Mutational signatures of redox stress in yeast and men

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DNA repair interest group videoconference
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Redox stress is linked to human disease and ageing

**A hallmark of cancer**

- Self-sufficiency in growth signal
- Insensitivity to anti-growth signals
- Limitless replicative potential
- Tissue invasion & metastasis
- DNA damage stress
- Mitotic stress
- Proteotoxic stress
- Metabolic stress
- Oxidative stress
- Evading immune surveillance
- Evading apoptosis
- Sustained angiogenesis

**Neurodegenerative diseases**

- Alzheimer’s, Parkinson’s, Huntington’s, Amyotrophic lateral sclerosis (ALS)
- Associated with cell loss/degeneration in high energy consuming cells

**Ageing**

- Redox theory of ageing

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Luo, Solimini, Elledge, *Cell*, 2009


Go and Jones, *Clin Sci*, 2017
Redox stress is a hallmark of cancer

Redox status is deregulated in majority of cancers due to:
- altered metabolism;
- mitochondrial dysfunction;
- inflammation;
- malfunctioning of peroxisomes;
- over- or under-expression of ROS-producing and ROS-scavenging enzymes.

Luo, Solimini, Elledge, *Cell*, 2009
How to assess the contribution of redox stress to disease and aging?

By analyzing the mutations accumulated following exposure to oxidizing agents and in tissues affected by a disease.
Mutational signature is a spectrum of mutations in the context of adjacent nucleotides.
Some of the mutational signatures reveal etiology of human cancers caused by dysregulation of specific enzymes attributed to activity of the AID/APOBEC family of cytidine deaminases.

ID17

Associated with TOP2A p.K743N mutation
Associated with tobacco smoking

... or by environmental exposures

Associated with tobacco smoking

... or by chemotherapeutic interventions

Chemotherapy treatment with platinum drugs
## Is there a signature of redox stress?

<table>
<thead>
<tr>
<th>Aging</th>
<th>Cytidine deaminases</th>
<th>Tobacco mutagenesis</th>
<th>Mismatch repair defects</th>
<th>UV exposure</th>
</tr>
</thead>
</table>

| Signature 1 | 40 | Signature 2 | 32 | Signature 3 | 13 | Signature 4 | 77 | Signature 5 | 49 | Signature 6 | 13 | Signature 7 | 3 |
| Signature 8 | 2 | Signature 9 | 2 | Signature 10 | 6 | Signature 11 | 3 | Signature 12 | 3 | Signature 13 | 22 | Signature 14 | 31 | Signature 15 | 22 |
| Signature 16 | 1 | Signature 17 | 6 | Signature 18 | 3 | Signature 19 | 41 | Signature 20 | 2 | Signature 21 | 14 | Signature 22 | 32 | Signature 23 | 13 | Signature 24 | 11 |
| Signature 25 | 1 | Signature 26 | 4 | Signature 27 | 3 | Signature 28 | 1 | Signature 29 | 10 | Signature 30 | 12 | Other signatures | 3 |

**Mutational signature present** | **Total validated mutational signatures in a cancer type** | **Total cancer types in which a signature is operative**

*Alexandrov et al., Nature, 2013*
Approach:

discerning the mutational signature of redox stress

-in the model organism *Saccharomyces cerevisiae*;

-in single strand DNA;

- selecting clustered (closely-spaced) mutations.
Why did we look for a signature of oxidative damage in single strand DNA?

Base Excision Repair requires second DNA strand *

Among published cancer signatures some are attributed to activity of enzymes that uses ssDNA as a substrate

* with some exceptions
Why did we look for a signature of oxidative damage in single strand DNA?

Localized hypermutability suggests persistent presence of ssDNA in cancer genomes

Nik-Zainal et al., 2012
The reporter system allows for the generation long stretches of ssDNA and selection for multiple mutations.

\[ \text{Telomere} \rightarrow 3'\text{Lys} \rightarrow \text{ADE2} \rightarrow \text{URA3} \rightarrow \text{CAN1} \rightarrow 5'\text{Lys} \rightarrow \text{Centromere} \]

\textit{cdc13-1ts} at 37\textdegree C

\[ \text{Telomere} \rightarrow 3'\text{Lys} \rightarrow \text{ADE2} \rightarrow \text{URA3} \rightarrow \text{CAN1} \rightarrow 5'\text{Lys} \rightarrow \text{Centromere} \]

\text{RED Can}^R \text{ clones = clustered mutations}

\textit{Chan et al., 2012}
Don’t walk away when I’m talking to you!

O₂

Don’t look now, but I think we’re on the downslope.
A Compendium of Mutational Signatures of Environmental Agents

Jill E. Kucab, Xueqing Zou, Sandro Morganella, Madeleine Joel, A. Scott Nanda, Eszter Nagy, Celine Gomez, Andrea Degasperi, Rebecca Harris, Stephen P. Jackson, Volker M. Arlt, David H. Phillips, Serena Nik-Zainal

Cell, 2019

Highlights

- 41 of 79 environmental agents yielded substitution signatures
- 6 agents produced double-substitution signatures and 8 produced indel signatures
- Several signatures match or exhibit similarity with signatures found in human tumors
- Topographical mutational asymmetries reveal mechanistic insights

“...hydrogen peroxide, anticipated to create ROS, and peroxynitrite, which generates reactive nitrogen (nitric oxide) species, did not yield clear mutation patterns”...
At an equitoxic dose hydrogen peroxide induces fewer mutations, than UV and MMS.
Even if it is possible to discern a mutational signature of oxidative stress, what to expect?

8oxoG mis-pairing with A leads to G to T transversions
Oxidative stress-induced mutagenesis in ssDNA occurs primarily at C.
Expected: G to T enrichment in oxidative stress signature
C to T enrichment in oxidative stress signature?
Signature of hydrogen peroxide-induced oxidative stress

Significant enrichment in C at position +1 and -1

Significant enrichment in A at position +2

https://plogo.uconn.edu/
Confirmed signature of hydrogen peroxide - induced oxidative stress

\[
(E)\text{ntrichment} = \frac{\text{Mutations}_{\text{cCc}\to\text{cTc}} \times \text{Context}_{\text{c}}}{\text{Mutations}_{\text{C}\to\text{T}} \times \text{Context}_{\text{ccc}}}
\]

- Produces sample-specific P-values
- Not affected by “topography” preferences

Roberts et al., 2012

<table>
<thead>
<tr>
<th>Motif</th>
<th>Fold enrichment</th>
<th>Mutational load</th>
<th>Bonferroni-corrected Fisher P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cca to Tca</td>
<td>3.00</td>
<td>21</td>
<td>1.95E-08</td>
</tr>
<tr>
<td>cCca to cTca</td>
<td>3.40</td>
<td>4</td>
<td>1.04E-02</td>
</tr>
<tr>
<td>Cca to Tna</td>
<td>1.49</td>
<td>19</td>
<td>2.43E-04</td>
</tr>
</tbody>
</table>
Is this signature hydrogen peroxide-specific?
Paraquat (PQ)

- Widely used herbicide
- Toxic to humans and animals
- Exposure has been linked to Parkinson’s Disease

https://emergency.cdc.gov/agent/paraquat/basics/facts.asp

Is there a signature of paraquat – induced mutagenesis?

Blanco-Ayala et. al., Free Radical Research, 2014
Exposure to paraquat increases mutation frequencies in sod1 mutants

Median frequency Can\(^R\), x10\(^6\)

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>sod1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock treatment</td>
<td>189</td>
<td>197</td>
</tr>
<tr>
<td>50 micromolar paraquat</td>
<td>264</td>
<td>565</td>
</tr>
</tbody>
</table>

\(p<0.0002\)

Median frequency Can\(^R\) Red, x10\(^7\)

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<tr>
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</tr>
<tr>
<td>50 micromolar paraquat</td>
<td>3</td>
<td>23</td>
</tr>
</tbody>
</table>

\(p=0.0009\)

ns

Mock treatment

50 micromolar paraquat
WT spontaneous

Hydrogen peroxide -induced

Paraquat -induced

WT

ogg1

rtt109

gcn5

sod1

Hydrogen peroxide -induced

Paraquat -induced

C-T   C-G   C-A
Mutational signatures of $\text{H}_2\text{O}_2$ and paraquat in ssDNA are similar.

**Hydrogen peroxide**

**WT**

$c\text{Cca}$ to $c\text{Tca}$

**sod1**

$\text{Cca}$ to $\text{Tca}$

$c\text{Cca}$ to $c\text{Tca}$

$\text{O}_2^{-}$
Two different oxidizing agents, hydrogen peroxide and paraquat, generate similar mutational signatures in ssDNA in yeast.
From yeast to men:

Is oxidative stress a major contributor to aging-related somatic mutations?

Where to look for redox stress – related signatures in human DNA?
Mitochondria accumulate somatic mutations with age, but they are not oxidative stress-induced (?)
The major type of somatic mutations in aging mitochondria is C to T changes

“Surprisingly, comparison of the mutation spectra of the young and old samples reveals a notable absence of the mutational signature of oxidative damage”... “We failed to find either a preponderance of GtoT/CtoA substitutions or a proportionally greater increase with age in this type of mutation relative to other types, despite a span of 80 years between our sequenced sample groups”

Kennedy et al., 2013
Mutational spectra of redox stress in yeast ssDNA and of aging in human mitochondrial DNA share a common feature.

Kennedy et al., 2013
Is there a distinct signature of aging in mtDNA?

Motif: gCg to gTg
Fold enrichment: 2.44
Fisher P value: 3.26E-06
Bonferroni-corrected: 6.50E-05
ggCg to ggTg signature of mitochondria aging: (methyl)cytosine deamination?

Clock-like signature?

Activity of APOBEC family cytidine deaminases
Evidence for redox-stress related signature in human cancers
Conclusions

• The majority of hydrogen peroxide- and paraquat-induced mutations in ssDNA occurs at C;

• C to T is the main type of redox stress-induced substitutions in ssDNA in yeast;

• 8oxoG is not the major redox stress-induced mutagenic lesion in ssDNA;

• The mutational signatures of hydrogen peroxide and paraquat in ssDNA in yeast are similar;

• Mutational signatures of redox stress in yeast ssDNA and of aging in human mitochondrial DNA share a common feature

• Many cancer genomes are enriched for redox stress-related signatures.
Questions

• What is the underlying DNA lesion at C?
• What are the molecular mechanisms of protection of ssDNA from oxidative damage?
• Do antioxidants prevent oxidative damage in ssDNA?
• Is it possible to find mutational signatures of oxidative stress in cancers by utilizing the new, broadened databases?
• Is there a mutational signature of redox stress in cells exposed to chronic inflammation?
• Are there other mutational signatures attributable to oxidative stress?
Acknowledgements

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Cytosine deamination?

\[
\text{Ung1+} \quad \text{TLS} \quad \text{Ung1+}
\]

Ung1+ deaminates cytosine \( C \) to uracil \( U \), which is then replaced by thymine \( T \) during DNA replication. Frequencies of C>T and C>G mutations are:

- Frequency C to T = 6.1E-4
- Frequency C to G = 4.9E-4

**Frequency C to T = Frequency C to G**

*Chen et al., 2013*