Uncovering the mechanism of cell death induced by saporin delivered into cancer cells by an antibody fusion protein targeting the transferrin receptor

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Transferrin Receptor (TfR) mediates iron transport across the plasma membrane through receptor-mediated endocytosis of iron bound to transferrin.

The elevated expression of TfR on cancer cells, its ability to internalize, its central role in the cellular pathology of human cancer make this receptor an attractive target for the therapy of cancer.

T. R. Daniels et al., Clinical Immunology (2006) 121, 144-158
Anti-hTfR IgG3-Av

Exhibits intrinsic cytotoxic activity against malignant hematopoietic cell lines. Alters the trafficking of the TfR leading to downregulation of cell-surface TfR expression and lethal iron starvation.
Hypothesis

Loading anti-hTfR IgG3-Av with a biotinylated toxin as a warhead will increase the cytotoxicity of the fusion protein alone

and thus...

the antibody-avidin fusion protein is capable of a two pronged attack against malignant cells
Saponaria officinials toxin

Ribosome inactivating toxin that blocks protein synthesis

Saporin and ricin have the same catalytic activity (N-glycosidase that removes an adenine residue from the ribosomal RNA)

Conjugation of an anti–transferrin receptor IgG3-avid fusion protein with biotinylated saporin results in significant enhancement of its cytotoxicity against malignant hematopoietic cells

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Cytotoxicity of anti-hTfR IgG3-A alone and complexed to b-SO6

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>anti-hTfR IgG3 Av</th>
<th>anti-hTfR IgG3 Av /b-SO6</th>
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<tbody>
<tr>
<td>IM-9</td>
<td>0.7</td>
<td>5.8</td>
<td>5.2</td>
</tr>
<tr>
<td>U266</td>
<td>2.2</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Annexin V</td>
<td>3.3</td>
<td>4.3</td>
<td>4.6</td>
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<td>Propidium iodide</td>
<td>12.5</td>
<td>21.9</td>
<td>68.4</td>
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<td>3.8</td>
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<tr>
<td></td>
<td>9.7</td>
<td>7.8</td>
<td>40.2</td>
</tr>
</tbody>
</table>
**Mechanism of b-SO6 cytotoxicity**

b-SO6 delivered into malignant B cells:

1. **TfR targeting was required for delivery and cytotoxicity**

2. **In contrast to anti-hTfR IgG3-Av alone, saporin-induced cell death was iron-independent**

3. **Blocked protein synthesis in both IM-9 and U266 cells**

4. **Activated a broad spectrum of caspases**


**Goal:** Further explore the mechanism of saporin-induced cell death in our system
Microarray analysis

Time course (hours)

0 1 3 9 24

IM-9

Buffer control

Anti-hTfR IgG3-Av

U266

Buffer control

Anti-hTfR IgG3-Av

HumanRef-8 Expression BeadChips from Illumina:
50mer gene-specific probes
each bead contains several hundred thousand probe copies
22,000 genes

Clustering was conducted using the Cluster program and visualized using Java TreeView
Microarray Results

Inflammation

DNA structure

Stress

IM-9 9h

U266

IM-9 9h

U266
Microarray Results

Inflammation

- PHGDH
- PSAT1
- TNFAIP3
- AXUD1
- RYBP
- CNOT10
- TNIP2
- MGC15619
- IFRD1
- TNFRSF17
- SKP2
- TNFSF9
- CLEC2D
- NCOA7
- ICAM1
- CCL3
- TOPBP1

- DPYSL2
- DPYSL3
- HIST1H2BK
- HIST1H1C
- MFSD3

DNA structure

- GLDC
- ALDOC
- ENO3
- PGK1
- PRDX4
- CCL3L3
- CD55
- RAB9A
- LIPA
- CDC25B
- LRP5L
- HIBCH
- CBS
- PFKFB4
- KIF20A
- ZFP36
- NQO1
- PSCP1
- SFRS10
- NR6IP1

- ACTG1
- MAT2A
- DUSP5
- NEK8
- PIM3
- C1orf24
- DNAJB2
- PSME3
- SEC61A2
- RNF121
- ADAM8
- ANKRD37
- SERTAD1
- CDT1
- RORA
- BATF
- KLF2
- RFX5
- ATF4
- ZNF38

- NFKB2
- PPP1R15A
- HSPA8
- RP9
- GPR132

- ARL8B
- RND3
- SGK
- NINJ1
- RRN3
- TRIP13
- DDX26
- SLC7A5
- TRIT1
- UBE2J1
- CPNE3
- U2AF1L4
- PDRG1
- FLJ11021

Stress

- FYTTD1
- THUMPD2
- HIST2H4
- BHLHB2
- KLF6
- CDC14B
- RGS1
- NFKBIE
- TXNIP
- GADD45B

- IRAK2
- TRIP10

IM-9 24h

U266

IRAK2

U266
Conclusions

- This is the first analysis of its kind to evaluate saporin regulated genes. A subset of genes is differentially expressed in both IM-9 and U266 cells. This subset of genes may represent key genes involved in the cellular response to the plant toxin saporin.

- In the more sensitive cell line (IM-9) changes in gene expression triggered by treatment with anti-hTfR IgG3-Av/b-SO6 occur earlier (9h) compared to the more resistant cell line U266 (24h). In addition, changes in IM-9 are of greater magnitude compared to changes in U266 cells.

- Anti-hTfR IgG3-Av is capable of a two-pronged attack against malignant hematopoietic cells via its intrinsic cytotoxicity and its ability to deliver a biotinylated toxin.
Future Directions

1. Validate by qPCR of genes identified by the microarray analysis

2. Conduct functional validation studies

3. Evaluate the in vivo cytotoxicity of anti-hTfR IgG3-Av alone and complexed to b-S06 in mouse xenograft models
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