Molecular-Genetic Factors in the Local-Regional Management of Breast Cancer

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I have no financial relationships with a commercial entity producing healthcare-related products and/or services.
Factors Influencing Local Treatment

**Host**

- Genetic Factors
  - (BRCA1, ATM, CHEK2, SNP’s)
  - Age
  - Race
  - Comorbidity

**Tumor**

- Nodes
- Size
- Pathologic subtype
- Molecular Factors
  - (ER, PR, HER2, p53, gene profiling, etc)

• Susceptibility
• Prognosis
• Response
• Normal Tissue
Molecular and Genetic Markers in Local Control of Breast Cancer

- Molecular/genetic markers have been integrated into clinical management of breast cancer with respect to systemic disease and overall survival
  - ER/PR/Her2/neu
  - Onco-DX
  - Gene Profiling

- Integration of Molecular Markers into the local-regional management of breast cancer lags far behind applications in systemic disease.
Outline for Discussion

• Molecular Profiling Studies in Local-Regional Management of Breast Cancer
• Molecular markers-ER, PR, Her2/neu in local-regional management of BC
• Molecular Markers as Surrogates for Subtyping-Luminal A,B, Her2, Basal Triple Negative in local-regional management
• Current research efforts
• Future Directions
Potential uses of Molecular and Genetic Factors in Local Management of Breast Cancer

- Identification of subsets of patients with 1-3 nodes who benefit most from PMRT
- Identification of patients at high risk of failure treated with CS+RT
- Identification of patients most suitable for wide excision alone
- Identification of patients who may or may be more or less suitable for APBI
- Targeting molecular pathways to improve response to radiation and local-regional control
- Genetic/Host-Identification of patients at risk for radiation complications
Fig 2. Unsupervised two-dimensional cluster analysis of 258 genes in 62 patients revealed two distinct groups of tumors; their locoregional recurrence rates were 41.4% (12 of 29) compared with 18.2% (six of 33)
Genomic Predictors of LRR

Cheng SH et al. JCO 24:4594, 2006

<table>
<thead>
<tr>
<th>Category/Genomic Score</th>
<th># Patients/ # LRR</th>
<th>3 Year LRC Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Node neg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;0.8</td>
<td>24/1</td>
<td>96%</td>
</tr>
<tr>
<td>&lt;0.8</td>
<td>3/2</td>
<td>33%</td>
</tr>
<tr>
<td>1-3 + N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;0.8</td>
<td>34/1</td>
<td>100%</td>
</tr>
<tr>
<td>&lt;0.8</td>
<td>19/12</td>
<td>47%</td>
</tr>
<tr>
<td>&gt;=4 + N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;0.8</td>
<td>4/1</td>
<td>75%</td>
</tr>
<tr>
<td>&lt;0.8</td>
<td>10/10</td>
<td>0%</td>
</tr>
</tbody>
</table>
Fig 3. Kaplan-Meier survival estimates for locoregional control in validation data set by (A) 258-gene and (B) 34-gene prediction tree models.
Genetic profiling—Local relapse after CS+Rt

- 161 Patients treated with CS+RT/16 Local relapses
- Wound signature gene profile tested
- Hypoxia profile also tested and not significant
- Wound profile-10 year risk of local relapse 6% with favorable profile vs 35% with unfavorable profile

161 Patients treated with breast conserving therapy; 17 patient with LR, 144 without

Training set; 81 patients 72 without LR, 9 with LR
- Input Gene List
  - Wound Signature
  - Hypoxia Signature
  - 70-genes

Calculate LR-Centroid Average Expression per gene from Gene List across LR patients in training set (n=9)

Calculate Pearson Correlation to LR-centroid for all patients (n=81) in training set

Look for optimal correlation cut off value to separate LR and No LR patients

Validation set; 80 patients 72 without LR, 8 with LR
- Calculate Pearson Correlation to LR-centroid (that has been calculated in training set LR-patients) for all patients (n=80) in validation set

Apply optimal correlation cut off that has been established in training set on patients in validation set

Graphs showing local recurrence free probability over years after surgery with different risk groups.
Onco\textsuperscript{type} DX™ 21-Gene Recurrence Score (RS) Assay

16 Cancer and 5 Reference Genes From 3 Studies

**PROLIFERATION**
- Ki-67
- STK15
- Survivin
- Cyclin B1
- MYBL2

**ESTROGEN**
- ER
- PR
- Bcl2
- SCUBE2

**INVASION**
- Stromelysin 3
- Cathepsin L2

**REFERENCE**
- Beta-actin
- GAPDH
- RPLPO
- GUS
- TFRC

**Category** | **RS (0-100)**
--- | ---
Low risk | RS <18
Int risk | RS ≥18 and <31
High risk | RS ≥31
Relationship Between RS and LRF

• Population: Patients with RS Assay from NSABP node-negative, ER + adjuvant trials:
  – B-14 Placebo: 355 pts
  – B-14/B-20 Tamoxifen: 895 pts
  – B-20 Chemo + Tamoxifen: 424 pts

Mamounas et al. NSABP
Fig 4. Ten-year Kaplan-Meier estimates of the proportions of locoregional recurrence (LRR) according to recurrence score (RS), initial locoregional treatment, and age in the 895 tamoxifen-treated patients in National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14/B-20 trials.
Oncotype in DCIS

• DCIS Score was developed from subset of Oncotype DX 21 Gene Assay
• Used ECOG 5194 DCIS treated with CS and observation as data set
• Assay performed on 327 cases (49%)
• Primary endpoint: IBTR as a function of DCIS Score
DCIS Score and local recurrence

Solin et al. SABC, 2011

![Graph showing Kaplan-Meier Risk for different DCIS Score Groups.](image)
Molecular Profiling and Local-Regiona MGMT

• Several studies performed to date show promise
• Unfortunately these are generally smaller convenience type retrospective series, without the necessary accompanying validation studies
• At this time most of these would be considered hypothesis generating and not ready for clinical decision making
Breast Cancer Subtypes and Local Management

- Note: Majority of studies use molecular marker surrogate (ER, PR, Her2) to classify tumors and report clinical data
- Estrogen/Progesterone Receptor
- Her2/neu
- Triple Negative
- Intrinsic Subtypes
  - Luminal A
  - Luminal B
  - Her2/neu
  - Triple Negative-Includes both true basal-like triple negative breast cancers as well as non-basal like triple negative breast cancers
## Hormonal Receptors in Local Control

<table>
<thead>
<tr>
<th>Study</th>
<th>Local TX</th>
<th>Pat.Pop</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheng</td>
<td>MAST</td>
<td>83 Node+</td>
<td>Negative ER correlated with LRR (31% vs 11%)</td>
</tr>
<tr>
<td></td>
<td>No RT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sundquist</td>
<td>MAST</td>
<td>629</td>
<td>Trend toward higher local relapse with Negative ER (12% vs 6%)</td>
</tr>
<tr>
<td></td>
<td>CS +/- RT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zellars</td>
<td>MAST +/-RT</td>
<td>1271</td>
<td>Higher LR in ER Negative (16% vs 12%), p = .04, but not significant in multivariate analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>259</td>
<td></td>
</tr>
<tr>
<td>Fisher</td>
<td>MAST</td>
<td>150</td>
<td>Higher LR in patients with both ER and PR negativity</td>
</tr>
<tr>
<td></td>
<td>CS (No RT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silvestrini</td>
<td>MAST</td>
<td>1800</td>
<td>No correlation with ER and LRR</td>
</tr>
<tr>
<td></td>
<td>CS +RT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elkhuizen</td>
<td>CS +RT</td>
<td>195 Case Controls</td>
<td>Higher frequency of PR negativity in locally recurrent group</td>
</tr>
<tr>
<td>Provenzano</td>
<td>CS+/-RT</td>
<td>95 Case-Control DCIS</td>
<td>Local relapse associated with ER and PR negativity</td>
</tr>
<tr>
<td>Santiago</td>
<td>CS+RT</td>
<td>559 known PR status patients</td>
<td>Higher rate of local control in PR+ vs PR-patients (89% vs 83%, p = .04)</td>
</tr>
</tbody>
</table>
# Her2/Neu in Local Control

<table>
<thead>
<tr>
<th>Study</th>
<th>LocalTx</th>
<th>Pat Pop.</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ringberg</td>
<td>CS Only</td>
<td>187 All DCIS</td>
<td>Relative risk of relapse with overexpression 1.7, p = .20</td>
</tr>
<tr>
<td>Haffty</td>
<td>CS +RT</td>
<td>32 Case-Control</td>
<td>Higher expression of Her2/neu in patients sustaining local relapse (56% vs 18%, p = .03)</td>
</tr>
<tr>
<td>Elkhuizen</td>
<td>CS+RT</td>
<td>195 Case-Controls</td>
<td>Higher expression of Her2/neu in patients sustaining local relapse (19% vs 10%, p = .10)</td>
</tr>
</tbody>
</table>
| Stal          | MAST +/- RT or CMF | 152 | LRR high in patients expressing Her2/neu and not receiving XRT  
LRR low if H2N+ treated with RT                                              |
| Carr          | MAST    | 190 | No correlation with Her2/neu and LRR                                                                                                 |
|               | CS+/-RT |          |                                                                                                                                        |
| Pierce        | CS +RT  | 137 | No correlation with Her2/neu and LRR (H2N+ correlated with EIC)                                                                       |
| Provenzano    | CS+/-RT All DCIS | 95 Case Control | Significant correlation with local relapse in H2N+ cases-RR = 5.0, p = .01                                                            |
Effect of trastuzumab on sensitizing ionizing radiation-induced apoptosis in breast cancer cell lines with various levels of HER2 protein.

Liang K et al. Mol Cancer Ther 2003;2:1113-1120

©2003 by American Association for Cancer Research
HER2 Radiation Resistance and Local Control

• Strong in-vitro evidence that Her2/neu overexpression is associated with radiation resistance
• Modest evidence that HER2/neu overexpression is associated with higher local relapse rates
• Likely that widespread administration of Herceptin may overcome radiation resistant related local relapses in HER2/neu positive patients
Adjuvant trastuzumab reduces locoregional recurrence in women who receive breast-conservation therapy for lymph node-negative, human epidermal growth factor receptor 2-positive breast cancer.
## Table 2. Sites of First Events.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Control Group</th>
<th>Trastuzumab Group</th>
<th>Control Group</th>
<th>Trastuzumab Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients with follow-up</td>
<td>872</td>
<td>864</td>
<td>807</td>
<td>808</td>
</tr>
<tr>
<td>Patients alive and event-free</td>
<td>701</td>
<td>781</td>
<td>717</td>
<td>758</td>
</tr>
<tr>
<td>Patients with any first event</td>
<td>171</td>
<td>83</td>
<td>90</td>
<td>50</td>
</tr>
<tr>
<td>Local or regional recurrence</td>
<td>35</td>
<td>15</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Distant recurrence</td>
<td>111</td>
<td>60</td>
<td>63</td>
<td>30</td>
</tr>
<tr>
<td>Contralateral breast cancer</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Other second primary cancer</td>
<td>15</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Death with no evidence of disease</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
• Conflicting Reports, but some concerns regarding higher local relapse rates
• Need to separate issues of post-mastectomy (with or without RT), and BCS with RT
## Triple Negative and BCS+RT: Local Control

<table>
<thead>
<tr>
<th>Study</th>
<th>LR TN</th>
<th>LR other</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haffty</td>
<td>17%</td>
<td>17%</td>
<td>TN vs all others NS Did not separate Her2/Luminal subtypes</td>
</tr>
<tr>
<td>Nguyen</td>
<td>7.1%</td>
<td>1.8%</td>
<td>TN and Her2 significantly higher LRR</td>
</tr>
<tr>
<td>Freedman</td>
<td>3.2%</td>
<td>3.0%</td>
<td>TN Her2 and Luminal not significantly different</td>
</tr>
<tr>
<td>Voduc</td>
<td>14%</td>
<td>9%</td>
<td>Significant in UVA but not in MVA</td>
</tr>
<tr>
<td>Arvold</td>
<td>8.8%</td>
<td>3.1%</td>
<td>Age independent factor even with subtyping and systemic Tx.</td>
</tr>
<tr>
<td>Solin</td>
<td>8.0%</td>
<td>4.0%</td>
<td>Significant in UVA but not in MVA</td>
</tr>
<tr>
<td>Siponen</td>
<td>3.4%</td>
<td>2.0%</td>
<td>Not significant in MVA</td>
</tr>
</tbody>
</table>
## Triple Negative and Mastectomy Local Control

<table>
<thead>
<tr>
<th>Study</th>
<th>LR TN</th>
<th>LR others</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voduc</td>
<td>19%</td>
<td>8-10%</td>
<td>TN and H2N had significantly higher LRR</td>
</tr>
<tr>
<td>Meyers</td>
<td>14%</td>
<td>4%</td>
<td>TN significantly higher then others after neoadjuvant; includes some BCT patients</td>
</tr>
<tr>
<td>Mersin</td>
<td>3.7%</td>
<td>1.6%</td>
<td>TN and Her2 higher in UVA, but not significant in MVA</td>
</tr>
<tr>
<td>Gabos</td>
<td>17%</td>
<td>3.8-10%</td>
<td>TN had higher HR compared to other subtypes (4.72, 1.53-14.92, p = .007)</td>
</tr>
<tr>
<td>Li</td>
<td>38%</td>
<td>20%</td>
<td>Inflammatory Cases: TN higher LRR rate then other subtypes</td>
</tr>
</tbody>
</table>
## Local Control Triple Negative Studies 2006-2011


### Table 1 Details of eligible studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Country of origin</th>
<th>Year</th>
<th>Study duration</th>
<th>Median follow-up time (months)</th>
<th>Median age (years)</th>
<th>Number of patients analyzed</th>
<th>BCT (n)</th>
<th>Mastectomy (n)</th>
<th>Luminal (n)</th>
<th>Her2+ (n)</th>
<th>TN (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arvold et al.</td>
<td>USA</td>
<td>2011</td>
<td>1997–2006</td>
<td>85</td>
<td>*</td>
<td>1,434</td>
<td>1,434</td>
<td>0</td>
<td>1,208</td>
<td>55</td>
<td>171</td>
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<tr>
<td>Siponen et al.</td>
<td>Finland</td>
<td>2011</td>
<td>2001–2005</td>
<td>57</td>
<td>58</td>
<td>1,281</td>
<td>1,281</td>
<td>0</td>
<td>1,178</td>
<td>23</td>
<td>80</td>
</tr>
<tr>
<td>Mersin et al.</td>
<td>Turkey</td>
<td>2011</td>
<td>2004–2008</td>
<td>44</td>
<td>49</td>
<td>1,101</td>
<td>0</td>
<td>1,101</td>
<td>913</td>
<td>82</td>
<td>106</td>
</tr>
<tr>
<td>Meyers et al.</td>
<td>USA</td>
<td>2011</td>
<td>1997–2005</td>
<td>55</td>
<td>48</td>
<td>149</td>
<td>49</td>
<td>100</td>
<td>80</td>
<td>20</td>
<td>49</td>
</tr>
<tr>
<td>Wong et al.</td>
<td>Singapore</td>
<td>2011</td>
<td>1989–2007</td>
<td>72</td>
<td>49</td>
<td>413</td>
<td>413</td>
<td>0</td>
<td>323</td>
<td>34</td>
<td>56</td>
</tr>
<tr>
<td>Millar et al.</td>
<td>Australia</td>
<td>2009</td>
<td>*</td>
<td>84</td>
<td>61</td>
<td>482</td>
<td>482</td>
<td>0</td>
<td>417</td>
<td>13</td>
<td>52</td>
</tr>
<tr>
<td>Solin et al.</td>
<td>USA</td>
<td>2009</td>
<td>1990–2003</td>
<td>47</td>
<td>55</td>
<td>519</td>
<td>519</td>
<td>0</td>
<td>370</td>
<td>59</td>
<td>90</td>
</tr>
<tr>
<td>Kyndt et al.</td>
<td>Denmark</td>
<td>2008</td>
<td>1982–1990</td>
<td>204</td>
<td>*</td>
<td>996</td>
<td>0</td>
<td>996</td>
<td>724</td>
<td>120</td>
<td>152</td>
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<tr>
<td>Freedman et al.</td>
<td>USA</td>
<td>2008</td>
<td>1990–2006</td>
<td>44</td>
<td>54</td>
<td>753</td>
<td>753</td>
<td>0</td>
<td>600</td>
<td>55</td>
<td>98</td>
</tr>
<tr>
<td>Ihemeladu et al.</td>
<td>USA</td>
<td>2008</td>
<td>1998–2005</td>
<td>*</td>
<td>*</td>
<td>309</td>
<td>131</td>
<td>178</td>
<td>207</td>
<td>34</td>
<td>68</td>
</tr>
<tr>
<td>Hafty et al.</td>
<td>USA</td>
<td>2006</td>
<td>1980–2003</td>
<td>95</td>
<td>*</td>
<td>482</td>
<td>482</td>
<td>0</td>
<td>365</td>
<td>*</td>
<td>117</td>
</tr>
</tbody>
</table>

*BCT* breast conserving therapy, *Luminal* Luminal A and B subtype, *HER2+* HER2/neu-overexpressing, *TN* triple negative, % all percentages are of number of patients analyzed within the study, * data not reported or not extractable from publication
**MAST**

**LUM vs H2N**

**MAST**

**LUM vs TN**

**MAST**

**H2N vs TN**
Inflammatory Breast
Local Relapse in TN

Li et al. Oncologist, 16:12, 2011

Figure 2. Rate of locoregional recurrence according to breast cancer subtype.

Abbreviations: ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.
• While triple negative cancers may be at higher risk for local relapse than other subtypes when treated with CS+RT...

• They may not fare better treated by mastectomy
Locoregional recurrence–free survival in triple-negative breast cancer T1-2N0 treated with breast-conserving therapy (BCT) and modified radical mastectomy (MRM) for (A) unmatched and (B) matched data sets.

Abdulkarim B S et al. JCO 2011;29:2852-2858
Triple Negative Early Stage Breast Cancers
Editorial in JCO by Pignol commenting on prior study

• The role of adjuvant radiotherapy following mastectomy in early stage T1/2 N0 Triple Negative Breast Cancer Requires Further study
• Mastectomy as more aggressive treatment for TNBC may be wrong
• That PMRT is not indicated in T1-2 N0 TNBC may be an oversimplification
Randomized Trial of PMRT in TNBC

Wang et al. Radiother Oncol 2011

- 681 women with TNBC post mastectomy randomized to PMRT or No PMRT
- Majority of women (> 80%) were node negative
- All received conventional chemotherapy (CMF/CAF) with RT beginning 2-3 weeks after CTX.
- Standard PMRT to CW and regional nodes as indicated 50 Gy in 25 fractions
- Median Follow-up was 86.5 Months
Randomized Trial Results: PMRT in TNBC
Recurrence free Survival

Wang et al. Radiother Oncol 2011

Graph showing recurrence-free survival over time with different treatment regimens. The graph indicates that the combination of chemotherapy and radiation (Chemo plus Radio) has a better recurrence-free survival rate compared to chemotherapy alone (Chemorapy). The hazard ratio (HR) is 0.78 with a 95% confidence interval (CI) of 0.72 to 0.98, and the p-value is 0.02.

No. at Risk
Chemotherapy: 315 308 286 273 246 235
Chemo plus: 366 364 348 340 329 323
Radio: 315 308 286 273 246 235

The Cancer Institute of New Jersey
Bringing research to life.
Randomized Trial Results: PMRT in TNBC

Overall Survival

Wang et al. Radiother Oncol 2011

Graph showing the overall survival rates for chemotherapy, chemotherapy plus radiotherapy, with the following details:

- **HR**: 0.83
- **95% CI**: 0.74 to 0.97
- **P**: 0.03

By year:
- **Chemotherapy**: 315 311 292 279 256 248
- **Chemotherapy plus Radio**: 366 365 357 346 339 331
Molecular Subtypes and Local Relapse: 
Luminal

• The bulk of evidence suggests that ER/PR+ Her2/neu negative tumors, commonly classified as Luminal A fare well with standard therapy

• Luminal B also appears to fare well though there are conflicting reports depending on how one determines Luminal B status
• Her2/neu expression is likely a risk factor for local relapse and relative radiation resistance: HOWEVER...

• The risk of HER2/neu positive tumors for local relapse is likely reduced by trastuzumab and perhaps other anti-Her2 agents

• In the current era, early data indicates that any increased risk of local relapse is offset by Trastuzumab
Molecular Subtypes and Local Control: Triple Negative

- Post Mastectomy TN is likely a risk factor for LR and should be considered in decision making regarding RT
- Post BCS+RT TN likely do worse then luminal A but similar to Her2/neu in the pre-Trastuzumab era
• The weight of evidence suggests that triple negative breast cancers likely have a higher rate of local relapse in selected patients when compared to luminal subtypes or HER2 subtypes treated with trastuzumab

• Can we enhance local control by combining RT with other agents in selected triple negative breast cancers at high risk for local relapse
Strategies to enhance local control in addition to RT for BC Subtypes

- Estrogen/Progesterone Positive-Hormonal Therapy
- Her2/neu expressing-Trastuzumab
- Any Subtypes-Systemic chemo
- Triple Negative-???-Novel Agents, Parp inhibitors, platinum Based Chemo

Extrapolating from the HER2/neu-Herceptin story, a targeted agent against triple negative breast cancers could solve any issues related to higher local relapse rates among triple negative breast cancers.
Triple Negative (basal like) cancers and radiation sensitivity

• Triple Negative Cancers are likely more aggressive locally and systemically-But are they Less “Radiation Sensitive”??

• Triple negative breast cancers should in fact be “sensitive” or hypersensitive to RT and other DNA damaging agents if they are truly “BRCA-like” tumors

• Why would local relapse, particularly after RT be higher in triple negative basal-like or BRCA like cancers

• There are likely subsets of triple negative breast cancers that are at higher risk-identifying those cases is an area ripe for investigation.
BRCA1-like (TN) Cancers and radiation sensitivity
Triple Negative (basal like) cancers and radiation sensitivity

- Are there subsets of triple negative breast cancers that are more (or less) sensitive to radiation therapy
- Can we manipulate the tumor environment to sensitize resistant tumors to radiation
53BP1 and Radiation Repair

IR → γ-H2AX → 53BP1 → ATM → BRCA1

- Ser15
- Ser966
- S/T-Q
- Thr68

- p53
- SMC-1
- Other targets
- Chk2

G1-phase arrest
Replication origin firing
G2 checkpoint activation
53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers

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Loss of 53BP1 Is a Gain for BRCA1 Mutant Cells

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DOI 10.1016/j.cell.2010.04.021

Mutations in BRCA1 predispose to tumorigenesis presumably from the inability to accurately repair DNA double-strand breaks by homologous recombination. Two new papers shed light on how loss of the DNA damage response protein 53BP1 reverses phenotypes of BRCA1 mutant cells, with potential clinical implications.

53BP1 Inhibits Homologous Recombination in Brca1-Deficient Cells by Blocking Resection of DNA Breaks

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53BP1 loss makes basal tumors less sensitive to DNA damaging agents


Figure 1: Inactivation of 53BP1 rescues proliferation defects and drug sensitivity of Brca1-null ES cells. (a) Schematic overview of mutant alleles in R26CreERT2 Brca1SCola and R26CreERT2 Brca1Δ/Δ ES cells. Before 4OHT-mediated induction of the CreERT2 recombinase, R26CreERT2 Brca1SCola cells are BRCA1 proficient and puromycin sensitive. Addition of 4OHT leads to CreERT2-mediated deletion of Brca1 exons 5 and 6, resulting in BRCA1 inactivation and concomitant expression of puromycin from the PGK promoter, thereby enabling selection of BRCA1-deficient R26CreERT2 Brca1Δ/Δ ES cells. (b) Western blot analysis of 53BP1 expression in R26CreERT2 Brca1SCola ES cells nontransduced or transduced with two independent lentiviral shRNA vectors against 53bp1, after treatment with 4OHT to delete the Brca1SCola allele. (c) Crystal violet staining of nontransduced R26CreERT2 Brca1SCola ES cells treated with 4OHT and stably transduced with lentiviral vectors expressing a control nontargeting shRNA (NT) or two independent shRNAs against 53bp1. (d,e) Susceptibility of R26CreERT2 Brca1SCola ES cells untreated or treated with 4OHT to DNA cross-linking agents cisplatin (d) or mitomycin C (e). Cell viability was measured after 4 d. Mean ± s.d. is shown from three independent measurements.
Deletion 53BP1 Reverses Sensitivity of BRCA1 def cells to PARP inhibitors

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Figure 2. Deletion of 53BP1 Reverses Sensitivity of Brca1<sup>−/−</sup> Cells to PARPi and Camptothecin

(A) Analysis of genomic instability in metaphases from B cells treated with 0, 10 nM, and 1 μM PARP inhibitor. Charts show the number of radial chromosomes, chromatid breaks, and chromosome breaks per 100 metaphases (n = 50 metaphases analyzed in each case). Note that genomic instability in Brca1<sup>−/−</sup> cells is independent of p53 status, and equivalent results were seen in Brca1<sup>−/−</sup>p53<sup>−/−</sup> and Brca1<sup>−/−</sup>p53<sup>+/−</sup> cells.

(B) Western blot showing Kap1 phosphorylation in B cells from the indicated genotypes treated with PARP inhibitor or 5Gy ionizing radiation.

(C) Analysis of genomic instability in metaphases from B cells treated with 4 nM camptothecin (CPT). B cells were cultured overnight with CPT prior to fixation and preparation of metaphase slides.
53BP1 and Radiation/Chemosensitivity in TN/BRCA like Cancers

- Collectively these basic science studies suggest that BRCA like cancers with 53BP1 intact are highly sensitive to RT and other agents.
- Loss of 53BP1 in these cancers rescues the cells ability to repair DNA damage-making the cells theoretically more resistant to radiation therapy (and likely PARP inhibitors, MMC, Cis-Platin).
- Hypothesis: TN Cancers which are deficient in 53BP1 may be more resistant to radiation.
Methods: 53BP1 Immunohistochemistry

High expression vs Low Expression
Results: Local Recurrence by 53BP1 status

- Low 53BP1: 76.8%
- High 53BP1: 90.5%

P = .01

IBRFS (years)
• Among triple negative tumors 53BP1 was prognostic of local relapse
• Loss of 53BP was associated with a 27% risk of LR as opposed to a 6% risk with expression of 53BP1
• In patients with 53BP1 intact the risk of LR in triple negative was similar to luminal tumors
• Caveat: Small numbers and lack of a validation set
53BP1 and local relapse

• These preliminary hypothesis generating findings suggest that loss of 53BP1 may be an adverse prognostic factor for local and distant metastasis among triple negative breast cancers

• If confirmed, this may be considered in the selection and guidance of therapy for patients with triple negative cancers

• However, currently there are no clinically available agents to upregulate 53BP1 or specifically target 53BP1
Conclusions

• For a majority of triple negative cancers local control is acceptable with appropriate surgery, postoperative radiation and systemic chemotherapy...however

• Triple negative breast cancers remain at higher risk of local relapse in selected circumstances

• Research efforts and strategies to enhance local control in these cancers is needed
Acknowledgements

- Breast Cancer Research Foundation
- Haffty Laboratory: Hao Wu, Devora Schiff, Residents and Students
- Sridar Ganesan Laboratory
- Kim Hirshfield Laboratory
Thank you!

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