A Molecular Portrait of Asian Breast Cancer:

Multi-Omics and Immune Profiling of a Prospective Breast Cancer Cohort Enriched in Young, Premenopausal Patients

Zhengyan ‘George’ Kan
Pfizer Oncology Research, San Diego, CA
Disclosure

- I am an employee of Pfizer Inc.
- I own Pfizer stocks.
Collaboration between SMC and Pfizer

Dr. Yeon Hee Park

Associate Professor
Division of Hematology-Oncology,
Department of Medicine,
Samsung Medical Center,
Sungkyunkwan University School of Medicine,
Seoul, Korea
Outline

• Background on Asian young, pre-menopausal breast cancer (YBC)
• Genomic landscape of Asian YBC and OBC
• Comparing molecular characteristics between YBC and OBC
• Immune-oncology (IO) profiling using expression signature and histopathological analyses
The Proportion of YBC is Significantly Higher in East Asia than in the West

South Korea: KBCS registry (2008)
Japan: Cancer statistics in Japan (2007)
USA: SEER data (2004-2008)
Poor Outcome of HR+ Breast Cancer at Very Young Age is due to Tamoxifen Resistance

Introduction

• The proportion of YBC (age ≤ 40) among BC in East Asia is estimated to be 16-32%, significantly higher than the 7% reported in Western countries.

• Breast cancers (BC) in younger, premenopausal patients (YBC) tend to be more aggressive with worse prognosis, higher chance of relapse and poorer response to endocrine therapies compared to breast cancers in older patients (OBC).

• Genomic and molecular characterizations have deepened our understanding of breast cancer biology, however, the molecular bases of Asian YBC remains poorly characterized.
Study Work Flow

Clinic

Patient Consent → Tumor Tissue

Tumor Tissue → YBC (n = 74)

Tumor Tissue → OBC (n = 60)

Pathology

Pathology QC → DNA/RNA Extraction

DNA/RNA Extraction → Histopathology

Histopathology → IO Analysis

Multi-omics Profiling (SGI)

DNA/RNA QC

DNA/RNA QC → RNASEQ (n = 115)

DNA/RNA QC → WES (n = 133)

DNA/RNA QC → CancerScan

Data Analysis

Computational Pipelines

Gene Expression (RSEM)

Somatic Mutations (VarScan2)

Copy Number Variation (ExomeCNV)
• We identified molecular subtypes using three methods: ER and HER2 immunohistochemistry analyses (IHC); gene expression classifier called PAM50; naïve Bayesian classifier (NMC) based on *ESR1*, *PGR* gene expression and *ERBB2* copy number data.
• A consensus classification was derived based on all three classifications, which are 92% concordant.

[Consensus classification diagram showing OBC and YBC classification with corresponding subtypes like ER+, ER+/HER2+, HER2+, TN, N/A, LumA, LumB, Normal]
Molecular Subtype Comparison

**YBC**
- 31 (56.4%)
- 9 (16.4%)

**OBC**
- 20 (48.3%)
- 9 (15.0%)

ERBB2 copy number vs. ER/PR expression (TPM)
## Significantly Mutated Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th># Mut. samples</th>
<th>Mut. Freq. (n = 133)</th>
<th>Mut. Rate (Mb)</th>
<th>p-value</th>
<th>q-value</th>
<th>Mut. Freq. (TCGA)</th>
<th>Rank (TCGA)</th>
<th>Gene Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>63</td>
<td>47.37%</td>
<td>153.34549</td>
<td>0</td>
<td>0</td>
<td>36.9%</td>
<td>1</td>
<td>tumor protein p53</td>
</tr>
<tr>
<td>GATA3</td>
<td>18</td>
<td>13.53%</td>
<td>55.74866</td>
<td>8.88E-16</td>
<td>8.38E-12</td>
<td>10.7%</td>
<td>3</td>
<td>GATA binding protein 3</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>39</td>
<td>29.32%</td>
<td>40.9562</td>
<td>3.77E-15</td>
<td>2.37E-11</td>
<td>35.5%</td>
<td>2</td>
<td>phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha</td>
</tr>
<tr>
<td>CBFB</td>
<td>6</td>
<td>4.51%</td>
<td>30.50222</td>
<td>4.19E-08</td>
<td>0.000198</td>
<td>1.6%</td>
<td>13</td>
<td>core-binding factor, beta subunit</td>
</tr>
<tr>
<td>PTEN</td>
<td>4</td>
<td>3.01%</td>
<td>9.93236</td>
<td>1.39E-06</td>
<td>0.00525</td>
<td>3.4%</td>
<td>9</td>
<td>phosphatase and tensin homolog</td>
</tr>
<tr>
<td>NF1</td>
<td>7</td>
<td>5.26%</td>
<td>2.88505</td>
<td>6.55E-05</td>
<td>0.195</td>
<td>2.8%</td>
<td>25</td>
<td>neurofibromin 1</td>
</tr>
<tr>
<td>ARID1A</td>
<td>6</td>
<td>4.51%</td>
<td>3.28988</td>
<td>7.23E-05</td>
<td>0.195</td>
<td>2.4%</td>
<td>N/A</td>
<td>AT rich interactive domain 1A (SWI-like)</td>
</tr>
</tbody>
</table>
Landscape of Genomic Alterations

<table>
<thead>
<tr>
<th>Alteration Type</th>
<th>YBC</th>
<th>OBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>amplification</td>
<td>37%</td>
<td>60%</td>
</tr>
<tr>
<td>deletion</td>
<td>26%</td>
<td>33%</td>
</tr>
<tr>
<td>missense</td>
<td>28%</td>
<td>28%</td>
</tr>
<tr>
<td>nonsense</td>
<td>14%</td>
<td>15%</td>
</tr>
<tr>
<td>stop_lost</td>
<td>12%</td>
<td>12%</td>
</tr>
</tbody>
</table>

Alteration Type
- essential splice_site
- frameshift Coding
- inframe indel
- amplification
- deletion
- mutation
- missense
- nonsense
- stop lost
Mutations More Prevalent in OBC than in YBC

\[ p = 0.0246 \]
CNVs More Prevalent in OBC than in YBC

Chromosome instability (CIN) score

OBC

YBC

BRCA1/2 Mut.

WT

MUT

ER+

ER+/HER2+

HER2+

TN

14
CNV More Prevalent in OBC than in YBC
CNV More Prevalent in OBC than in YBC
OBC Tumors More Proliferative than YBC

$p = 0.00584$

Ki67 (%)

ER+  ER+/HER2+  HER2+  TN

n = 29  n = 9  n = 9  n = 13
n = 31  n = 9  n = 2  n = 13
What Pathways are Differentially Expressed in HR+ YBC vs. OBC?

MASRI RESISTANCE TO TAMOXIFEN AND AROMATASE INHIBITORS UP
Endocrine Therapy Resistance Signatures Up-regulated in YBC

Research Article

Genome-Wide Analysis of Aromatase Inhibitor-Resistant, Tamoxifen-Resistant, and Long-Term Estrogen-Deprived Cells Reveals a Role for Estrogen Receptor

Selma Masri,¹ Sheryl Phung,¹ Xin Wang,¹ Xiwei Wu,² Yate-Ching Yuan,² Lawrence Wagman,³ and Shiuan Chen¹

¹Department of Surgical Research, ¹Division of Information Sciences, and ²Department of General Oncologic Surgery, Beckman Research Institute of the City of Hope, Duarte, California
Oxidative Phosphorylation Pathways Up-regulated in HR+ YBC vs. OBC
Cell Cycle and Proliferation Pathways Up-regulated in HR+ OBC vs. YBC
Immune and Inflammatory Pathways Up-regulated in HR+ OBC vs. YBC
Immuno-oncology (IO) Therapies

- Tumor antigens may be recognized by immune surveillance and activates cell killing by tumor infiltrating cytotoxic T lymphocytes (CTL).
- T-cell responses are inhibited by immune checkpoint pathways mediated by CTLA4, PD1/PD-L1 etc.
- Immune checkpoint blockade by IO therapies amplifies anti-tumor immune responses - Nivolumab (αCTLA4), Pembrolizumab (αPD1), Avelumab (αPDL1).

Chen & Mellman. *Oncology meets Immunology.* 2013. Immunity

Classification of TIL Subtypes based on Immune Expression Signature
Cytolytic Activity Varies across TIL Subtypes, Normal Breast Tissues and Cell lines

The diagram illustrates the CYT score (GZMA, PRF1) distribution across different immune classes (high, low, medium, quiet) for Normal Tissues and Cell lines.
OBC Seem More Immunogenic than YBC
- We performed H&E staining and immunohistochemistry (IHC) analyses of three TIL markers (CD4, CD8 and CD45) on 111 tumors.

- TIL score was calculated as $\log_{10} T$, where $T$ is the average TIL count from 5 separate regions of the H&E image.

- Digital imaging analysis was performed to quantify the relevant tissue area and the number of marker positive cells within those regions for each IHC slide.
  - Cell density is calculated as the number of marker positive cells in each mm$^2$ of analyzed tissue.
Histopathological and Expression IO Analyses are Highly Concordant
Histopathological and Expression IO Analyses are Highly Concordant
Summary

• We have performed the first large-scale multi-omics study of Asian breast cancer that would significantly contribute to the compendium of molecular data available for young, premenopausal breast cancer.

• The molecular landscape of Asian BC cohort is similar to Western BC studies in terms of major landmarks – ER over-expression, ERBB2 amplification and mutations in TP53, PIK3CA and GATA3.
  – We have identified ARID1A as a significantly mutated gene in breast cancer.

• There are potentially significant molecular distinctions between Asian YBC vs. OBC.
  – BRCA1/BRCA2 germline loss-of-function mutations are enriched in HR+ YBC.
  – YBC tumors appear to be less proliferative and smaller in size than OBC while OBC tumors harbor more mutations and copy number alterations than YBC.
  – Endocrine resistance signatures are up-regulated in HR+ YBC than in OBC, pointing to a molecular mechanism for tamoxifen resistance previously reported for Korean YBC.
  – Within the HR+ subtype, energy metabolism pathways such as oxidative phosphorylation appears to be up-regulated in YBC while cell cycle/proliferation and immune/inflammatory pathways appear to be up-regulated in OBC.

• Gene expression signature analyses have identified four subtypes of varying tumor-infiltrating lymphocyte (TIL) and cytolytic activities.
  – YBC seems to be less immunogenic than OBC with a lower mutation burden.
  – Expression-based and histopathological analyses of IO markers are strongly correlated.
Acknowledgements

Samsung Medical Center

• Yeon Hee Park
• Hae Hyun Jung
• Woosung Chung (SGI)
• Yoon-la Choi
• Jinho Kim (SGI)
• Woong-Yang Park (SGI)
• Se Kyung Lee
• Seok Won Kim
• Jeong Eon Lee
• Ji-Yeon Kim
• Jin Seok Ahn
• Young-Hyuck Im
• Seok Jin Nam

Pfizer Oncology Research

• Ying Ding
• Soonweng Cho (Johns Hopkins U.)
• Soo-Hyeon Lee (Pfizer Korea)
• Eric Powell
• Shibing Deng (Pfizer Statistics)
• Pamela Vizcarra
• Julio Fernandez
• Tim Nichols (Pfizer DSRD)
• Sripad Ram (Pfizer DSRD)
• Keith A. Ching
• Jadwiga Bienkowska
• Paul Rejto
• Yuan-Hua Ding (Pfizer ERDI)
Back Up


Clinical Characteristics of Western YBC

Box 3 | Clinical characteristics in women <40

- High risk of local recurrence
- Short median time from diagnosis to local recurrence
- High risk of mortality following local recurrence
- High risk of contralateral breast cancer
- High proportion oestrogen receptor-negative or progesterone receptor-negative disease
- High proportion of HER2-positive disease
- High proportion TP53-positive tumours
Contrary to previous reports, Asian YBC is enriched in Luminal A and HER2+ subtypes. In addition, Asian YBC is not significantly enriched in TNBC. Asian YBC is dominated by HR+ diseases - 77% including both ER+ and ER+/HER2+.

### IHC/Clinical

<table>
<thead>
<tr>
<th></th>
<th>OBC</th>
<th>OBC (%)</th>
<th>YBC</th>
<th>YBC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+</td>
<td>28</td>
<td>46.7%</td>
<td>46*</td>
<td>62.2%</td>
</tr>
<tr>
<td>ER+HER2+</td>
<td>7</td>
<td>11.7%</td>
<td>10</td>
<td>17.9%</td>
</tr>
<tr>
<td>HER2+</td>
<td>10</td>
<td>16.7%</td>
<td>2</td>
<td>3.6%</td>
</tr>
<tr>
<td>TN</td>
<td>13</td>
<td>21.7%</td>
<td>13</td>
<td>23.2%</td>
</tr>
<tr>
<td>UA</td>
<td>2</td>
<td>3.3%</td>
<td>3</td>
<td>5.4%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( p = 0.08 \)

\( p = 0.006 \)

### PAM50

<table>
<thead>
<tr>
<th></th>
<th>OBC</th>
<th>OBC (%)</th>
<th>YBC</th>
<th>YBC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LumA</td>
<td>7</td>
<td>11.7%</td>
<td>21</td>
<td>38.2%</td>
</tr>
<tr>
<td>LumB</td>
<td>28</td>
<td>46.7%</td>
<td>16</td>
<td>29.1%</td>
</tr>
<tr>
<td>Her2</td>
<td>12</td>
<td>20.0%</td>
<td>3</td>
<td>5.5%</td>
</tr>
<tr>
<td>Basal</td>
<td>12</td>
<td>20.0%</td>
<td>14</td>
<td>25.5%</td>
</tr>
<tr>
<td>Normal</td>
<td>1</td>
<td>1.7%</td>
<td>1</td>
<td>1.8%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( p = 0.0011 \)

\( p = 0.06 \)

### Consensus

<table>
<thead>
<tr>
<th></th>
<th>OBC</th>
<th>OBC (%)</th>
<th>YBC</th>
<th>YBC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+</td>
<td>29</td>
<td>48.3%</td>
<td>44</td>
<td>59.5%</td>
</tr>
<tr>
<td>ER+HER2+</td>
<td>9</td>
<td>15.0%</td>
<td>10</td>
<td>17.9%</td>
</tr>
<tr>
<td>HER2+</td>
<td>9</td>
<td>15.0%</td>
<td>4</td>
<td>7.1%</td>
</tr>
<tr>
<td>TN</td>
<td>13</td>
<td>21.7%</td>
<td>16</td>
<td>28.6%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( p = 0.225 \)

\( p = 0.08 \)

\( p = 0.58 \)

### Consensus (RNASEQ n = 115)

<table>
<thead>
<tr>
<th></th>
<th>OBC</th>
<th>OBC (%)</th>
<th>YBC</th>
<th>YBC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+</td>
<td>29</td>
<td>48.3%</td>
<td>31</td>
<td>56.4%</td>
</tr>
<tr>
<td>ER+HER2+</td>
<td>9</td>
<td>15.0%</td>
<td>9</td>
<td>16.4%</td>
</tr>
<tr>
<td>HER2+</td>
<td>9</td>
<td>15.0%</td>
<td>2</td>
<td>3.6%</td>
</tr>
<tr>
<td>TN</td>
<td>13</td>
<td>21.7%</td>
<td>13</td>
<td>23.6%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( p = 0.456 \)

\( p = 0.056 \)

\( p = 0.83 \)
Genomic alterations enriched in YBC or OBC.

<table>
<thead>
<tr>
<th>Type</th>
<th>Gene</th>
<th>YBC (n=73)</th>
<th>OBC (n=60)</th>
<th>p-value</th>
<th>q-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic Mutation</td>
<td>TP53</td>
<td>27 (37.0%)</td>
<td>36 (60.0%)</td>
<td>0.0093</td>
<td>0.1395</td>
</tr>
<tr>
<td>Somatic Mutation</td>
<td>NF1</td>
<td>1 (1.4%)</td>
<td>6 (10.0%)</td>
<td>0.0456</td>
<td>0.293143</td>
</tr>
<tr>
<td>Somatic Mutation</td>
<td>CBFB</td>
<td>1 (1.4%)</td>
<td>5 (8.3%)</td>
<td>0.0903</td>
<td>0.507938</td>
</tr>
<tr>
<td>Somatic Mutation</td>
<td>PIK3CA</td>
<td>19 (26.0%)</td>
<td>20 (33.3%)</td>
<td>0.4444</td>
<td>1</td>
</tr>
<tr>
<td>Somatic Mutation</td>
<td>GATA3</td>
<td>11 (15.1%)</td>
<td>7 (11.7%)</td>
<td>0.6191</td>
<td>1</td>
</tr>
<tr>
<td>Somatic Mutation</td>
<td>PTEN</td>
<td>3 (4.1%)</td>
<td>1 (1.7%)</td>
<td>0.6266</td>
<td>1</td>
</tr>
<tr>
<td>Somatic Mutation</td>
<td>ARID1A</td>
<td>3 (4.1%)</td>
<td>3 (5.0%)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Amplification</td>
<td>MYC</td>
<td>0</td>
<td>5 (8.3%)</td>
<td>0.017</td>
<td>0.136</td>
</tr>
<tr>
<td>Amplification</td>
<td>ERBB2</td>
<td>10 (13.7%)</td>
<td>17 (28.3%)</td>
<td>0.0507</td>
<td>0.2028</td>
</tr>
<tr>
<td>Somatic Mutation</td>
<td>BRCA1</td>
<td>1 (1.4%)</td>
<td>0</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Germline LOF</td>
<td>BRCA1</td>
<td>3 (4%)</td>
<td>0</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Germline LOF</td>
<td>BRCA2</td>
<td>6 (8%)</td>
<td>3 (5.0%)</td>
<td>0.3533</td>
<td></td>
</tr>
<tr>
<td>Somatic/Germline</td>
<td>BRCA1/2</td>
<td>10 (13.7%)</td>
<td>3 (5.0%)</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>

TP53, NF1 protein-altering somatic mutations and MYC, ERBB2 amplifications are enriched in OBC. Loss-of-function (LOF) mutations affecting BRCA1 or BRCA2 are enriched in YBC.
### What Pathways are Differentially Expressed in HR+ YBC vs. OBC?

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Up-regulated</th>
<th>Database</th>
<th>Geneset</th>
<th># Genes</th>
<th>NES</th>
<th>FDR q-val</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR+ YBC</td>
<td>Hallmark</td>
<td>OXIDATIVE_PHOSPHORYLATION</td>
<td>199</td>
<td>-2.81478</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HR+ YBC</td>
<td>KEGG</td>
<td>PARKINSONS_DISEASE</td>
<td>113</td>
<td>-2.58741</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HR+ YBC</td>
<td>REACTOME</td>
<td>RESPIRATORY_ELECTRON_TRANSPORT</td>
<td>65</td>
<td>-2.52911</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HR+ YBC</td>
<td>Biocarta</td>
<td>PROTEASOME_PATHWAY</td>
<td>28</td>
<td>-2.48144</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HR+ YBC</td>
<td>KEGG</td>
<td>OXIDATIVE_PHOSPHORYLATION</td>
<td>116</td>
<td>-2.47474</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HR+ YBC</td>
<td>REACTOME</td>
<td>FORMATION_OF_ATP_BY CHEMIOSMOTIC_COUPLING</td>
<td>13</td>
<td>-2.32748</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HR+ YBC</td>
<td>Hallmark</td>
<td>ESTROGEN_RESPONSE_EARLY</td>
<td>200</td>
<td>-2.32048</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HR+ YBC</td>
<td>REACTOME</td>
<td>TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT</td>
<td>117</td>
<td>-2.21898</td>
<td>0.00328</td>
<td></td>
</tr>
<tr>
<td>HR+ YBC</td>
<td>KEGG</td>
<td>DRUG_METABOLISM_CYTOCHROME_P450</td>
<td>72</td>
<td>-2.21243</td>
<td>0.00285</td>
<td></td>
</tr>
<tr>
<td>HR+ YBC</td>
<td>Hallmark</td>
<td>ESTROGEN_RESPONSE_LATE</td>
<td>199</td>
<td>-2.13894</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>KEGG</td>
<td>LEISHMANIA_INFECTION</td>
<td>70</td>
<td>1.73803</td>
<td>0.03707</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>KEGG</td>
<td>PRIMARY_IMMUNODEFICIENCY</td>
<td>35</td>
<td>1.61149</td>
<td>0.09367</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>KEGG</td>
<td>INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION</td>
<td>46</td>
<td>1.60056</td>
<td>0.08103</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>KEGG</td>
<td>ALLOGRAFT_REJECTION</td>
<td>35</td>
<td>1.58956</td>
<td>0.07455</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>KEGG</td>
<td>NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY</td>
<td>61</td>
<td>1.56616</td>
<td>0.08697</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>KEGG</td>
<td>SYSTEMIC_LUPUSERYTHEMATOSUS</td>
<td>134</td>
<td>1.5536</td>
<td>0.09184</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>Hallmark</td>
<td>ALLOGRAFT_REJECTION</td>
<td>200</td>
<td>1.49757</td>
<td>0.06265</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>Hallmark</td>
<td>G2M_CHECKPOINT</td>
<td>200</td>
<td>1.46906</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>Hallmark</td>
<td>MYC_TARGETS_V2</td>
<td>58</td>
<td>1.4509</td>
<td>0.04217</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>Hallmark</td>
<td>E2F_TARGETS</td>
<td>199</td>
<td>1.41913</td>
<td>0.05067</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>Hallmark</td>
<td>MITOTIC_SPINDLE</td>
<td>199</td>
<td>1.41661</td>
<td>0.04119</td>
<td></td>
</tr>
<tr>
<td>HR+ OBC</td>
<td>Hallmark</td>
<td>INFLAMMATORY_RESPONSE</td>
<td>198</td>
<td>1.40403</td>
<td>0.04037</td>
<td></td>
</tr>
</tbody>
</table>

- **Energy metabolism**
- **Immune/inflammatory**
- **Estrogen response**
- **Cell cycle/proliferation**
GSEA Analyses Revealed Differentially Expressed Pathways in YBC vs. OBC

The enrichment plot ranks genes based on relative overexpression from OBC (left) to YBC (right) and marks the positions of the pathway genes by vertical lines.
GSEA Analyses Revealed Differentially Expressed Pathways in YBC vs. OBC

Expression patterns of all pathway genes in OBC and YBC samples are shown in the heatmap (F-G).
Classification of TIL Subtypes based on Immune Cell Expression Signature

- Tumor infiltrated lymphocytes (TIL) play important roles in tumor suppression and immuno-oncology (IO) therapies such as checkpoint inhibitors (αPD-L1).

- Using gene expression signatures representing distinct immune cell types [REF], we classified the cohort into four subtypes of varying TIL activities: high, medium, low and quiet.

- OBC tumors are significantly enriched (p-value: 0.01291) in the TIL-high subtype than YBC tumors, suggesting that OBC tumors are more immunogenic than YBC tumors.

- This is consistent with the observation that OBC exhibits higher burden of protein-altering somatic mutations than YBC samples (40 vs. 26, p-value: 0.034), presumably giving rise to more neoantigens.
“Big” Questions

• What are the molecular drivers of Asian BC?

• What are the differences between YBC and OBC?

• Can we learn something new about Breast Cancer in general?
What Pathways are Differentially Expressed in HR+ YBC vs. OBC?

KEGG_PROPOANOATE_METABOLISM
KEGG_BILE_ACID_METABOLISM
BILE_ACID_BILE_SALT_METABOLISM
MASRI RESISTANCE TO TAMOXIFEN AND AROMATASE INHIBITORS UP

KEGG_RETINOL_METABOLISM
KEGG_GLUTATHIONE_METABOLISM
INTEGRATION_OF_ENERGY_METABOLISM
KEGG_XENOBIOTIC_METABOLISM
Cytolytic Activity Varies with Chemotherapy Treatment Statuses

\[ p = 1 \times 10^{-5} \]

\[ p = 0.48 \]

TIL Subtype

- 1.high
- 2.medium
- 3.low
- 4.quiet

Oncology
A Pfizer Research Unit
IHC Cell Density vs. Gene Expression

CD8

CD163

CD4

CD45
Comparing IHC Cell Densities in YBC vs. OBC

- **CD8**: T test; \( P = 0.303929 \)
- **CD163**: T test; \( P = 0.009938 \)
- **CD4**: T test; \( P = 0.085674 \)
- **CD45**: T test; \( P = 0.313851 \)
TIL Score Higher in HR+ OBC vs. YBC

$P = 0.074754$