Gene expression differences in lung cancer cell lines after treatment with a Notch inhibitor

DATA-ANALYSIS AND INTERPRETATION OF AN AFFYMETRIX MICROARRAY DATASET

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- Aim
- Study design
- Results and discussion
  - Array analysis
  - Statistical analysis
  - Pathway analysis
  - Gene ontology
  - Network analysis
- Conclusion
Introduction

- Leading cause of cancer-related deaths worldwide
- Lung cancer: NSCLC, SCLC
- Notch signaling
- Notch-1, Notch-3
- γ-secretase
- Cell lines: A549, H460a
Leading cause of cancer-related deaths worldwide: Lung cancer

Notch signaling: Notch-1, Notch-3

γ-secretase

Cell lines: A549, H460
Aim

- To identify differentially expressed genes and biological processes after treatment of the lung cancer cell lines A549 and H460a with a Notch inhibitor for 6 and 24 hours.
Study design

- A549 and H460a treated with Notch inhibitor or vehicle for 6 and 24 hours
- Quadruplicate
- 32 samples subdivided into 8 groups

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Duration (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 (A549)</td>
<td>RO492909</td>
<td>24</td>
</tr>
<tr>
<td>C1 (A549)</td>
<td>RO492909</td>
<td>6</td>
</tr>
<tr>
<td>C1 (A549)</td>
<td>Vehicle</td>
<td>24</td>
</tr>
<tr>
<td>C1 (A549)</td>
<td>Vehicle</td>
<td>6</td>
</tr>
<tr>
<td>C2 (H460a)</td>
<td>RO492909</td>
<td>24</td>
</tr>
<tr>
<td>C2 (H460a)</td>
<td>RO492909</td>
<td>6</td>
</tr>
<tr>
<td>C2 (H460a)</td>
<td>Vehicle</td>
<td>24</td>
</tr>
<tr>
<td>C2 (H460a)</td>
<td>Vehicle</td>
<td>6</td>
</tr>
</tbody>
</table>
Results and discussion
Array analysis (1)

- RNA degradation plot
Array analysis (2)

- PCA analysis after normalisation
PCA analysis after GCRMA normalization
Array analysis (3)

- Cluster dendrogram after normalisation
Cluster dendrogram of GCRMA normalized data

- C1 control for 24 hours
- C1 control for 6 hours
- C1 treated for 24 hours
- C1 treated for 6 hours
- C2 control for 24 hours
- C2 control for 6 hours
- C2 treated for 24 hours
- C2 treated for 6 hours
Array analysis (4)

- Before normalisation some outliers, but corrected after normalisation
- No arrays were excluded
Three comparisons:

1. A549_vs_H460a_t6_treated_corrected_for_control
2. A549_vs_H460a_t24_treated_corrected_for_control
3. t24_vs_t6_treated_A549_corrected_for_control_and_H460a

- P-value < 0.05
  - LogFC < -0.4 or LogFC > 0.4

- Adjusted p-value not used
A549 treated \leftrightarrow H460a treated

A549 control \leftrightarrow H460a control

6 h. corrected for

A549 treated \leftrightarrow H460a treated

A549 control \leftrightarrow H460a control

24 h. corrected for
A549 treated
A549 control
A549 control
A549 treated
6 h.

H460a treated
H460a control
H460a control
H460a treated
24 h.

corrected for

corrected for
Pathway analysis (1)

- First (combined) comparison
- 17 significant pathways (z-score >1.96)

<table>
<thead>
<tr>
<th>Pathway</th>
<th>positive (r)</th>
<th>measured (n)</th>
<th>total</th>
<th>%</th>
<th>Z Score</th>
<th>p-value (permuted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAPK Cascade</td>
<td>10</td>
<td>28</td>
<td>33</td>
<td>35.71%</td>
<td>4.5</td>
<td>0</td>
</tr>
<tr>
<td>Blood Clotting Cascade</td>
<td>6</td>
<td>20</td>
<td>25</td>
<td>30.00%</td>
<td>2.95</td>
<td>0.007</td>
</tr>
<tr>
<td>RB in Cancer</td>
<td>18</td>
<td>96</td>
<td>104</td>
<td>18.75%</td>
<td>2.83</td>
<td>0.005</td>
</tr>
<tr>
<td>Complement and Coagulation Cascades</td>
<td>10</td>
<td>50</td>
<td>67</td>
<td>20.00%</td>
<td>2.33</td>
<td>0.022</td>
</tr>
<tr>
<td>Oncostatin M Signaling Pathway</td>
<td>12</td>
<td>65</td>
<td>66</td>
<td>18.46%</td>
<td>2.24</td>
<td>0.029</td>
</tr>
</tbody>
</table>
Pathway analysis (2)

- Second (2x corrected) comparison
- 8 significant pathways (z-score >1.96)

<table>
<thead>
<tr>
<th>Pathway</th>
<th>positive (r)</th>
<th>measured (n)</th>
<th>total</th>
<th>%</th>
<th>Z Score</th>
<th>p-value (permuted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB in Cancer</td>
<td>15</td>
<td>96</td>
<td>104</td>
<td>15.63%</td>
<td>3.45</td>
<td>0.001</td>
</tr>
<tr>
<td>Interferon type I signaling pathways</td>
<td>8</td>
<td>50</td>
<td>57</td>
<td>16.00%</td>
<td>2.59</td>
<td>0.01</td>
</tr>
<tr>
<td>miRNA Biogenesis</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td>33.33%</td>
<td>2.58</td>
<td>0.02</td>
</tr>
<tr>
<td>Membrane Trafficking</td>
<td>10</td>
<td>73</td>
<td>173</td>
<td>13.70%</td>
<td>2.35</td>
<td>0.016</td>
</tr>
<tr>
<td>Sulfation Biotransformation Reaction</td>
<td>3</td>
<td>14</td>
<td>29</td>
<td>21.43%</td>
<td>2.17</td>
<td>0.027</td>
</tr>
<tr>
<td>DNA Damage Response (only ATM dependent)</td>
<td>11</td>
<td>89</td>
<td>101</td>
<td>12.36%</td>
<td>2.09</td>
<td>0.037</td>
</tr>
<tr>
<td>Leptin signaling pathway</td>
<td>8</td>
<td>60</td>
<td>62</td>
<td>13.33%</td>
<td>2.01</td>
<td>0.035</td>
</tr>
<tr>
<td>EGF/EGFR Signaling Pathway</td>
<td>17</td>
<td>158</td>
<td>163</td>
<td>10.76%</td>
<td>1.99</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Pathway analysis (3)

- Rb in cancer pathway
Examples of RB-E2F Complexes
inhibiting E2F-driven gene expression

Examples of RB-E2F Complexes
promoting E2F-driven gene expression
Pathway analysis (4)

- Downregulation of CDK2 $\rightarrow$ no inactivation of Rb
- Downregulation of CCND3 $\rightarrow$ no inactivation of Rb
- Notch inhibitor $\rightarrow$ cell cycle exit and active Rb
Pathway analysis (5)

- MAPK cascade pathway
MAPK Cascade

Growth and Mitogenesis

Nucleus

Title: MAPK Cascade
Availability: CC BY 2.0
Last modified: 10/17/2013
Organism: Homo sapiens
MAPK cascade plays a role in cancer
Interaction between Notch signaling and MAPK cascade
Pathway Analysis (7)

- Blood clotting cascade
- Oncostatin M and complement and coagulation cascades pathway
- Cytoscape
Blood clotting cascade

- Oncostatin M and complement and coagulation cascade pathway

Cytoscape
Pathway analysis (8)

- Downregulation of SERPINE1 $\Rightarrow$ tumor suppression, prevention of blood clotting
- Downregulation of JUNB $\Rightarrow$ involved in growth factor response
- Notch inhibition may lead to downregulation of SERPINE1 and JUNB
### Pathway analysis (9)

- Notch signaling pathways
- Z-score < 1.96

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Comparison 1</th>
<th>Comparison 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notch Signaling pathway WP61_70097</td>
<td>0.10</td>
<td>0.59</td>
</tr>
<tr>
<td>Notch Signaling pathway WP268_70096</td>
<td>-0.60</td>
<td>0.13</td>
</tr>
</tbody>
</table>
Pathway analysis (10)

- Downregulation of HES1 $\Rightarrow$ decreased proliferation and cell cycle exit
- Upregulation of PSEN2 $\Rightarrow$ reactivation of Notch signaling
Gene ontology (3)

- t24_vs_t6_treated_A549_corrected_for_control_and_H460a
- Significant processes, but not specific enough to use
- Results not used because too broad processes colored and results do not correspond to PathVisio
Significant processes, but not specific enough to use. Results not used because too broad. Processes colored and results do not correspond to PathVisio.
Network analysis (2)

- A549_vs_H460a_t24_treated_corrected_for_control
- Hub proteins: JUNB and SULT2B1
- JUNB: growth factor response, downregulation in cancer
- SULT2B1: sulfate conjugation, also significant pathway in PathVisio
- HES and SERPINE1
Hub proteins: JUNB and SULT2B1

JUNB: growth factor response, downregulation in cancer

SULT2B1: sulfate conjugation, also significant pathway in PathVisio

HES and SERPINE1
Network analysis (3)

- t24_vs_t6_treated_A549_corrected_for_control_and_H460a
- Hub proteins: SDF2L1 and UBAC1
- SDF2L1: induced by ER stress → associated with cancer
- UBAC: E3 ubiquitin-protein ligase, important in cancer
t24_ vs _t6_ treated _A549_ corrected for control and H460.

Hub proteins: SDF2L1 and UBAC1.

SDF2L1: induced by ER stress → associated with cancer.

UBAC: E3 ubiquitin-protein ligase, important in cancer.
Conclusion (1)

- QC report: no arrays excluded
- Statistical analysis: P-value < 0.05 logFC < -0.4 or > 0.4. Adjusted p-value not used.
- Pathway analysis: a lot differentially expressed genes. Association with cancer.
- Gene ontology: not specific enough to use.
- Network analysis: hub proteins associated with cancer.
Inhibition of Notch signaling affects cell cycle progression and causes a reduction in cancer development.

Further research is needed

- Role of Notch signaling in cancer
- Cell lines
- Notch inhibitors in cancer treatment
Thank you for your attention!

Questions?
Gene ontology (1)

- A549_vs_H460a_t6_treated_corrected_for_control
- No specific significant processes
No specific significant processes
Gene ontology (2)

- A546_vs_H460a_t24_treated_corrected_for_control
- Three quite significant processes
Three quite significant processes corrected for control.
Network analysis (1)

- A549\_vs\_H460a\_t6\_treated\_corrected\_for\_control
- Hub proteins: ZFP36 and JUNB
- ZFP36: RNA binding protein, interacts with AU-rich elements
- JUNB: role in growth factor response, downregulation in cancer
- HES1 and SERPINE1
A549 versus H460a treated for control

Hub proteins: ZFP36 and JUNB

ZFP36: RNA binding protein, interacts with AU-rich elements

JUNB: role in growth factor response, downregulation in cancer

HES1 and SERPINE1