Is genomic grading killing histological grading?

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Histological Grade and Breast Cancer Biology

**PHENOTYPE**

**Low Grade**
- Polarized groups of cells that form tubular or duct-like structures

**High Grade**
- No tubular structures
- Nuclear pleomorphism
- Mitotic activity+++
## Histological Grade and Breast Cancer Biology

### PATTERN of TUMOR MARKERS

<table>
<thead>
<tr>
<th>Low Grade</th>
<th>Positively correlated to ER + status:</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGR</td>
<td></td>
</tr>
<tr>
<td>TFF1</td>
<td></td>
</tr>
<tr>
<td>CDH1</td>
<td></td>
</tr>
<tr>
<td>DSP</td>
<td></td>
</tr>
<tr>
<td>MDM2</td>
<td></td>
</tr>
<tr>
<td>NME1</td>
<td></td>
</tr>
<tr>
<td>CCND1</td>
<td></td>
</tr>
<tr>
<td>TJP1...</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>High Grade</th>
<th>Positively correlated to ER - status:</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDKN2A</td>
<td></td>
</tr>
<tr>
<td>CCNE</td>
<td></td>
</tr>
<tr>
<td>EGFR</td>
<td></td>
</tr>
<tr>
<td>ERBB2</td>
<td></td>
</tr>
<tr>
<td>SERPINE1</td>
<td></td>
</tr>
<tr>
<td>PLAU</td>
<td></td>
</tr>
<tr>
<td>HXB</td>
<td></td>
</tr>
<tr>
<td>CDH3...</td>
<td></td>
</tr>
</tbody>
</table>

Lacroix et al, ERC 2004
Histological Grade and Breast Cancer PROGRESSION

Low Grade DCIS  High Grade DCIS

Tumor Markers: p53, erbb2, Ki-67, ER, PR, bcl-2, angiogenesis

• No marker was clearly associated with progression
• Correlation with grade

DCIS ► ID occurs independently of tumor grade

Warnberg et al, BJC 2001
65% of grade 1 tumors lost the long arm of Chromosome 16 compared with only 16% of grade 3 tumors

Roylance et al., Cancer Research 1999
Histological Grade and Clinical Outcome

Figure 3. Relationship between histological grade (□ = I; ◆ = II; ■ = III) and overall survival in 1830 patients with primary carcinoma of the breast: \( \chi^2 = 198.06, 2 \ text{df}; P < 0.0001. \)

Elston and Ellis, Histopathology 1991
LOW AND HIGH GRADE TUMORS
TWO DISTINCT DISEASES

Distinct cell type of origin?
Histological Grade

PROBLEMS

GRADE 2
Difficult treatment decision making: under- or over-treatment likely

Poor inter observer reproducibility
Can we better characterize histological grade?
Gene Expression Profiling in Breast Cancer: Understanding the Molecular Basis of Histologic Grade To Improve Prognosis

Christos Sotiriou, Pratyaksha Wirapati, Sherene Loi, Adrian Harris, Steve Fox, Johanna Sneds, Hans Nordgren, Pierre Farmer, Viviane Praz, Benjamin Haibe-Kains, Christine Desmedt, Denis Larsimont, Fatima Cardoso, Hans Peterse, Dimitry Nuyten, Marc Buyse, Marc J. Van de Vijver, Jonas Bergh, Martine Piccart, Mauro Delorenzi

*J Natl Cancer Inst. 2006 Feb 15;98(4):262-72*

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### Table 1. Microarray datasets used in this study

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Institution</th>
<th>No. of samples</th>
<th>Grade ratio*</th>
<th>% Grade 2</th>
<th>ER ratio*</th>
<th>Systemic treatment</th>
<th>Microarray platform</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>KJX64</td>
<td>Uppsala</td>
<td>24</td>
<td>11/0/13</td>
<td>0</td>
<td>0/24</td>
<td>Yes†</td>
<td>Affymetrix U133A</td>
<td>Training set (this study)</td>
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<tr>
<td>KJ125</td>
<td>John Radcliffe</td>
<td>40</td>
<td>22/0/18</td>
<td>0</td>
<td>0/40</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Uppsala</td>
<td>64</td>
<td>26/28/10</td>
<td>44</td>
<td>13/54</td>
<td>No</td>
<td>Affymetrix U133A</td>
<td>Validation set (this study)</td>
</tr>
<tr>
<td>NCI</td>
<td>John Radcliffe</td>
<td>61</td>
<td>8/18/18</td>
<td>41</td>
<td>24/32</td>
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<td></td>
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<tr>
<td>STNO</td>
<td>John Radcliffe</td>
<td>99</td>
<td>16/38/45</td>
<td>38</td>
<td>34/65</td>
<td>Yes‡</td>
<td>cDNA (NCI)</td>
<td>Soiriou et al. (14)</td>
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<tr>
<td>NKI2</td>
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<td>Stanford</td>
<td>85§</td>
<td>9/33/33</td>
<td>44</td>
<td>18/56</td>
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<td>Nederlands Kanker Institutu</td>
<td>165 (untreated)</td>
<td>40/49/76¶</td>
<td>30</td>
<td>43/122</td>
<td>No</td>
<td>Agilent</td>
<td>Van de Vijver et al. (15)</td>
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<tr>
<td>Total</td>
<td></td>
<td>668</td>
<td>35/52/43¶</td>
<td>40</td>
<td>26/104</td>
<td>Yes#</td>
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<tr>
<td>No. of patients in validation set**</td>
<td>597</td>
<td>134/218/225</td>
<td>38</td>
<td>158/433</td>
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</table>
Developing Genomic Grade in the training set

Identify genes correlated with grade 1 vs grade 3

128 probe sets of “grade signature” (97 genes) FDC>0

Define GGI score (gene-expression grade index):

\[
GGI = scale \left( \sum_{j \in G_3} x_j - \sum_{j \in G_1} x_j - \text{offset} \right)
\]

- Concordance with histological grade
- Prognostic value of GGI
Genomic Grade (GG) in the Validation Set N=125

Grade 1

Grade 2

Grade 3

GG1

GG3

GG1 vs grade 1 or 3: p = nan (t-test)
Consistent Distribution of GG in Different Populations and Microarray Platforms

Sorlie et al. PNAS 2001

Van de Vijver et al. NEJM 2002
Central Pathology Review!

Sotiriou et al. PNAS 2003
GG and Clinical Outcome

**Histological Grade**
- Grade 1
- Grade 2
- Grade 3

**Grade 2**
- GG1
- GG3

**Genomic Grade**
- GG1
- GG3

Number at risk:

<table>
<thead>
<tr>
<th>Dataset</th>
<th>n</th>
<th>Hazard Ratio</th>
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<tbody>
<tr>
<td>KJ125</td>
<td>62</td>
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<tr>
<td>NKI2(U)</td>
<td>116</td>
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<td>NKI2(T)</td>
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<tr>
<td>STNO</td>
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<td></td>
</tr>
<tr>
<td>NCI</td>
<td>61</td>
<td></td>
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<tr>
<td>Total</td>
<td>354</td>
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</table>

Hazard ratios for different datasets and grade levels are shown in the diagram.
Genomic Grade genes

Pre-replication complex

MCM2
MCM4
MCM6
MCM10
CDK4,6
CDC45L
CDKN3
MYBL2
MCM3
G1 Phase
G2 Phase
Mitosis
S Phase
Growth Factors
Restriction Point
Ras, Raf, Myc, Fos, Jun
Myc
CDK 4,6
PCNA
Cyclin D1, 2 & 3
Rb
E2F
Rb
Cyccln A & B
Cdc2
Cdc2
Cyclin B
Cdc2
Cdc2
Cyclin A
CDK2
Cyclin E
CDK2
CDK7
CDK2
Cyclin H
Cdc25A
CDK2
p107 & E2F
p107 & E2F
p53
p16
p15
p15
p27
p21
p21
TGFβ
TGFβ Contact Inhibition
Weel
Cdc25C
Weel
Proteins implicated in cancer
How important are proliferation genes in prognostic gene signatures?
Almost Identical!
TRANSBIG VALIDATION SERIES

Using Affymetrix® (VERIDEX)
76-genes (ROTTERDAM) versus Genomic Grade

Almost Identical!
Genomic Grade and Molecular Subtypes
Sotiriou et al. SABCS 2005

Sorlie et al. PNAS 2001

Clinical Outcome

Genomic grade genes

GGI: High

GGI: Low

High

Low

Basal ERBB2 Normal LC LB Luminal A

p<0.01
Hypothesis

- **BRCA1 mutation**
- **ER negative**
- **ERBB2 amplification**
- **p53 mutation**
- Size (large)
- Node (positive)
- **Proliferation (high)**
- **Poor Prognosis**
Is genomic grading killing histological grading?
Acknowledgements

Prof M. Piccart

Collaborators:

Marc Buyse
Fanny Piette

Asa
Mauro

Benjamin

Christine
Sherene
Françoise
Virginie
Backup
META ANALYSIS
11 studies
1623 patients

GG3 vs. GG1:
HR = 2.47 [CI 2.05 – 2.99]
p < 0.001 (logrank test)

number at risk
GG1 925 676 404 240 106 40 10 3
GG3 621 456 271 133 56 18 3
total 1623 1291 860 511 239 96 28 3

RT-PCR
The relationship between ER, grade and prognosis

<table>
<thead>
<tr>
<th>counts</th>
<th>HG1</th>
<th>HG2</th>
<th>HG3</th>
<th>total</th>
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</thead>
<tbody>
<tr>
<td>ER−</td>
<td>8</td>
<td>38</td>
<td>107</td>
<td>153</td>
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<tr>
<td>ER+</td>
<td>123</td>
<td>179</td>
<td>116</td>
<td>418</td>
</tr>
<tr>
<td>total</td>
<td>131</td>
<td>217</td>
<td>223</td>
<td>571</td>
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</table>

<table>
<thead>
<tr>
<th>percentage</th>
<th>HG1</th>
<th>HG2</th>
<th>HG3</th>
<th>total</th>
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</thead>
<tbody>
<tr>
<td>ER−</td>
<td>1.4</td>
<td>6.7</td>
<td>18.7</td>
<td>26.8</td>
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<tr>
<td>ER+</td>
<td>21.5</td>
<td>31.3</td>
<td>20.3</td>
<td>73.2</td>
</tr>
<tr>
<td>total</td>
<td>22.9</td>
<td>38.0</td>
<td>39.1</td>
<td>100.0</td>
</tr>
</tbody>
</table>

(a) Table of counts and percentages for ER and grade.

(b) Histogram showing the distribution of GGI with ER+ and ER−.

(c) Kaplan-Meier curves for ER− vs. ER+.

(d) Kaplan-Meier curves for ER− vs. ER+.

(e) Kaplan-Meier curves for ER+ vs. GG1.

(f) Kaplan-Meier curves for GG3 vs. GG1.

Number at risk:
- GG1: 287, 251, 211, 127, 64, 30, 12, 3
- ER+GG1: 255, 229, 196, 116, 56, 26, 10, 2
- total: 427, 349, 230, 162, 81, 38, 14, 2
- GG3: 296, 141, 84, 49, 17, 4
- ER+GG3: 172, 120, 85, 46, 25, 12, 4
- total: 233, 193, 141, 64, 40, 17, 4
Relationship with the 70-gene Amsterdam Signature

Based on 113 probe (93 genes) mapped on the Agilent arrays