Disclosure Information

I hereby declare that I have had business or personal interests in the following industrial enterprises since 1 September 2016:

<table>
<thead>
<tr>
<th>Name of the enterprise / Nature of the interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
</tr>
</tbody>
</table>
Association between LAPTM4B gene copy number alterations and anthracyclin based chemotherapy in hormone receptor negative breast carcinomas

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2. University of Szeged, Department of Oncotherapy, Szeged-Hungary
3. MTA-SE-NAP, Brain Metastasis Research Group, 2nd Department of Pathology, Semmelweis University, Budapest- Hungary.
4. Harvard Medical School, Boston, MA, 02215, USA.
Introduction

Yulong Yin et al.  
Chem. Commun  
2015;51:14454-14457

Xiaojun Tan et al.  
EMBO J.  
2015;34:475-490
LAPTM4B in breast carcinomas – references

- LAPTM4B and YWHAZ contribute to de novo chemoresistance to anthracyclines and are permissive for metastatic recurrence. Overexpression of these two genes may predict anthracycline resistance and influence selection of chemotherapy. *Nat Med.* 2010 Feb;16(2):214-8. doi: 10.1038/nm.2090.


Aim of study

- To determine whether the presence of extra copies of LAPTM4B gene have negative predictive value to the efficacy of anthracycline based treatment in patients with hormone receptor negative (HR-) breast carcinomas.
Patients and methods

Tumor samples of 139 HR- breast cancer patients were enrolled in this study.
The study was ethically approved by the Semmelweis University Institutional Review Board (SE-IKEB 120/2013)

Cohort I.
• 69 core biopsies of HR- primary breast cancer patients treated with neoadjuvant chemotherapy
  – 50/69 patients were treated with anthracycline based neoadjuvant chemotherapy (mainly in combination with taxane).
  – 19/69 breast cancer patients representing the control group and receiving non-anthracycline based (mostly platinum in combination with taxane) chemotherapy.
Evaluation of the pathological response to neoadjuvant chemotherapy

Primary tumor (TR)
1: Pathological complete remission (pCR)
   a. no residual carcinoma or
   b. no residual invasive tumour but DCIS present.
2: Partial response to neoadjuvant therapy
   a. minimal residual disease (<10 % of tumour remaining)
   b. evidence of response with 10–50 % of tumour remaining
   c. >50 % of tumour cellularity remaining with some features of regression.
3: No evidence of response to therapy

Lymph nodes (NR)
1. No evidence of metastasis or regression.
2. Metastases not present but evidence of regression.
3. Metastasis present with evidence of regression.
4. Metastasis with no sign of regression.

pCR=TR1a,b and NR1,2

Cohort II.

- 4 TMA of 70 formalin fixed, paraffin embedded (FFPE) HR-breast carcinoma samples from surgically removed specimens.
  - 57/70 patients received anthracycline based (22.8% in combination with taxane) chemotherapy.
  - 13/70 as the control group received non-anthracycline based therapies.

Distant metastasis free survival (DMFS) was assessed and defined as the time elapsed between the first diagnosis of primary breast carcinoma to the date of appearance of any distant metastasis.
Interphase FISH reaction

- Interphase FISH technique was used to analyze the copy number status of *LAPTM4B* gene.
- Hybridization was performed with custom-made, Texas Red/FITC dual labelled LAPTM4B/CEN8q FISH probes (Abnova Corp., Taoyuan City, Taiwan).
FISH evaluation

- The total number of tumor cells analyzed in FISH reactions ranged from 50 to 210.
- Data derived from the FISH analysis included:
  - average LAPT M4B copy number/cell;
  - average CEN8 copy number/cell;
  - LAPT M4B/CEN8 ratio;
  - average LAPT M4B copy number/cell in amplified cell population;
  - percentage of polysomic or amplified cells.
Results
No extra copy of LAPT M4B gene in a TNBC case
Higher average *LAPTM4B* gene copy number observed in two TNBC cases treated with anthracycline
Higher average *LAPTM4B* gene copy number and higher CEN8 observed in a TNBC case
The association of average *LAPTM4B* copy number/cell with response to neoadjuvant chemotherapy

**AC-based**

- pCR (N=16)
- pPR (N=30)
- pNR (N=4)

**non AC-based**

- pCR (N=10)
- pPR (N=8)
- pNR (N=1)

- p=0.029
- p=0.035
- p>0.05
Average *LAPTM4B* copy number/cell in the adjuvant setting

**AC-based**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample Size (N)</th>
<th>Average Gene Copy Number</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-metastatic</td>
<td>34</td>
<td>2.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Distant metastatic</td>
<td>23</td>
<td>4.0</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Non AC-based**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample Size (N)</th>
<th>Average Gene Copy Number</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-metastatic</td>
<td>9</td>
<td>2.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Distant metastatic</td>
<td>4</td>
<td>2.0</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
DMFS in the AC-treated adjuvant cohort

- Cut-off value for poor prognosis was defined as follows: the ratio of amplified cell ($LAPTM4B/c8 > 2.0$) population is more than 15% and the average gene copy number is more than 2.5 per sample.

$\text{p}=0.044$

$\text{Exp(B)}: 3.794$
Conclusions

• Our results confirm the possible role of the \textit{LAPTM4B} gene in anthracycline resistance in HR- breast cancer.

• Alternative drug combinations without anthracyclines might be considered for those HR- breast cancer patients whose cancer harbors \textit{LAPTM4B} extra copies.
Thank you!

- Laura Vízkeleti
- Zoltán Szállási
- Orsolya Rusz
- Janina Kulka
- Orsolya Papp
- Gábor Lotz
- Kristóf Bende
- Csilla Szundi

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Anthracycline based group</th>
<th>Non-anthracycline based group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>50±15 (range: 26-79)</td>
<td>52±15 (range: 29-76)</td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>5 (11.4%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>39 (88.6%)</td>
<td>16 (94.1%)</td>
</tr>
<tr>
<td>IHC-based molecular types</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNBC</td>
<td>42 (95.5%)</td>
<td>15 (88.2%)</td>
</tr>
<tr>
<td>HER2+</td>
<td>2 (4.5%)</td>
<td>2 (11.8%)</td>
</tr>
<tr>
<td>Regional lymph node</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>11 (25.0%)</td>
<td>9 (52.9%)</td>
</tr>
<tr>
<td>N1</td>
<td>13 (29.5%)</td>
<td>6 (35.3%)</td>
</tr>
<tr>
<td>N2</td>
<td>15 (34.1%)</td>
<td>2 (11.8%)</td>
</tr>
<tr>
<td>N3</td>
<td>5 (11.4%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Pathological response to neoadjuvant therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCR</td>
<td>15 (34.1%)</td>
<td>10 (58.8%)</td>
</tr>
<tr>
<td>pPR</td>
<td>25 (56.8%)</td>
<td>6 (35.3%)</td>
</tr>
<tr>
<td>pNR</td>
<td>4 (9.1%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Chemotherapy regimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TE/TA/TAX+E/TAX+A/TAC</td>
<td>15 (34.1%)</td>
<td>-</td>
</tr>
<tr>
<td>TEX/FEC+T</td>
<td>25 (56.8%)</td>
<td>-</td>
</tr>
<tr>
<td>FEC</td>
<td>4 (9.1%)</td>
<td>-</td>
</tr>
<tr>
<td>T+CDDP</td>
<td>-</td>
<td>12 (70.6%)</td>
</tr>
<tr>
<td>T+CBP/TAX+CBP</td>
<td>-</td>
<td>5 (29.4%)</td>
</tr>
<tr>
<td>Therapy response</td>
<td>p (2-sided)</td>
<td>pCR (TR1)</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>avg. CN</td>
<td>0.029</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>avg. 8c</td>
<td>0.048</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>ratio</td>
<td>0.905</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>amplified cells (%)</td>
<td>0.384</td>
<td>21.4 ± 4.1</td>
</tr>
<tr>
<td>avg. CN in ampl.cells</td>
<td>0.140</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>ratio in ampl.cells</td>
<td>0.084</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>polysome cells (%)</td>
<td>0.023</td>
<td>28.0 ± 5.3</td>
</tr>
<tr>
<td>avg. 8c in poly.cells</td>
<td>0.077</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>deletion (%)</td>
<td>0.335</td>
<td>13.6 ± 3.1</td>
</tr>
<tr>
<td>diploid (%)</td>
<td>0.099</td>
<td>31.0 ± 3.0</td>
</tr>
<tr>
<td>monoploid (%)</td>
<td>0.027</td>
<td>7.6 ± 1.3</td>
</tr>
<tr>
<td>apparent ampl. (%)</td>
<td>0.050</td>
<td>13.4 ± 2.4</td>
</tr>
<tr>
<td>CN gain (%)</td>
<td>0.118</td>
<td>13.0 ± 2.1</td>
</tr>
<tr>
<td>CN gain + ampl (%)</td>
<td>0.820</td>
<td>34.4 ± 4.9</td>
</tr>
</tbody>
</table>

| N                | 16         | 4               |