S4-05
Exome sequencing identifies shift in TP53 and PIK3CA mutation status after paclitaxel-based neoadjuvant chemotherapy in breast cancer

Dr. Shao: Nothing to disclose.
Dr. Jiang: Nothing to disclose.
Dr. Yu: Nothing to disclose.

Loss of TP53 and PIK3CA mutations after neoadjuvant chemotherapy defines favorable prognostic biomarkers in breast cancer

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Background

- **Neoadjuvant chemotherapy (NCT)**
  - Standard of care for locally advanced breast cancer
  - Both pre- and post-chemotherapy samples can be obtained

- **Genetic changes in tumor after NCT**:
  - Different study designs have been employed
    - Genomes sequencing: after NCT (Shao et al. 2012 SABCS)

- **Genetic evolution** might occur in breast cancer under NCT and could have impact on **prognosis**

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**Background**

- **TP53** and **PIK3CA** are the most frequently mutated genes in breast cancer

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**Comprehensive molecular portraits of human breast tumours**

The Cancer Genome Atlas Network

We analysed primary breast cancers by genomic DNA copy number arrays, DNA methylation, exome sequencing, messenger RNA arrays, microRNA sequencing and reverse-phase protein arrays. Our ability to integrate information across platforms provided key insights into previously defined gene expression subtypes and demonstrated the existence of four main breast cancer classes when combining data from five platforms, each of which shows significant molecular heterogeneity. Somatic mutations in only three genes (TP53, PIK3CA and GATA3) occurred at >10% incidence across all breast cancers; however, there were numerous subtype-associated and novel gene mutations including the enrichment of specific mutations in GATA3, PIK3CA and MAP3K1 with the luminal A subtype. We identified two novel TCGA. Comprehensive molecular portraits of human breast tumors. (2012) *Nature*
**Background**

- Whole exome sequencing revealed *loss of TP53 and PIK3CA mutations* after NCT in two pts

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locally advanced basal-like BC</td>
<td>Decrease to 50% in diameter</td>
</tr>
</tbody>
</table>

**Weekly PC regimen**

- Cancer tissues microdissection
- Compare DNA exomes

**Unpublished data**

**Hypothesis**

- Loss of *TP53 and PIK3CA mutations* might be a *common phenomenon* after NCT and serve as prognostic biomarkers
Methods

Patients

- Neoadjuvant cohorts (locally advanced breast cancer)
  - Cohort 1: 206 non-pCR pts — Training cohort
  - Cohort 2: 158 non-pCR pts — Validation cohort
    - 4 cycles of weekly PC (paclitaxel and carboplatin) regimen
    - Pathologic response (the Miller-Payne scoring system):
      - Grade 5 = pCR
      - Grade 1-4 = non-pCR

- Adjuvant cohort (operative breast cancer)
  - Cohort 3: 81 pts
    - Prior surgical resection + adjuvant chemotherapy

Methods

- Sample collection and processing
  - Cohort 1 & 2:
    - Matched samples before and after NCT
    - Macrodisssection to avoid the influence of stroma → tumor cells
      - > 80% → mutation analysis
  - Cohort 3:
    - Surgical specimens
    - Laser-captured microdissection → 30 to 40 tumor foci for each case → mutation analysis

- Mutation analysis
  - DNA from tumor tissues and paired blood DNA
  - Sanger sequencing of all exons of TP53 and PIK3CA
  - Detected mutations were confirmed by pyrosequencing
Methods

Statistical analysis
- Change in mutation status
  - McNemar test
- Association between mutation loss and NCT response
  - Cochran-Armitage trend test

Survival
- Kaplan-Meier plot
- Cox proportional hazards model

Results

Part I
- Prevalence of mutation shift

Part II
- Influence of mutation shift on tumor response

Part III
- Influence of mutation shift on patient survival

Part IV
- Intratumoral heterogeneity of mutations
PART I: Prevalence of mutation shift

Distribution of mutations in pre-treatment tumors
- Basal-like status was associated with \( TP53 \) mutations while luminal status correlated with \( PIK3CA \) mutations.
- Mutation status of \( TP53 \) or \( PIK3CA \) had no influence on patient survival.

**TP53 mutations**

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1</th>
<th>Cohort 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>10%</td>
<td>12%</td>
</tr>
<tr>
<td>Luminal B</td>
<td>20%</td>
<td>25%</td>
</tr>
<tr>
<td>HER2+</td>
<td>10%</td>
<td>15%</td>
</tr>
<tr>
<td>Basal-like</td>
<td>40%</td>
<td>30%</td>
</tr>
</tbody>
</table>

**PIK3CA mutations**

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1</th>
<th>Cohort 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
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<tr>
<td>HER2+</td>
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<td>15%</td>
</tr>
<tr>
<td>Basal-like</td>
<td>40%</td>
<td>40%</td>
</tr>
</tbody>
</table>

**PART I: Prevalence of mutation shift**

Significant decrease in mutation rates after NCT in both cohorts \( (P<0.001 \) for both cohorts).

**Cohort 1**

<table>
<thead>
<tr>
<th></th>
<th>Pre-NCT</th>
<th>Post-NCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT</td>
<td>51</td>
<td>25</td>
</tr>
<tr>
<td>WT</td>
<td>156</td>
<td>181</td>
</tr>
</tbody>
</table>

**Cohort 2**

<table>
<thead>
<tr>
<th></th>
<th>Pre-NCT</th>
<th>Post-NCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT</td>
<td>42</td>
<td>20</td>
</tr>
<tr>
<td>WT</td>
<td>116</td>
<td>138</td>
</tr>
</tbody>
</table>
PART II: Mutation shift & tumor response

- Loss of mutation was significantly associated with a better pathological response (Miller-Payne grade 3 or 4) ($P<0.001$ for both cohorts)

![](image1)

PR: partial response, Miller-Payne grade 3 or 4

PART III: Mutation shift & patient survival

- Loss of mutation indicates better survival

Cohort 1 (training)

- Kaplan-Meier plot

![](image2)

- Cox proportional hazards model (MT-to-WT vs WT-to-WT)
  - HR=0.62; 95% CI, 0.23 to 0.84; $P=0.004$
PART III: Mutation shift & patient survival

Validated association between loss of mutation and improved survival in Cohort 2

Cohort 2 (validation)

Kaplan-Meier plot

B

DFS log-rank $P = .038$

Cohort 2
Percent survival (%)

0 20 40
Disease-free survival (months)
60 80 100
0 24 48 72 96

OS log-rank $P = .058$

Cohort 2
Percent survival (%)

0 20 40
Overall survival (months)
60 80 100
0 24 48 72 96

Cox proportional hazards model (MT-to-WT vs WT-to-WT)

HR = 0.71; 95% CI, 0.34 to 0.92; $P = 0.011$

Loss of TP53 and PIK3CA mutations is associated with improved survival

QUESTION: What is the biological nature of mutation shift?
PART IV: Intratumoral heterogeneity

- Microdissection and Sanger sequencing

28.4% of the samples (23 of 81) had intratumoral heterogeneity in TP53 or PIK3CA mutations

Both mutant and non-mutant cancer cells coexist in the same tumor

Partially explain the genetic basis of mutation shift after chemotherapy
Discussion

Tumor
Somatic change

MT-to-MT

MT-to-WT

Neoadjuvant chemotherapy

Unfavorable prognosis

Favorable prognosis

- Tumor cells with chemo-resistant TP53 or PIK3CA mutations
- Tumor cells with chemo-sensitive TP53 or PIK3CA mutations
- Tumor cells without TP53 or PIK3CA mutation

Limitations

- Lack of sufficient pre-NCT tumor tissues for microdissection and mutation analysis
- Molecular mechanisms resulting in the chemotherapy-related loss of mutation
  - taxanes or platinum?
  - Clonal evolution?
- Validation in a prospective cohort or a clinical trial
Preliminary conclusions

Chemotherapy may affect somatic mutation status of breast cancer, lowering the number of \textit{TP53} and \textit{PIK3CA} mutations.

Loss of \textit{TP53} and \textit{PIK3CA} mutations are associated with better response to NCT and improved survival.

Acknowledgement

- Yi-Zhou Jiang
- Ke-Da Yu (experiments)
- Jing Bao
- Jin Shen (study samples)

Thanks! Welcome to Shanghai!