: 80 patients, 20 events
- ER pos/Placebo: 274 patients, 81 events
- ER pos/Tamoxifen: 284 patients, 58 events

10-years
50% Relative Reduction
p-value for TRT x ER interaction = 0.09
Problem with Histological Classification of DCIS

Intra-Tumor Diversity

Cribriform Low Grade

≥ 50% of all DCIS

Solid/Comedo High Grade
Enormous Histological and Molecular Diversity with DCIS (and IBC)

**Histological Features**

*Case #012*

- 30% Grade=1
- 60% Grade=2
- 10% Grade=3

**Diversity Extends to:**

- Standard Prognostic Biomarkers
- Intrinsic Molecular Subtypes
- Specific Mutations
- Other…

**Example (Case 089)**

- Grade 1
- Grade 2
- IHC for ER
- IHC for HER2
Advances in Understanding the Progression of DCIS to IBC

Myoepithelial cells are essential for suppressing invasion.

DCIS and IBC tumor cells almost identical (gene expression).

DCIS and IBC stromal cells (e.g. fibroblasts) very different and they are essential for regulating tumor invasion.

There are Important Genetic Differences Between DCIS and IBC

74 differentially expressed genes in ≥ 2 studies (meta-analysis of 14 studies)
Classification of DCIS vs. IBC using 74-gene profile.
(based on hierarchical clustering)


96% Accuracy
Some of the 74 Genes Directly Regulate Progression.

Example:
Suppression (shRNAi) of 10 genes elevated in DCIS identified 4 genes which normally function to suppress invasion:

CSTA
DST
FAT1
TMEM45

Natural History of DCIS

DCIS (Non-Lethal) → Overall Proportion Unknown… ≥ 30-40% over 30 years

JAMA 239:1863, 1978
Cancer 46:919, 1980
Cancer 49:751, 1982
Sem Diag Pathol 11:223, 1994

IBC (Potentially Lethal)

Absolute proportion too high… All IBCs evolved from DCIS precursor
Importance of Premalignant Breast Lesions

Precursors of Potentially Lethal IBC

High Cost (Economical and Personal)

Targets for Prevention of IBC
ER Very High in Nearly all Early Premalignant Lesions

Prevention with hormonal therapies a good idea.
Whole-genome analysis informs breast cancer response to aromatase inhibition

Matthew J. Ellis1,2,3,4, Ji Ding1,2,4, Dong Shen1,2,4, Jingjin Luo1,2,4, Vera J. Sunnar1,4, John W. Wallis1,2,4,5, Brian A. Van Tine1, Jeremy Hoop2, Reece J. Golfin2,8,9,10, Theodore C. Goldstein1,10, Sam Ng1,11, Ili Lin1, Robert Crowder1, Jacqueline Snider1, Karla Ballman1, Jason Weber1,4,11, Ken Chen1,11, Daniel C. Koholdt4,5,11, Cyril R. Kandoth4,11, William S. Schierding4,5, Joshua F. McMichael1,6,5, Christopher A. Miller1,6,5, Charles L.u4,5, Christopher C. Harris1,6,5, Michael D. McLellan1,6,5, Michael C. Wendt1,6,5, Katherine DeSchevry1,6,5,10, Craig Alfred1,6,5, Laura Esserman1,6,5, I. Unzeitig6,5, Julie Margenthaler6,5, G. V. Babiker7,4, P. Kelly Marcom7,4, J. M. Guenther7,4, Marilyn Leitch7,4, Kelly Hunt7,4, John Olson7,4, Yu Tao7,4, Christopher A. Maher7,4, Lucinda L. Fulton4,5, Robert S. Fulton4,5, Michelle Harrison4,5, Ben Obeek8,5, Feiyu Du8,5, Ryan Demeter8,5, Tammi L. Vickers4,5, Adrian Elhammali8,9,20, Helen Pwnica-Worms8,9,12,20,21, Sandra McDonald8,20,21, Mark Watson8,9,20,21, David J. Dooling4,5, David Ota8,5, Li-Wei Chang4,5, Rom Bose4,5, Timothy J. Ley4,5,4, David Pwnica-Worms8,9,10,12,21, Joshua M. Stuart8,5, Richard K. Wilson2,4,6, & Elaine R. Mardis4,5

Top 10 Most Frequent Recurring Mutations (n=77)

- **PIK3CA**: 58%
- **TP53**: 23%
- **MAP3K1**: 17%
- **GATA3**: 10%
- **CDH1**: 10%
- **ATR**: 7%
- **MLL3**: 6%
- **RUNX1**: 5%
- **RB1**: 5%
- **TBX3**: 3%

Only 10 of 3355 of all mutations in ≥ 3% of all tumors

Which mutations are most important?

3355 Total Tier 1 Somatic Mutations (average = 44/tumor)
Theoretical Limits to Genetic Diversity in Breast Cancer

Ellis et al. *Nature, June, 2012*

Sequencing ER+ human breast cancers (n=77)
3355 tier 1 mutations ⇒ average 44/ tumor

Assume 10 mutations from 3000 cancer genes in each tumor
~6 x 10^{34} combinations!

Estimated 10^{21} stars in the visible universe.

Assume just 3 mutations from 100 cancer genes in each tumor
⇒ 1 x 10^6 combinations!

Every IBC is essentially unique
⇒ we need to be realistic about targeted therapy.

Priority: More Focus on Prevention (esp. Molecular)