**Ductal Carcinoma in Situ**

Morphology-Based Knowledge and Molecular Advances

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Attending Pathologist
Director of Breast Pathology

1. Morphology
2. Molecular studies
3. Immunoreactivity
4. Prediction studies

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**Ductal Carcinoma In Situ (DCIS)**

- Historically, DCIS accounted for only 1-2% of all breast cancer
- At present, DCIS accounts for 20-30% of all newly diagnosed breast cancer

*Jemal et al., CA Cancer J Clin 2009;59:225

**Incidence of DCIS**

Adapted from Silverstein & Lagios, Oncology 11:393 (1997)

**DCIS: A CHANGING ENTITY**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Before 1985</th>
<th>After 1985</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>Low vs. High</td>
<td></td>
</tr>
<tr>
<td>Presentation</td>
<td>Palpable vs. Nonpalpable</td>
<td></td>
</tr>
<tr>
<td>Classification</td>
<td>Architectural vs. Grade</td>
<td></td>
</tr>
<tr>
<td>Margin assessment</td>
<td>Gross vs. Microscopic</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>Mastectomy vs. Lumpectomy</td>
<td></td>
</tr>
<tr>
<td>Axillary metastases</td>
<td>Not uncommon vs. Very rare</td>
<td></td>
</tr>
<tr>
<td>Confusion</td>
<td>None vs. Great</td>
<td></td>
</tr>
</tbody>
</table>

**Ductal Carcinoma In Situ (DCIS)**

Neoplastic intraductal lesion characterized by
- increased epithelial proliferation
- subtle to marked cellular atypia
- an inherent, but not necessarily obligate tendency for progression to invasive breast cancer.

WHO 2003
**DCIS nuclear grade**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Grade I (low)</th>
<th>Grade II (intermediate)</th>
<th>Grade III (high)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleomorphism</td>
<td>monotonous</td>
<td>intermediate</td>
<td>markedly</td>
</tr>
<tr>
<td></td>
<td>(monomorphic)</td>
<td></td>
<td>pleomorphic</td>
</tr>
<tr>
<td>Size</td>
<td>1X to 2X RBC</td>
<td>intermediate</td>
<td>&gt;2.5X RBC or</td>
</tr>
<tr>
<td></td>
<td>or normal ductal cell</td>
<td></td>
<td>normal ductal cell</td>
</tr>
<tr>
<td></td>
<td>nucleus</td>
<td></td>
<td>nucleus</td>
</tr>
<tr>
<td>Chromatin</td>
<td>diffuse, finely dispersed</td>
<td>intermediate</td>
<td>vesicular with irregular chromatin distribution</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>occasional</td>
<td>intermediate</td>
<td>prominent, often multiple</td>
</tr>
<tr>
<td>Mitoses</td>
<td>occasional</td>
<td>intermediate</td>
<td>may be frequent</td>
</tr>
<tr>
<td>Orientation</td>
<td>polarized toward luminal space</td>
<td>intermediate</td>
<td>usually not polarized toward luminal space</td>
</tr>
</tbody>
</table>

Lester S, DCIS protocol, Arch Pathol Lab Med, 2009

**Nuclear Grade**

![Low nuclear grade](image1)

![High nuclear grade](image2)

**DCIS architecture**

Many different patterns
- Cribriform
- Solid
- Papillary
- Micropapillary
- Spindle
- Flat (clinging)
  - low NG excluded
- “Pagetoid”

![ER staining](image3)

![E-cadherin staining](image4)
Necrosis

- Absent
- Extensive "comedo" type

Common in high or intermediate nuclear grade DCIS
- Often associated with coarse Ca^{2+}
- Linear branching pleomorphic Ca^{2+} on mammogram

Small laminated Ca^{2+}
((calcified secretions)
Coarse Ca^{2+} in necrosis

Increased incidence of DCIS due to increase of non-comedo type

Virnig BA, JNCI 2010 (modified)

DCIS size

- Important to estimate
  - Probability of residual cancer in the breast
  - Margin status
  - Risk of local recurrence

DCIS size

Only one slide with DCIS → report largest microscopic span
**DCIS size**

**Serial sequential sampling**
- Entire specimen is sectioned in sequence and block location recorded.
- The size of DCIS can be determined from the location of the involved blocks.
- The number of blocks containing DCIS is used to estimate the extent:
  
  \[
  \text{# blocks} \times 0.4 \text{ cm thickness of tissue} = \text{extent}
  \]

Lester S, DCIS protocol, Arch Pathol Lab Med, 2009

**Nonsequential sampling**
- The specimen is sampled, but not in sequence.
- The number of blocks containing DCIS is used to estimate the extent:

  \[
  \text{# blocks} \times 0.4 \text{ cm thickness of tissue} = \text{extent}
  \]

Lester S, DCIS protocol, Arch Pathol Lab Med, 2009

**Margins**
- If DCIS involves two opposing margins, the distance between the margins can be used as the extent of the DCIS in the specimen.

Lester S, DCIS protocol, Arch Pathol Lab Med, 2009

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**DCIS size correlates with residual disease at re-excision and margin status**

Cheng L, JNCI 1997, 89:1356-1360

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**The New York Times**

January 31, 2012

**Breast Cancer Surgery Rules Are Called Unclear**

- Positive margin = tumor at ink
- Close margin?
  - Usually <2mm, but also <1mm or <3mm
- Negative margin?
  - NSABP definition = no tumor at ink >2 mm? >5 mm? >10 mm?

---

**Margins**

No need for RT if margin > 10 mm

Prospective Study of Wide Excision Alone for Ductal Carcinoma in Situ of the Breast

N Engl Journal Medicine 1999

158 pts with DCIS Nuclear Grade 1 or 2; size <2.5 cm
Wide excision (margin>10 mm or re-excision w/o DCIS)
no radiotherapy, no tamoxifen
local recurrence rate 2.4% per patient-year (12% at 5 y)

---

Journal Clinical Oncology 2006
margin status and local recurrence of DCIS treated with excision + RT

- 1103 pts
- 10 centers
- Pos margin = tumor at ink (10)
- Neg margin
  - >2 or ≥2 mm (8)
  - >2-3 mm (1)
  - >3 mm (1)

Solin LJ, Cancer 2005

margin status and local recurrence of DCIS treated with excision +/- RT

<table>
<thead>
<tr>
<th>Margin Status</th>
<th>Patients</th>
<th>HRTR 10yr (95% CI)</th>
<th>Patients</th>
<th>HRTR 10yr (95% CI)</th>
<th>HR (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>250</td>
<td>0.36 (0.21-0.61)</td>
<td>100</td>
<td>1.13 (0.60-2.09)</td>
<td>2.61 (1.49-4.67)</td>
<td>0.002</td>
</tr>
<tr>
<td>&gt;10 mm</td>
<td>45</td>
<td>0.37 (0.12-1.09)</td>
<td>20</td>
<td>1.12 (0.46-2.69)</td>
<td>3.33 (1.96-5.70)</td>
<td>0.003</td>
</tr>
<tr>
<td>1-9 mm</td>
<td>44</td>
<td>0.27 (0.15-0.51)</td>
<td>26</td>
<td>1.12 (0.46-2.69)</td>
<td>3.33 (1.96-5.70)</td>
<td>0.003</td>
</tr>
<tr>
<td>&lt;1 mm</td>
<td>46</td>
<td>0.27 (0.15-0.51)</td>
<td>29</td>
<td>1.11 (0.46-2.69)</td>
<td>3.33 (1.96-5.70)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

RT overcomes the benefits of wider excision margins

margin status and volume of disease near margin

- Volume of disease as # of foci close (<1 mm) to margin
  - 1 duct vs 2 ducts vs 3 ducts
- ≥2 foci of DCIS at <1 mm from margin
  - high risk of LR w/o RT
  - HR=3.37; p=0.002
  - greater benefit of RT
  - HR 0.14; p=0.004

Rudloff U, Ann Surgery 2010; 251:583-591

DCIS and IHC

ER, PR, HER2

Etc...

ER

- About 70% of DCIS is ER positive
  - mean expression rate in 36 studies
  - range 49-96.6%
- Significant positive correlation with PR
- Inverse correlation with nuclear grade
- Inverse correlation with HER2

Lari SA and Kuerer MH, J Cancer, 2011

ER in DCIS and risk of local recurrence

- 329 pts
- excision alone between 1983-1994
- median F/U time 8.2 years
- Follow-up
  - 72 pts with invasive recurrence
  - 71 pts with DCIS recurrence
  - 186 pts w/o recurrence
- ER neg status associated with DCIS recurrence
- No association in pts with invasive recurrence

Kerlikowske K, J Natl Cancer Institute, 2010
Benefit of tamoxifen
4 randomized trials with excision of DCIS +/- RT

**Correa C, JNCI monograph 41, 2010**

**HER2**
- About 40% of DCIS is HER2 positive
  - mean expression rate in 36 studies
  - range 9-67%
  - assessment based on IHC and/or FISH
- HER2 more common in high grade DCIS with necrosis
- HER2+ DCIS more likely to recur
  - recurrence 42% HER2+ vs 12% HER2-
  - Provenzano E, Eur J Cancer, 2003

**DCIS and ER, PR**

**Table 1. Classification of Immunohistochemical Results for Estrogen and Progesterone Receptor for Ductal Carcinoma In Situ (DCIS)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER positive</td>
<td>in most cases of DCIS, the majority of tumor cells will be positive for ER. In cases of DIP, ER expression may not be predictive of clinical outcome.</td>
</tr>
<tr>
<td>PR positive</td>
<td>in most cases of DCIS, the majority of tumor cells will be positive for PR. In cases of DIP, PR expression may not be predictive of clinical outcome.</td>
</tr>
<tr>
<td>Basal-like</td>
<td>these categories should be used for cases of DCIS with no cells showing immunoreactivity for hormone receptors.</td>
</tr>
<tr>
<td>Luminal A</td>
<td>these cases should be used for cases of DCIS with no cells showing immunoreactivity for hormone receptors.</td>
</tr>
<tr>
<td>Luminal B</td>
<td>these cases should be used for cases of DCIS with no cells showing immunoreactivity for hormone receptors.</td>
</tr>
<tr>
<td>HER2 positive</td>
<td>these categories should be used for cases of DCIS with no cells showing immunoreactivity for hormone receptors.</td>
</tr>
<tr>
<td>HER2 negative</td>
<td>these categories should be used for cases of DCIS with no cells showing immunoreactivity for hormone receptors.</td>
</tr>
</tbody>
</table>

Assessment of ER suggested, but not required
Assessment of PR not required
HER2; no specific mention

**CK 5/6**
- Basal CK
- Present in normal epithelium and UDH
- Not present in FEA, ADH, LG-DCIS
- Present in basal DCIS (discussed later)

- Can be useful in DDX of low grade epithelial proliferation
  - CK14 and 34BE12 show similar reactivity

**Biomarker Expression and Risk of Subsequent Tumors After Initial Ductal Carcinoma In Situ Diagnosis**

**Figure 1. Ductal epithelial proliferations of the breast: a biological continuum? Comparative genomic hybridization and high-molecular-weight cytokeratin expression patterns**

**J Pathol 2001**
DCIS
one disease?  many?

Nuclear Grade
low  high

Low grade nuclei have smooth outline
fine and uniform chromatin

Morphologic and cytologic criteria for diagnosis of
Low grade DCIS vs ADH vs UDH

<table>
<thead>
<tr>
<th></th>
<th>UDH</th>
<th>ADH</th>
<th>Low Grade DCIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUCLEAR ATYPIA</td>
<td>absent</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>MORPHOLOGY</td>
<td>not polarized</td>
<td>polarized</td>
<td>polarized</td>
</tr>
<tr>
<td>ARCHITECTURE</td>
<td>irregular, not rigid</td>
<td>some complex and rigid</td>
<td>complex and rigid</td>
</tr>
<tr>
<td>EXTENT</td>
<td>not relevant</td>
<td>&lt;2 ducts or &lt;2mm</td>
<td>≥2 ducts or ≥2 mm</td>
</tr>
</tbody>
</table>

CARCINOID TUMOR
Columnar cell change

- Alteration of terminal duct lobular units
- Often detected due to associated Ca²⁺
- BIRADS 3 indeterminate Ca²⁺

Columnar cell change immunoprofle

<table>
<thead>
<tr>
<th>POSITIVE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ER, PR</td>
<td></td>
</tr>
<tr>
<td>Bcl-2, cyclin D1</td>
<td></td>
</tr>
<tr>
<td>Mib-1 Low (&lt;3%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NEGATIVE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2</td>
<td></td>
</tr>
<tr>
<td>GCDFP-15</td>
<td></td>
</tr>
<tr>
<td>Basal CKs (CK 5/6)</td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td></td>
</tr>
</tbody>
</table>

Carcinoid tumor

Carcinoid Cell Lesions of the Breast: The Missing Link in Breast Cancer Progression?
A Morphological and Molecular Analysis

Peter H. Songrane, PhD,* Veen Cadee, BSc,* Dwayne S. Bata, BPha, MSc, Chris Jones, PhD†
Sawhney, Barry, MSc,* John P. Means, FRCPath,† Andrew Hardy, FRCPath,‡
Sarah E. Palmer, FRCPath,* Andrew M. S. Lee, FRCPath,* Simeon Houghton, FRCPath,*
Jan O. Ellis, FRCPath,* and Sandi K. Lohkbaar, FRCPath***
Am J Surg Pathol 2005
FLAT EPITHELIAL ATYPIA

- Single layer of mildly atypical cells
- Stratification of uniform, cuboidal to columnar cells up to 3-5 cell layers
- Often contains microcalcifications

WHO 2003

MORPHOLOGY OF DCIS ASSOCIATED WITH FEA

<table>
<thead>
<tr>
<th>Morphological Feature</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Nuclear Grade</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cribriform and/or Micropapillary</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No comedo necrosis</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No stromal desmoplasia</td>
<td>0.02</td>
</tr>
<tr>
<td>No stromal inflammation</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Collins L, Mod Pathol, 20, 1149-1155; 2007

FEA

Tubular carcinoma

ALH and LCIS
Low grade mammary epithelial neoplasia

- Low grade atypia
- Immunophenotype
  - ER+, PR, bcl-2, cyclin D1 positive
  - Mib1 low (<10% for inv ca)
  - CK5/6, CK14, HER2 and p53 negative
  - No HER2 gene amplification
- Distinct from high grade epithelial lesions
  - “Intrinsic” Luminal A subtype


Progression of breast carcinoma what we believed before…

Lopez-Garcia M, Histopathology, 2010

Classification of ductal carcinoma in situ by gene expression profiling

J Clin Cancer Res 2008

Molecular Grading of Ductal Carcinoma In situ of the Breast

Clin Cancer Res, 2008

Gene array profiles of 46 carcinoma in situ associated with invasion

45 DCIS
1 LCIS
Clin Cancer Res, 2008

Correlation with nuclear grade
ER, PR
HER2
p53
Ki67

"Intrinsic" Molecular Subtypes of Invasive Breast Carcinoma

<table>
<thead>
<tr>
<th>intrinsic molecular subtypes</th>
<th>&quot;molecular&quot; immunoprofile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>+ + -</td>
</tr>
<tr>
<td>Luminal B</td>
<td>+ + +</td>
</tr>
<tr>
<td>HER2</td>
<td>- - +</td>
</tr>
<tr>
<td>basal</td>
<td>CK5/6+ and/or EGFR+</td>
</tr>
</tbody>
</table>

Perou CM, Nature 2000
Sorlie T, PNAS 2001

"basal" DCIS

- 6% to 8% of DCIS
- Intermediate to high nuclear grade
- ER-, PR-, HER2-
- CK5/6 pos and/or EGFR pos
- High Ki67

Bryan BB, Mod Pathol 2006
Livasy CA, Mod Pathol, 2006

"basal" DCIS

- DCIS found in BRCA1 germline mutation carriers
  - DCIS in BRCA2 germline mutation carriers is usually luminal type

Van De Groep P, J Clin Pathol 2009
CK 5/6 immunoreactivity is not uniform

Basal DCIS

Follow-up study

- 392 women with DCIS
- ER, PR, HER2, CK 5/6 and EGFR
  - 32/392 (8.2%) women had "basal" DCIS
- Median F/U 122 months (3-130)

Basal DCIS and LOCAL recurrence

Basal DCIS and ANY recurrence

What is the morphologic precursor of basal DCIS?
**“molecular” subtypes of DCIS**

- Same subtypes as invasive carcinoma
- Each DCIS subtype likely precursor of same invasive carcinoma subtype
  - Lum B DCIS → Lum A invasive most common exception

**DCIS**

Prediction of future events

**NATURAL HISTORY OF DCIS TREATED WITH BIOPSY ALONE**

<table>
<thead>
<tr>
<th>Study</th>
<th>average F/U yrs</th>
<th>average time to recurrence yrs</th>
<th>recurrences</th>
<th>% of invasive recurrences</th>
<th>overall progression to invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betsill, 1978</td>
<td>21.6</td>
<td>9.7</td>
<td>7/10 (70%)</td>
<td>6/7 (86%)</td>
<td>6/10 (60%)</td>
</tr>
<tr>
<td>Rosen, 1980</td>
<td>18</td>
<td>9.7</td>
<td>10/15 (68%)</td>
<td>8/10 (80%)</td>
<td>8/15 (53%)</td>
</tr>
<tr>
<td>Eusebi, 1989</td>
<td>16</td>
<td>5</td>
<td>1/7 (14%)</td>
<td>1/1 (100%)</td>
<td>1/7 (14%)</td>
</tr>
<tr>
<td>Page, 1982</td>
<td>16</td>
<td>6.1</td>
<td>7/25 (28%)</td>
<td>7/7 (100%)</td>
<td>7/25 (28%)</td>
</tr>
<tr>
<td>Page, 1995</td>
<td>29</td>
<td>10.8</td>
<td>10/26 (40%)</td>
<td>9/10 (90%)</td>
<td>9/26 (36%)</td>
</tr>
<tr>
<td>Sanders, 2005</td>
<td>31</td>
<td>13.8</td>
<td>12/28 (43%)</td>
<td>11/12 (92%)</td>
<td>11/28 (39%)</td>
</tr>
<tr>
<td>Collins, 2005</td>
<td>8.6</td>
<td>10.7</td>
<td>10/13 (77%)</td>
<td>6/10 (60%)</td>
<td>6/13 (46%)</td>
</tr>
</tbody>
</table>
**NATURAL HISTORY OF LOW GRADE DCIS TREATED WITH BIOPSY ALONE**

Invasive carcinoma develops within 15 years in 30% of pts with untreated LG-DCIS

Sanders ME, Cancer 2005

---

**Overview of 4 randomized trials with excision of DCIS +/- RT**

<table>
<thead>
<tr>
<th>Study</th>
<th>Years</th>
<th>Patients eligible for analysis</th>
<th>Median F/U (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSABP B-17</td>
<td>1985-90</td>
<td>798</td>
<td>16.5</td>
</tr>
<tr>
<td>EORTC 10853</td>
<td>1986-96</td>
<td>918</td>
<td>10.4</td>
</tr>
<tr>
<td>SweDCIS</td>
<td>1987-99</td>
<td>1011</td>
<td>8.4</td>
</tr>
<tr>
<td>UK/ANZ</td>
<td>1990-98</td>
<td>1002</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Correa C, JNCI monograph 41, 2010

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**Benefit of radiotherapy**

4 randomized trials with excision of DCIS +/- RT

- Half of all LRs are invasive
- Radiation reduces LR by 50%
- RT benefit independent of any other factor

Correa C, JNCI monograph 41, 2010

---

**Benefit of tamoxifen**

4 randomized trials with excision of DCIS +/- RT

Correa C, JNCI monograph 41, 2010

---

**% OF ACADEMIC PHYSICIANS RECOMMENDING RADIOTHERAPY AFTER WIDE EXCISION OF DCIS**

Ceilley et al., Cancer 2004;101:1958
**MSKCC DCIS NOMOGRAM**

Rudloff et al., 2010 J Clin Oncol 28:3762

**MSKCC DCIS NOMOGRAM CALIBRATION CURVES**

Rudloff et al., 2010 J Clin Oncol 28:3762

**MSKCC nomogram tested on MD Anderson DCIS Cohort concordance index of 0.63**

Yi M. JCO February 20, 2012 vol. 30 no. 6

**NOMOGRAM VALIDATION WITH HARVARD/KAISER POPULATION (N=495)**

Collins et al., abstract 118 2012 USCAP meeting

**12 gene RT-PCR assay**

to assess DCIS score and estimate risk of local recurrence

<table>
<thead>
<tr>
<th>Proliferation Genes</th>
<th>Hormone Receptor Group</th>
<th>Reference Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki67</td>
<td>PR</td>
<td>beta actin</td>
</tr>
<tr>
<td>STK15</td>
<td></td>
<td>GADPH</td>
</tr>
<tr>
<td>Survivin</td>
<td></td>
<td>RPLPO</td>
</tr>
<tr>
<td>cyclin B1</td>
<td></td>
<td>GUS</td>
</tr>
<tr>
<td>MYBL2</td>
<td>GSTM1</td>
<td>TFRC</td>
</tr>
</tbody>
</table>
ECOG E5194
Prospective multicenter study DCIS treated by excision alone

n = 670
Cohort 1: Low/intermediate grade, size < 2.5 cm
Cohort 2: High grade, size < 1 cm
Treatment:
  - Surgical excision with at least 3 mm margin
  - No radiotherapy
  - Some pts received tamoxifen

Hughes LL, JCO 2009

12 gene RT-PCR assay to assess DCIS score and estimate risk of local recurrence

347 patients with 10 year follow-up

ANALYSIS
  - Continuous variable
  - 3 risk groups
    - Low risk (score < 39)
    - Intermediate risk (score 39 – 54)
    - High risk (score > 55)
  - Data presented at SABCS 2011, not yet published

DCIS

- heterogeneous disease
  - “intrinsic” molecular subtypes +/- IHC correlation
  - no mention of “molecular” classification in dx
- Breast conservation
  - RT reduces local recurrence by half
  - Hormonal therapy benefit in ER+ DCIS
- Tools for prediction of local recurrence
  - MSKCC DCIS nomogram
  - 12 gene assay
References


