The Keap1-Nrf2 Pathway in Toxicology

Curtis Klaassen
University of Kansas Medical Center
## Cd tolerance from Cd pretreatment

<table>
<thead>
<tr>
<th>Time after Cd pretreatment</th>
<th>n</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pretreatment</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>2 hr</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>4 hr</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>6 hr</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>8 hr</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>24 hr</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2 day</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>4 day</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>8 day</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>16 day</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Rats were pretreated with CdCl₂ (2.0 mg Cd/kg, sc) and at indicated time challenged with a lethal dose of CdCl₂ (4.0 mg/kg, iv), and mortality was recorded within 48 hrs after Cd challenge.

*Goering and Klaassen JTEH 14:803, 1984*
Cd pretreatment protected against acute Cd hepatotoxicity

Rats were pretreated with CdCl$_2$ (2.0 mg Cd/kg, sc) and 24 hrs later were challenged with hepatotoxic doses of CdCl$_2$ (2.0-4.0 mg/kg, iv), and serum SDH was determined 10 hrs later. Data are Mean ± SE of 4-6 rats.

Goering and Klaassen TAAP 74:308, 1984
Cd pretreatment alters subcellular Cd distribution

Rats were pretreated with CdCl₂ (2.0 mg Cd/kg, sc) and 24 hrs later were challenged with hepatotoxic doses of CdCl₂ (3.5 mg Cd/kg, iv), and subcellular Cd distribution was determined 2 hrs later. Data are Mean ± SE of 6 rats.

Goering and Klaassen TAAP 70:195, 1983
Cd pretreatment alters cytosolic Cd distribution

Rats were pretreated with CdCl₂ (2.0 mg Cd/kg, sc) and 24 hrs later were challenged with hepatotoxic doses of CdCl₂ (3.5 mg Cd/kg, iv), and cytosolic Cd distribution was determined 2 hrs later.

Goering and Klaassen TAAP 70:195, 1983
MT-null mice are highly susceptible to chronic Cd-induced nephrotoxicity

Wild-type control and MT-null mice were given CdCl2 (0.05-2.4 mg/kg, sc) daily for 6 weeks, and Blood urea nitrogen (BUN) and renal MT were determined. N = 6-8 mice.

The relationship between human exposure to cadmium and renal injury

Klaassen et al., Ann Rev Pharmacol Toxicol 39, 267, 1999
In 1987, a post-doctoral fellow, Jie Liu, came to my Lab with a “suitcase” of chemicals in Traditional Chinese Medicine that he thought would protect against liver injury.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Chemical Name</th>
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<tbody>
<tr>
<td>OA</td>
<td>Oleanolic acid</td>
</tr>
<tr>
<td>UA</td>
<td>Ursolic acid</td>
</tr>
<tr>
<td>UV</td>
<td>Uvaol</td>
</tr>
<tr>
<td>α-H</td>
<td>α-Hederin</td>
</tr>
<tr>
<td>HD</td>
<td>Hederagenin</td>
</tr>
<tr>
<td>GL</td>
<td>Glycyrrhizin</td>
</tr>
<tr>
<td>18β-GA</td>
<td>18 β-Glycyrrhetinic acid</td>
</tr>
<tr>
<td>18 α-GA</td>
<td>18 α-Glycyrrhetinic acid</td>
</tr>
<tr>
<td>HAG</td>
<td>19 α-Hydroxyl asiatic acid 29-0- β-glucoside</td>
</tr>
<tr>
<td>HA</td>
<td>19 α-Hydroxyl asiatic acid</td>
</tr>
</tbody>
</table>
Oleanolic acid
How?

We spent a considerable amount of time tying to determine how Oleanolic Acid protects against this large panel of hepatotoxicants, unfortunately, with little success.
Anticarcinogenic enzyme inducers are of two types:
(a) bifunctional inducers (e.g., TCDD, polycyclic aromatics) that elevate both Phase II enzymes, NAD(P)H: quinone oxidoreductase, and certain Phase I enzymes;
(b) monofunctional inducers (e.g., diphenols, thiocarbamates, 1,2-dithiol-3-thiones, beta-naphthoflavone) that elevate primarily Phase II enzymes.

Prochaska and Talalay
*Cancer Res 1988*
Phenolic antioxidants

Phenolic antioxidants

GST-Ya

GST-Ya GST-Pi Nqo1

Itoh et al., BBRC 1997.

Ho-1 (Alam et al., JBC 1999)
Gclc (Moinova et al., BBRC 1999)

Antioxidants

Maf

Nrf2

Nrf2

ARE

ARE

ARE

ARE

ARE

ARE
Oxidative stress and activators

- CUL3
- Keap1
- Nrf2
- ARE
- Maf
- Anti-oxidant and inflammation
- Cell survival and proliferation
- Drug metabolism
- Lipid metabolism
<table>
<thead>
<tr>
<th>Nrf2 activator</th>
<th>Structure</th>
<th>Keap1 cysteine residues</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>dexamethasone 21-mesylate</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>257, 273, 288, 297, and 613 (human)</td>
<td>(Dinkova-Kostova et al., 2002)</td>
</tr>
<tr>
<td>iodoacetyl-N-biotinylhexylenediamine</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>196, 226, 241, 257, 288, and 319 (mouse)</td>
<td>(Hong et al., 2005b)</td>
</tr>
<tr>
<td>sulforaphane</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>77, 226, 249, 257, 489, 513, 518 (human)</td>
<td>(Hong et al., 2005)</td>
</tr>
<tr>
<td>xanthohumol</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>151, 319, and 613 (human)</td>
<td>(Luo et al., 2007)</td>
</tr>
</tbody>
</table>
Michael Sporn, “the father of chemoprevention” asked for our Oleanolic Acid so he could make more potent analogues.
2-cyano-3,12-dioxoooleana-1,9(11)-dien-28-oic acid (CDDO)
CDDO compounds are potent Nrf2 activators

A single dose of CDDO-Im (1mg/kg, i.p.) increases Nrf2-dependent genes in wild-type but not Nrf2-null mice

CDDO-Im protection against acetaminophen hepatotoxicity is Nrf2-dependent

Oleanolic acid is also a Nrf2 activator

A single dose of oleanolic acid (30mg/kg, i.p.) increases Nrf2 translocation into nucleus and induce Nrf2 target genes in wild-type but not Nrf2-null mice.
What Pathways Might the Keap1-Nrf2 Pathway Alter?
Generation of “Gene dose-response” Model

Nrf2-null  Wild-type  Keap1-KD  Keap1-HKO

β-actin

Keap1

mRNA expression

Protein expression

Nrf2

Nrf2-null  Wild-type  Keap1-KD  Keap1-HKO

N.A.

β-actin
Messenger RNA and Protein Levels of Nrf2 Target Genes, as well as GSH Content were Increased in “Gene dose-response” Model

![Graphs showing mRNA and Protein expression of Nqo1 and Gclc in different conditions](image)

- **Nqo1** mRNA and Protein expression
- **Gclc** mRNA and Protein expression

Nrf2-null WT Keap1-KD Keap1-HKO

- Nqo1
- Gclc
- B-actin

![Graph showing Cytosol GSH levels in different conditions](image)

- Cytosol GSH in Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO

Note: N.A. indicates not available.
Transcriptional Profiling by Microarray Analysis

Nrf2-null
n=3

Wild-type
n=3

Keap1-KD
n=3

Keap1-HKO
n=3

Liver

Total RNA

Microarray
Data Analysis from Microarray

Raw data from microarray analysis (CEL file)

- imported into “R” program using affy package

Processed by Robust Multichip Averaging (gcRMA) package

- The mean probe signal intensities higher than $\log_2 100$
in at least one genotype group were selected for further analysis

- Analysis of variance by Linear Models for Microarray Data (Limma) Package

- Gene symbols and other annotations were obtained from the mouse 4302 and anaffy packages
Constitutively Induced or Suppressed Genes

WT>Nrf2-null Keap1-KD>WT

Keap1-HKO>Keap1-KD

WT<Nrf2-null Keap1-KD<WT

Keap1-HKO<Keap1-KD

Suppressed genes

Induced genes

Fold-induction

WT

Wild-type Keap1-KD Keap1-HKO

Nrf2-null

Fold-induction

Nrf2-null Wild-type Keap1-KD Keap1-HKO

* *
## Annotation clustering using DAVID analysis

<table>
<thead>
<tr>
<th>Term</th>
<th>Enrichment Score</th>
<th>Gene Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Top annotation cluster for the set of genes induced through Nrf2 activation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione transferase and glutathione metabolism</td>
<td>8.2</td>
<td>Gstm1, Gss, Gsta2, Gstm2, Gsta3, Gstm3, Gclc, Gstm4, Gsta4, Gpx4, Gstm6</td>
</tr>
<tr>
<td>Oxidation reduction and NADPH bind enzymes</td>
<td>6.0</td>
<td>Cyp2g1, Xdh, Ptgr1, Htatip2, Ugdh, Coq7, Fth1, Akr1c13, Akr1a4, Cryl1, Fmo1, Gpx4, Aox1, Cyp2a5, Txnrd1, Nqo1, Srxn1</td>
</tr>
<tr>
<td>Carboxylesterase</td>
<td>3.9</td>
<td>Ces2, Ces1, BC015286, Ces5</td>
</tr>
<tr>
<td>Chemical and iron homeostasis</td>
<td>3.2</td>
<td>Xdh, Ftl1, Gclc, Fech, Ftl2, Hexa, Hexb, Bnip3, Afg3I2, Fth1, Abcg8, Anxa7</td>
</tr>
<tr>
<td>Cofactor biosynthetic process</td>
<td>2.4</td>
<td>Gss, Fech, Gclc, Coq7, Gch1</td>
</tr>
<tr>
<td><strong>Top annotation cluster for the set of genes suppressed through Nrf2 activation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response to nutrient and extracellular stimulus</td>
<td>2.3</td>
<td>Avpr1a, Adipor2, Cp, Apom, Cbs</td>
</tr>
</tbody>
</table>
What Drug Processing Genes are Altered by Nrf2 activation

- Uptake transporters
- Phase-I enzymes
- Phase-II enzymes
- Efflux transporters
Drug metabolizing genes
Major P450 Drug Metabolizing Genes in Human

<table>
<thead>
<tr>
<th>Human Gene</th>
<th>Mouse ortholog</th>
<th>Inducers</th>
<th>Nuclear receptor</th>
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</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Cyp1a2</td>
<td>Omeprazole</td>
<td>AhR</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Cyp2a4</td>
<td>Barbiturates</td>
<td>CAR</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Cyp2b10</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>CYP2C8</td>
<td>Cyp2c65</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>CYP2C9</td>
<td>N.A.</td>
<td>Rifampin</td>
<td>PXR</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Cyp2c50</td>
<td>Rifampin</td>
<td>PXR</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Cyp2d22</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Cyp2e1</td>
<td>Ethanol</td>
<td></td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Cyp3a41a</td>
<td>Rifampin</td>
<td>PXR</td>
</tr>
</tbody>
</table>
Phase-I Drug Processing Enzymes (Cytochrome P450)

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Human ortholog</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyp2a5</td>
<td>CYP2A6</td>
<td>Nicotine, halothane, aflatoxin</td>
</tr>
<tr>
<td>Cyp2c50</td>
<td>N.A.</td>
<td>Arachidonic Acid</td>
</tr>
<tr>
<td>Cyp2c54</td>
<td>N.A.</td>
<td>Arachidonic Acid</td>
</tr>
<tr>
<td>Cyp2g1</td>
<td>CYP2G1P</td>
<td>Sex hormones, acetaminophen</td>
</tr>
<tr>
<td>Cyp2u1</td>
<td>CYP2U1</td>
<td>Fatty acids</td>
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</tbody>
</table>
Non-P450 Phase-I Enzymes: Aldo-keto Reductase

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Human ortholog</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akr1a4</td>
<td>AKR1A1</td>
<td>Trans-muconaldehyde (cytotoxic metabolite of benzene)</td>
</tr>
<tr>
<td>Akr1b3</td>
<td>AKR1B1</td>
<td>Acrolein, 4-hydroxy-trans-2-nonenal (HNE)</td>
</tr>
<tr>
<td>Akr1c13</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Akr1c19</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Akr7a5</td>
<td>AKR7A2</td>
<td>Aflatoxin B1</td>
</tr>
</tbody>
</table>
Non-P450 Phase-I Enzymes: Carbonyl Reductase and Carboxylesterase

**Carbonyl Reductase**

- **Cbr1**
  - Substrates: Doxorubincin, S-nitroglutathione
- **Cbr3**
  - Substrates: Doxorubincin

**Carboxylesterase**

- **Ces1**
  - Substrates: ACE inhibitors (imidapril)
- **Ces2**
  - Substrates: Propionyl propranolol
Non-P450 Phase-I Enzymes: Other

**Gene** | **Enzyme** | **Substrate**
--- | --- | ---
Aldh1a1 | Aldehyde dehydrogenase | Acetaldehyde
Aox1 | Aldehyde oxidase | Famciclovir
Ephx1 | Epoxide hydrolase | Polycyclic aromatic hydrocarbons
Fmo1 | Flavin monoxygenase 1 | Cimetidine
Nqo1 | NAD(P)H quinone oxidoreductase | Quinones
Xdh | Xanthine dehydrogenase | 6-mercaptopurine
Phase-II enzymes: Glutathione Conjugation

Not altered: Gstk1, Gstm5, Gstm7, Gsto1, Gstp1, GSTT1, GSTT2
Phase-II enzymes: Glucuronidation

**Cytosol**

Glucose $\xrightarrow{\text{Ugp2}}$ UDP-glucuronic acid $\xrightarrow{\text{Slc35d1}}$ Glucuronidation

**ER**

Ugdh $\xrightarrow{\text{Slc35d1}}$ Glucuronidation

---

**Graph**

Fold-induction

- Nrf2-null
- Wild-type
- Keap1-KD
- Keap1-HKO

Enzymes: Ugp2, Ugdh, Slc35d1, Ugt1a6a, Ugt1a9, Ugt2b35, Ugt2b36, Ugt2b5, Ugt3a1

* Denotes statistical significance.
Uptake and Efflux Transporters

Uptake Transporters

- Oatp1a1
- Atp8b1
- Ntcp
- Oat2

Efflux Transporters

- Mrp2
- Mrp3
- Mrp4
- Mrp9
- Bcrp
- Abcg5
- Abcg8

Legend:
- Nrf2-null
- Wild-type
- Keap1-KD
- Keap1-HKO

Fold-induction
**UPTAKE**

Phase I

Nucleophiles

**Phase II**

Hydrophilicity

Conjugates

Hydrophilicity

Phase I

Nqo1

Eh-1

Ugts, Sults

**EFFLUX**

Mrps

**Diffusion**

Cyps

Detoxification

Electrophiles

Oxidative Stress and Formation of Adducts

Gsts

GSH Conjugation

Glycine

Gcl

Gs

Nrf2

Cysteine

Glutamate

\( \gamma \)-glutamylcysteine

GSH
AhR and Nrf2 interactions

Yeager et al., Toxi Sci 2009.
Expression of Which Antioxidant Genes are altered by Nrf2
Antioxidant Genes were Induced with Graded Nrf2 Activation

1) GSH synthesis and regeneration

2) Reduction of hydrogen peroxide

3) Reduction of oxidized protein

4) Reduction of bilirubin and ion sequester
\[ \text{NADPH} \rightarrow \text{Prdx (red)} \rightarrow \text{Prdx (oxi)} \rightarrow \text{GSH} \rightarrow \text{GSSG} \rightarrow \text{NADP}^+ \rightarrow \text{Prdx (oxi)} \rightarrow \text{Prdx (red)} \rightarrow \text{NADPH} \]

\[ \text{Fe}^{2+} \rightarrow \text{Ferritin} \]

\[ \text{H}_2\text{O}_2 \rightarrow \text{OH}^- \]

\[ \text{Gcl, Gs} \rightarrow \text{GSH} \rightarrow \text{Gpx} \rightarrow \text{NADP}^+ \rightarrow \text{GSSG} \rightarrow \text{NADPH} \]

\[ \text{glutamate, cysteine, glucine} \]

\[ \text{Biliverdin} \rightarrow \text{Bilirubin} \rightarrow \text{NADP}^+ \rightarrow \text{Bvrb} \rightarrow \text{NADPH} \]
Does Nrf2 activation alter the toxicity of chemicals?
Diquat: ‘Model Chemical’ to Study Oxidative Stress

- Diquat does not bind covalently with biological molecules, and minimally metabolized in rodent.

- Diquat treatment can cause lipid peroxidation, increased ALT level, BUN level and necrosis in rats and mice.

Experimental Design

125 mg/kg Diquat (i.p.)

Nrf2-null                      Wild-type                        Keap1-KD

Serum                        Lung                                Liver

ALT                          H&E                                 H&E

Lung GSH                     Liver GSH                            Gclc mRNA
Lipid Peroxidation

![Graph showing lipid peroxidation over time for different conditions.](image)
Liver Injury

Serum ALT

- Control
- Diquat
  - Nrf2-null
  - Wild-type
  - Keap1-kd

ALT (U/L)

Time (h)

0 1 2 3 4 5 6

0 200 400 600

* indicates significant difference.

ALT (U/L)
GSH and GSSG Concentrations in Liver

\[ \text{O}_2^\cdot \rightarrow \text{Peroxiredoxin} \rightarrow \text{H}_2\text{O} \]

\[ \text{GSH} \xrightarrow{\text{Gpx}} \text{GSSG} \]

\[ \text{Catalase} \]

\[ \text{GSH} \]

\[ \text{GSSG} \]

**GSH**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Wild-type</th>
<th>Nrf2-null</th>
<th>Keap1-kd</th>
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<tbody>
<tr>
<td>0</td>
<td>10</td>
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</tr>
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**GSSG**

<table>
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<tr>
<th>Time (h)</th>
<th>Wild-type</th>
<th>Nrf2-null</th>
<th>Keap1-kd</th>
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<td>0.8</td>
<td>1.0</td>
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<td>1</td>
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<td>0.5</td>
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<tr>
<td>6</td>
<td>0.0</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>7</td>
<td>0.0</td>
<td>0.1</td>
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</tbody>
</table>
Lung Toxicity

Relative Lung Weight

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diquat</th>
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</thead>
<tbody>
<tr>
<td>Nrf2-null</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Wild-type</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Keap1-kd</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Conclusions on diquat

• Diquat treatment induced lung and liver injury.

• This injury was enhanced in Nrf2-null mice, and attenuated in Keap1-kd mice.

• The difference of the injury appears to be caused by the difference in the basal levels of total GSH and GSH synthesis.
Acute Alcoholic Liver Disease: when oxidative stress meets disrupted lipid metabolism

Hypothesis: Nrf2 activation prevents alcohol-induced hepatic oxidative stress and lipid accumulation.
Does Nrf2 alter the ethanol disappearance in mice?

- Nrf2-null
- Wild-type
- Keap1-KD
- Keap1-HKO

“Binge drinking” model (5g/kg ethanol)
Or isocaloric glucose solution

Collect 20 µL of the blood from the tail at 1, 2, 3, 4, 5, 6 h after treatment

Serum ethanol concentration
Ethanol disappearance

![Graph showing Ethanol disappearance over time for different genotypes: Nrf2-null, WT, Keap1-KD, and Keap1-HKO. The x-axis represents time (h) from 0 to 7, and the y-axis represents Serum Ethanol (mg/ml) from 3.0 to 0.0. The lines show a downward trend as time increases, indicating the rate at which Ethanol disappears from the serum.]
Does Nrf2 prevent ethanol-induced liver injury?

Nrf2

Nrf2-null  Wild-type  Keap1-KD  Keap1-HKO

“Binge drinking” model (5g/kg ethanol)
Or isocaloric glucose solution

Sacrifice after 6 hours

Serum

ALT, TBARS, and lipid profile

Liver

Histology, GSH, and lipid profile
Markers of liver injury

![Bar graph showing ALT and LDH levels across different genotypes and treatment conditions.]

*: different between control and cadmium treatment in the same genotype.
†: different between wild-type and other genotype groups after ethanol treatment.
Markers of oxidative stress *in vivo*

![Graph showing TBARS, Cytosol GSH, and Mitochondrial GSH levels in control and ethanol groups, along with Nrf2-null, Wild-type, Keap1-KD, and Keap1-HKO conditions.](image)
Indicator of oxidative stress *in vitro*

2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (H₂DCFDA) → cellular esterase → H₂DCF → ROS → DCF (green fluorescent)

![Images of fluorescence staining for different genotypes](image)

**Graphs:**
- Time course of ROS over 24h for different genotypes.
- Concentration response of ROS to ethanol at different concentrations.

**Legend:**
- Nrf2-null
- Wild-type
- Keap1-KD
- Keap1-HKO
**Nqo1**

- **mRNA relative to wild-type control**
  - Control: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol treatment significantly increases mRNA levels in Nrf2-null and Keap1-HKO compared to wild-type control.

**Gclc**

- **mRNA relative to wild-type control**
  - Control: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol treatment significantly decreases mRNA levels in Nrf2-null compared to wild-type control.

**Nrf2-null**

- **Protein relative to wild-type control**
  - Control: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol treatment significantly decreases protein levels in Nrf2-null compared to wild-type control.

**Wild-type**

- **Protein relative to wild-type control**
  - Control: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol treatment significantly increases protein levels in Wild-type compared to wild-type control.

**Keap1-KD**

- **Protein relative to wild-type control**
  - Control: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol treatment significantly decreases protein levels in Keap1-KD compared to wild-type control.

**Keap1-HKO**

- **Protein relative to wild-type control**
  - Control: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
  - Ethanol treatment significantly increases protein levels in Keap1-HKO compared to wild-type control.
Lipid profiles in serum and liver

**Serum TG**
- Serum TG levels are shown for different genotypes: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO.
- Control vs. Ethanol groups are compared.
- An asterisk (*) indicates statistical significance compared to control, and a dagger (†) indicates significance compared to Wild-type.

**Liver TG**
- Liver TG levels are shown for different genotypes: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO.
- Control vs. Ethanol groups are compared.
- An asterisk (*) indicates statistical significance compared to control, and a dagger (†) indicates significance compared to Wild-type.

**Serum FFA**
- Serum FFA levels are shown for different genotypes: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO.
- Control vs. Ethanol groups are compared.
- An asterisk (*) indicates statistical significance compared to control, and a dagger (†) indicates significance compared to Wild-type.

**Liver FFA**
- Liver FFA levels are shown for different genotypes: Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO.
- Control vs. Ethanol groups are compared.
- An asterisk (*) indicates statistical significance compared to control, and a dagger (†) indicates significance compared to Wild-type.
Srebp1 mRNA, protein, and target gene expression

**Srebp1 mRNA**

- Control vs. Ethanol
- Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO

**Scd1 mRNA**

- Control vs. Ethanol
- Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO

**Srebp1 Protein**

- Control vs. Ethanol
- Nrf2-null, Wild-type, Keap1-KD, Keap1-HKO
Keap1-Nrf2 pathway plays an important role in protecting against ethanol-induced hepatic alterations \textit{in vitro} and \textit{in vivo}.

The protective effect of Nrf2 may result from elevation of cellular GSH concentrations and suppression of the Srebp1 signaling pathway.
• We showed earlier that MT protects against Cd hepatotoxicity
• Might Nrf2 also protect against Cd toxicity
Summary

Nrf2 protects against:

• Diquat
• Ethanol
• Cd

Conclusion:

• Nrf2 appears to an important pathway to protect against many chemicals.
• Presently: Take antioxidants

• Future: Have body make antioxidants
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