Aldehydes: What Are They and Why Should They be Avoided?

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FA Adult meeting,
Baltimore, 2014
4 parts of my talk

- Overview of Fanconi anemia
- What are Aldehydes?
- Why Aldehydes should be avoided by FA patients?
- Potential therapeutics for FA based upon our knowledge of the Aldehyde toxicity and FA
Fanconi Anemia (FA): An inherited Chromosome Instability Syndrome

Rare Autosomal Recessive Disease: 1/100,000 births

Characterized by
- Developmental defects
- Bone marrow failure (aplastic anemia by age 5)
- Cancer susceptibility (leukemia, squamous cell carcinoma, gynecologic cancers)
- Hypersensitivity to DNA crosslinking agents (Cisplatin, MitomycinC)
- Sixteen different complementation groups of FA have been defined by somatic cell fusion studies (All sixteen FA genes have been identified)

Fanconi anemia

Mutation in any of 16 FA complementation groups

Developmental Abnormalities

Bone marrow failure

Cancer/Leukemia

Healthy

Leukemia

Age

Percentage
Fanconi Anemia
Cells have a characteristic Cellular phenotype:

Hypersensitivity to DNA cross-linking agents, e.g. Mitomycin C
Other phenotypes of Fanconi Anemia Cells

DNA cross-link sensitivity

Delayed growth, G2 arrest

### The Sixteen Fanconi Anemia Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>FA patients, estimated (%)</th>
<th>Chromosome</th>
<th>Protein product, Kd</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>60%</td>
<td>16q24.3</td>
<td>163</td>
</tr>
<tr>
<td>B</td>
<td>2%</td>
<td>Xp22.31</td>
<td>95</td>
</tr>
<tr>
<td>C</td>
<td>10%</td>
<td>9q22.3</td>
<td>63</td>
</tr>
<tr>
<td>D1/BRCA2</td>
<td>4%</td>
<td>13q12.3</td>
<td>380</td>
</tr>
<tr>
<td>D2</td>
<td>4%</td>
<td>3p25.3</td>
<td>155</td>
</tr>
<tr>
<td>E</td>
<td>10%</td>
<td>6p21-22</td>
<td>60</td>
</tr>
<tr>
<td>F</td>
<td>rare</td>
<td>11p15</td>
<td>42</td>
</tr>
<tr>
<td>G</td>
<td>10%</td>
<td>9p13</td>
<td>68</td>
</tr>
<tr>
<td>I</td>
<td>rare</td>
<td>15q26</td>
<td>150</td>
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<tr>
<td>J/BRIP1</td>
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<td>17q23.2</td>
<td>130</td>
</tr>
<tr>
<td>L</td>
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<td>2p16.1</td>
<td>52</td>
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<tr>
<td>M</td>
<td>rare</td>
<td>14q21.2</td>
<td>250</td>
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<tr>
<td>N/PALB2</td>
<td>rare</td>
<td>16p12</td>
<td>130</td>
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<tr>
<td>O/RAD51C</td>
<td>rare</td>
<td>17q25.1</td>
<td>42</td>
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<tr>
<td>P/SLX4</td>
<td>rare</td>
<td>16p13.3</td>
<td>200</td>
</tr>
<tr>
<td>Q/XP-F</td>
<td>rare</td>
<td>16p13.12</td>
<td>104</td>
</tr>
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</table>
Sixteen FA proteins Cooperate in a common pathway for ICL repair

FANCP/SLX4 is a multidomain protein complex that interacts with the XPF/ERCC1 nuclease

SLX4 has a UBZ4 (ubiquitin binding site).

Translesion Synthesis (TLS)
Progressive loss of blood stem cells leads to the bone marrow failure in FA patients early in life.
CAUSES of bone marrow failure in FA patients

FA-deficient bone marrow cell

- Inflammatory cytokines (TNF-α), Interferon-gamma, Oxygen
- DNA cross-linking agents
- Endogenous genotoxin?
- Cytokinesis failure

Apoptosis, DNA damage or Cell cycle arrest?
A big puzzle in the FA research

Mitomycin C and Cisplatin are NOT the DNA-damaging agents in vivo in FA.

- Which endogenous genotoxins are a threat to Fanconi anemia?
Aldehydes are organic compounds

O'Brien PJ et al,
Critical Reviews in Toxicology, 2005
Aldehydes:

- Reactive chemicals that can injure cells
- Can interact with (and crosslink) DNA molecules
- Some aldehydes (like formaldehyde) are endogenous and are formed within the body during normal metabolism
- Some are exogenous (say, from alcoholic beverages)
- Aldehydes are broken down by a family of enzymes (including ALDH2 and ALDH3)
Exogenous Sources of Aldehydes

Environmental:
- Air through phytochemical degradation, automobiles
- cooking fumes
- cigarette smoke
- hospitals, laboratories
- cosmetics, perfumes, hair saloon
- raw materials in factories

Dietary:
- ripe fruits and vegetables, coffee, soy-sauce
- alcoholic beverages
Endogenous Sources of Aldehydes

• Some are produced in our body during normal metabolism (e.g. Formaldehyde, Acetaldehyde, 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) etc.).

• Acetaldehyde is formed in the body from the breakdown of ethanol—a source of acetaldehyde among those who consume alcoholic beverages.

• Formaldehyde is produced close to DNA as a byproduct of histone demethylation.
Acetaldehyde metabolism in our body

Alcohol → Acetaldehyde → Acetate

Alcohol dehydrogenase (ADH) → Aldehyde dehydrogenase 2 (ALDH2)
Formaldehyde metabolism in our body

Formaldehyde (Toxic) → Formate → CO₂ (Non toxic)

Alcohol dehydrogenase 5 (ADH5)
Acetaldehyde and Formaldehyde

- Highly reactive aldehydes, form DNA adducts *in vitro* and *in vivo*, carcinogens

- Potential genotoxins (endogenous DNA/protein cross-linking agents) responsible for the Fanconi anemia phenotypes.

- Mouse models with the genetic deficiency of enzymes required for aldehyde metabolism (e.g. Aldh2, Adh5) have been used.

*work from Dr. KJ Patel’s laboratory, UK*
Acetaldehyde is toxic for FA

- FA pathway deficient chicken B cells and mouse bone marrow cells are hypersensitive to Acetaldehyde *in vitro.*

- *ALDH2,* an Acetaldehyde detoxifying enzyme is of critical importance in individuals with FA

- A mouse model with defect in *ALDH2* and FA gene:
  - has spontaneous bone marrow failure
  - has severely reduced blood stem cells
  - develops leukemia
  - has developmental abnormalities
  - is highly susceptible to alcohol toxicity
Formaldehyde is highly toxic for FA

- FA pathway deficient human and chicken B cells are hypersensitive to Formaldehyde \textit{in vitro}.

- \textit{ADH5}, a Formaldehyde detoxifying enzyme is also of critical importance in individuals with FA

- FA pathway deficient chicken B cells die if AHD5 gene is deleted.

- A mouse model with defect in ADH5 and FA gene:
  - succumbs to death few weeks after birth
  - has severe spontaneous bone marrow failure
  - has severely reduced blood stem cells
  - develops leukemia
  - has liver and kidney failure
Maternal alcohol exposure aborts the development of Aldh2-/-Fancd2-/- embryos

Aged Aldh2-/-Fancd2-/- mice succumb to bone marrow failure

Young Aldh2−/−Fancd2−/− mice have severely low amount of the blood forming stem cells

Natural ALDH2 gene mutation

- ALDH2 is mutated in approximately 1 billion people, most common in Southeast Asia.

- The mutant gene (E487K) causes a significant decrease in ALDH2 activity.

- Asian flushing syndrome
Genetic mutation in ADLH2 gene increases the risk of squamous cell carcinoma in Japanese population

Asian Flushing Syndrome

Brooks PJ et al, PLoS Medicine, 2009
Mutant ALDH2 is associated with accelerated bone marrow failure in Japanese FA patients.

Total 64 patients:
n=3 for AA, n=25 for GA, n=36 for GG

Hira A et al. Blood 2013
Acetaldehyde poses a threat to blood stem cells when both ALDH2 and FA pathway are absent.
Increase patient’s capacity to detoxify Aldehydes: FA therapy?

Acetate

Acetaldehyde

Genotoxic to FA cells

BM failure, Cancer, developmental defects

Formate

Formaldehyde

Genotoxic to FA cells

BM failure, Cancer, developmental defects

ALDH2

ADH5
Implications/Recommendations:

- FA patients should limit alcohol consumption

- Alcohol and aldehydes can cross the placenta (i.e., a pregnant mother carrying an FA fetus should limit alcohol consumption)
Implications/Recommendations:

- We should:
  1) develop drugs to stimulate ALDH2 activity
  2) develop drugs to detoxify (sponge up) aldehydes from blood

- Early evidence suggests that low ALDH2/ALDH3 levels may correlate with increased incidence of Squamous Cell Carcinoma of the head and neck.
Future Studies:

- Why are only the blood stem cells specifically affected by loss of ALDH2 and FA gene?

- Why do some FA cell lines have more ALDH2 than others?

- Mice with ALDH2/FA deficiency or ADH5/FA deficiency have spontaneous bone marrow failure (helpful experimental models)

- Can we suppress aldehyde production? Or suppress its Accumulation? Dietary Effects?

- Are some aldehydes more toxic than others to FA patients?
Possible treatments
Thiol “Sponges” for removing reactive aldehydes from the blood

N-Acetyl Cysteine (NAC)  Cysteamine

FDA approved
Therapeutic approaches to treat FA

- Androgen, G-CSF
- Bone marrow transplant
- Tempol
- Resveratrol
- Anti-oxidants (NAC), anti-inflammatory agents
- Gene therapy?
- Anti-apoptotic compounds?
- DNA-PK inhibitor?
- CHK1 inhibitor? P53 inhibitor?
A new drug candidate: Alda-1

- Alda-1 is a novel small molecule agonist of ALDH2 enzyme.
- Alda-1 stimulates the activity of ALDH2 enzyme and promotes the removal of aldehydes from blood in vivo.
Summary

- Aldehydes are highly toxic to the FA bone marrow and they may be responsible for the bone marrow failure and leukemia in FA.

- FA mouse models with deficiency in aldehyde metabolism exhibit many pathophysiological features of FA and are therefore useful for testing the therapeutic agents.

- Importance of monitoring aldehyde levels in the blood of FA patients

- Treatment options:
  - avoid dietary aldehydes
  - drugs which can “sponge” up aldehydes from the blood (e.g. NAC, Cysteamine)
  - drugs which can stimulate the enzymatic removal of aldehydes from the blood (e.g. ALDA1).