OXIDATIVE STRESS AND CARCINOGENESIS

Alexander V. Ivanov

May 25, 2016
### REACTIVE OXYGEN SPECIES

<table>
<thead>
<tr>
<th>ROS</th>
<th>Half-life (s)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO•</td>
<td>$10^{-9}$</td>
<td>Highly reactive</td>
</tr>
<tr>
<td>O$_2$•⁻</td>
<td>$10^{-6}$</td>
<td>Poor oxidant</td>
</tr>
<tr>
<td>$^1$O$_2$</td>
<td>$10^{-6}$</td>
<td>Highly reactive</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>$10^{-3}$ - minutes</td>
<td>Poor oxidant</td>
</tr>
</tbody>
</table>

Adapted from *K. Krumova and G. Cosa (2016)*
**Reactive Oxygen Species (ROS)**

- Electron transfer chain
  - Catabolism of polyamines, lipids...
- Protein folding
- NADPH oxidases (NOX)
- Fenton / Haber / Weiss reactions

**Fenton Reaction**

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^\cdot + \text{OH}^- 
\]

**Haber-Weiss Reaction**

\[
\text{O}_2^\cdot + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{OH}^\cdot + \text{OH}^- 
\]
ROS damage DNA, lipids, and proteins

Adapted from www.qiagen.com
NEUTRALIZATION OF ROS

Adapted from H.Zhu et al., Exp Biol Med 2008
Oxidative stress is an imbalance between ROS production and antioxidant defenses.

Prevalence of reducing agents over ROS is referred to as reductive stress.
Detection of ROS and oxidation products

Direct ROS detection

Detection of oxidation products

**Proteins**
- HS-groups
- GSH/GSSG
- 8-nitroTyr
- 8-chloroTyr
- Carbonylated proteins

**Lipids**
- Malondialdehyde (MDA)
- 4-Hydroxy-nonenal (HNE)
- F₂-Isoprostane
- TBARS

**DNA**
- 8-oxoG
- 5-hydroxyC
- 8-nitroG
**Detection of ROS: DCFH₂DA**

- DCFH₂DA does not react with hydrogen peroxide. It reacts with hydroxyl-radical and other types of ROS/RNS. Thus it reflects general redox status.

- DCFH₂DA generates ROS
DETECTION OF ROS: DHE AND MITO SOX

- DHE and MitoSOX react only with superoxide and no other type of ROS
- HPLC or mass-spec analysis is required for analysis of oxidation products

**Detection of ROS: Ratiometric Sensors**

- **HyPER proteins** respond only to hydrogen peroxide.
- Since plasmids are used, HyPER proteins allow measurement of H2O2 in cytoplasm, mitochondria, nucleus etc. The exception is endoplasmic reticulum.
- Suitable for real-time measurements.
- Drawback: pH-sensitive proteins. A control SyPER should be used.

Adapted from V. Belousov et al., Nat Protocols 2006, ARS 2016
**Nrf2/ARE Pathway**

Nrf2/ARE pathway controls expression of genes encoding:
- Enzymes of glutathione biosynthesis
- Enzymes that scavenge ROS directly (Nqo1, Prdx etc)
- Heme oxygenase (HO-1)
- Phase III proteins (multidrug-resistant proteins etc)
- Metabolic enzymes

**Redox-Sensitive Modifications of Proteins**

[Diagram of redox-sensitive modifications of proteins]
HCV LIFE CYCLE
In the liver of chronic hepatitis C patients ROS levels are increased by 2-5 log, as estimated by EPR technique.

### CHC patients exhibit elevated levels of oxidative stress markers and decrease antioxidant status...

<table>
<thead>
<tr>
<th>Control group (n:28)</th>
<th>CHC Patients (n:19) Before Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>4.20 ± 1.47</td>
<td>9.28 ± 1.61</td>
</tr>
<tr>
<td>CuZn-SOD</td>
<td>285.78 ± 96.46</td>
<td>213.84 ± 71.61</td>
</tr>
<tr>
<td>GSH-Px</td>
<td>8.01 ± 1.79</td>
<td>6.52 ± 1.86</td>
</tr>
<tr>
<td>ALT</td>
<td>21.53 ± 6.02</td>
<td>95.84 ± 22.68</td>
</tr>
<tr>
<td>AST</td>
<td>22.50 ± 4.91</td>
<td>80.52 ± 19.27</td>
</tr>
</tbody>
</table>

### ...whereas elimination of infection reverts oxidative stress

<table>
<thead>
<tr>
<th></th>
<th>CHC Patients Before Treatment</th>
<th>CHC Patients After treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>9.28 ± 1.61</td>
<td>4.88 ± 1.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CuZn-SOD</td>
<td>213.84 ± 71.61</td>
<td>357.94 ± 82.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSH-Px</td>
<td>6.52 ± 1.86</td>
<td>9.47 ± 1.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT</td>
<td>95.84 ± 22.68</td>
<td>26.73 ± 10.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST</td>
<td>80.52 ± 19.27</td>
<td>25.52 ± 8.68</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
MECHANISMS OF ROS PRODUCTION IN HCV-INFECTED CELLS
Hepatitis B and C viruses promote liver cancer

Liver cancer is the 2d most common type of cancer (2012: app 745,000 cases – WHO)

Incidence of liver cancer increases by 2.3% per year (as was estimated for 2003-2012)

Therapy is ineffective, poor survival of patients

Hepatitis B and C viruses (HBV, HCV) account for 60-80% of all cases of hepatocellular carcinoma (HCC)
  - Incidence rate in chronic hepatitis C patients – 1-5% per year
  - Chronic hepatitis C increases HCC risk by 62.9 fold (Denmark)
  - Elimination of infection reduces risk by 4.7 fold only

Genotypes of HCV affect rate of HCC incidence:
  - Bruno et al (Hepatol, 2007): gt1 (4.26%) vs gt2a/b (1.69)
  - Nikontchou et al (JVIH, 2011): in pts with cirrhosis gt3 (34%) vs non-gt3 (17%)
MARKERS OF OXIDATIVE STRESS CORRELATE WITH LIVER PATHOLOGIES

8-OxoG vs liver disease

Inflammation & tissue damage

Fibrosis

Liver cancer

Adapted from Cardin et al., BMC Cancer 2012

Yadav et al., Am J Gastro 2002

Tanaka et al., Br J Cancer 2008
HCV-ASSOCIATED LIVER CANCER IS ASSOCIATED WITH CHRONIC INFLAMMATION, ADVANCED FIBROSIS, AND OXIDATIVE STRESS,

Tanaka et al., Br J Cancer 2008

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HCC group (N = 36)</th>
<th>Non-HCC group (N = 82)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.3 ± 8.2</td>
<td>54.7 ± 11.4</td>
<td>0.3718a</td>
</tr>
<tr>
<td>Laboratory data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (IU L⁻¹)</td>
<td>91.9 ± 50.4</td>
<td>65.6 ± 52.9</td>
<td>0.0021a</td>
</tr>
<tr>
<td>AST (IU L⁻¹)</td>
<td>91.4 ± 42.7</td>
<td>60.5 ± 38.3</td>
<td>0.0003a</td>
</tr>
<tr>
<td>Liver histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory activity (0/1/2/3)⁵</td>
<td>0/4/18/14</td>
<td>1/37/31/13</td>
<td>0.0015b</td>
</tr>
<tr>
<td>Fibrosis staging (0/1/2/3/4)⁶</td>
<td>0/1/3/10/22</td>
<td>1/28/23/17/1</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>Total iron score</td>
<td>11.09 ± 4.75</td>
<td>6.23 ± 5.62</td>
<td>&lt;0.0001a</td>
</tr>
<tr>
<td>8-OHdG-positive hepatocytes (per 10⁵ μm²)</td>
<td>65.2 ± 20.2</td>
<td>40.0 ± 23.5</td>
<td>&lt;0.0001a</td>
</tr>
</tbody>
</table>

Tanaka et al., Br J Cancer 2008

Oxidative Stress in the Absence of Inflammation in a Mouse Model for Hepatitis C Virus-associated Hepatocarcinogenesis¹

Kyoji Moriya, Kiyotaka Nakagawa, Tomofumi Santa, Yoshizumi Shintani, Hajime Fujie, Hideyuki Miyoshi, Takeya Tsutsumi, Teruo Miyazawa, Kotaro Ishibashi, Toshiharu Horie, Kazuhiro Imai, Toru Todoroki, Satoshi Kimura, and Kazuhiro Koike²

Department of Internal Medicine, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655 [K. M., Y. S., H. F., H. M., T. T., S. K., K. K.]; Biodynamic Chemistry Laboratory, Tohoku University Graduate School of Life Science and Agriculture, Sendai 980-8575 [K. N., T. M.]; Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo 113-0033 [T. S., K. I.]; Daichi Pharmaceuticals, Tokyo 134-0081 [K. I.]; Department of Pharmacology, University of Chiba, Chiba 260-8670 [T. H.]; and Department of Laboratory Medicine, Keio University School of Medicine, Tokyo 160-8582 [T. T.], Japan.

ABSTRACT

The mechanism of hepatocarcinogenesis in hepatitis C virus (HCV) infection is still undefined. One possibility is the involvement of oxidative stress, which can produce genetic mutations as well as cause chromosomal hepatitis (3). If this is the case, HCV would only be indirectly associated with hepatocarcinogenesis. Another possibility is the direct involvement of HCV in hepatocarcinogenesis, whereby the product of the virus may be oncogenic and involved in cell transformation.
HCV CORE, NS3, AND NS5A PROTEINS PROMOTE CARCINOGENESIS

<table>
<thead>
<tr>
<th>HCV transgene</th>
<th>Genotype</th>
<th>Promoter</th>
<th>Genetic background</th>
<th>Pathology</th>
<th>Cancer frequency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyprotein</td>
<td>1b</td>
<td>Albumin</td>
<td>C57BL/6</td>
<td>Steatosis, HCC</td>
<td>5/37</td>
<td>(Lerat et al., 2002)</td>
</tr>
<tr>
<td>Core</td>
<td>1b</td>
<td>HBV</td>
<td>C57BL/6</td>
<td>Steatosis, HCC</td>
<td>14-31%</td>
<td>(Moriya et al., 1998)</td>
</tr>
<tr>
<td>Core-E1-E2</td>
<td>1a</td>
<td>CMV</td>
<td>B6C3F1</td>
<td>Steatosis, variety of tumors of hepatic and non-hepatic origin</td>
<td>17/185</td>
<td>(Naas et al., 2005)</td>
</tr>
<tr>
<td>NS5A</td>
<td>1b</td>
<td>HBV</td>
<td>C57BL/6J × CBA/J</td>
<td>Steatosis, tumors</td>
<td>10/163</td>
<td>(Wang et al., 2009a)</td>
</tr>
</tbody>
</table>

Adapted from McGivern and Lemon, Oncogene 2011

Different transformation pathways of murine fibroblast NIH 3T3 cells by hepatitis C virus core and NS3 proteins

Irina S. Smirnova a,b,*, Nikolai D. Aksenov a, Maksim S. Vonsky a, Maria G. Isaguliants b

a Institute of Cytology RAS, Tikhoretsky Avenue 4, 194064 St. Petersburg, Russian Federation
b Swedish Institute for Infectious Disease Control, Stockholm, Sweden

Received 25 April 2005; revised 14 April 2006; accepted 8 June 2006

Abstract

The oncogenic potential of both Hepatitis C virus (HCV) core and HCV NS3 proteins has been demonstrated, but these proteins induce transformation of immortal murine fibroblasts NIH 3T3 via different pathways. As long-term expression (50–100 passages) of HCV core triggers neoplastic transformation of NIH 3T3 through crisis of growth, HCV NS3 induces transformation shortly after transfection. We explain this distinction by different effects of core and NS3 on p53-mediated transactivation: inhibition by NS3 and activation by core protein.
© 2006 International Federation for Cell Biology. Published by Elsevier Ltd. All rights reserved.
HCV-induced oxidative stress contributes to production of TGFb1 and proinflammatory cytokines in hepatocytes, may lead to cell death followed by production of cytokines by Kupffer cells etc...

ROS produced by NADPH oxidases also play crucial ROS in activation of HSCs during fibrogenesis.
HCV inhibits removal of 8-oxoG by base-excision reparation

Adapted from Nickson & Parsons, Front Genet 2014

Pal et al., J Gastro Hepatol 2010
Another transcription factors that are heavily implicated in control of metabolic pathways are HIF1a and c-Myc, both of which are activated by HCV.

A growing number of evidence suggest that metabolic changes in the cell can trigger tumour progression.
ROS can affect cell cycle
(Experience from Hepatitis-unrelated studies)

Adapted from Menon & Goswani et al., Oncogene 2007, Sarsour et al, Cancer Res 2012
INTERPLAY BETWEEN BIOGENIC POLYAMINES AND HCV-INDUCED OXIDATIVE STRESS

ODC
SSAT
SMO
**Summary**

1. Hepatitis C (HCV) virus is a carcinogenic virus, elimination of infection does not reduce risk for development of liver cancer to the baseline level
2. HCV triggers massive oxidative stress as shown both in vitro and in vivo
3. Markers of oxidative stress correlate with liver inflammation, fibrosis score and risks for liver cancer
4. Reactive oxygen species (ROS) are contribute to tumorigenesis even in the absence of inflammation
5. ROS are involved in fibrogenesis
6. Induction of ROS in the infected cells contribute to dysregulation of signaling pathways, metabolic changes, genome instability etc

**Future Directions**

1. Estimation of role of different ROS-producing systems in HCV-associated pathologies
2. Detailed analysis of compartmentalization of ROS production in the infected cells
3. Analysis of HCV impact of ROS-scavenging systems (such as peroxiredoxins)
4. Explore possible redox-sensitive modification of viral and host cell proteins and their impact in pathologies
ACKNOWLEDGEMENTS

Olga Smirnova
Olga Ivanova
Vladimir Valuev-Elliston
Olga Khomich
Alex Khomutov
Marina Kukhanova
Sergey Kochetkov

This work was supported by Russian Ministry of Education and Science (Agreement 14.616.21.0043)