**Hypothesis**

**Persistent oxidative stress in cancer**

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**Abstract** DNA of cancers such as renal cell carcinoma and mammary invasive ductal carcinoma, is persistently exposed to more oxidative stress than that of adjacent normal tissue. This indicates that DNA of neoplasm, especially adenocarcinoma.

We have recently reported that human renal cell carcinomas have a higher content of 8-OH-dG than adjacent non-tumorous renal tissue [9]. Tables 1 and 2 summarize the published data. Similar results have been found in tumors of other organs. Four independent groups of investigators showed a higher content of 8-OH-dG in human tumors by the use of two different methods. This indicates that DNA of neoplasm, especially adenocarcinoma, is more exposed to ROS than its non-tumorous counterpart.

It is reasonable to suppose that membrane lipids near the ROS generation site are the most severely damaged.

The metabolism of ROS in cancer cells is a research area that has not been intensively pursued. The first important question is whether cancer cells produce larger amounts of ROS than non-neoplastic cells or whether the antioxidant system of cancer cells is suppressed. There is evidence favoring both mechanisms. Large amounts of hydrogen peroxide are reportedly produced in vitro without exogenous stimulation in several human carcinoma cell lines, including malignant melanoma, colon carcinoma, pancreatic carcinoma, neuroblastoma, breast carcinoma and ovarian carcinoma [10]. A flavoprotein and/or a nucleotide oxidase may be involved in the mechanism of H2O2 production [10]. Hydrogen peroxide freely passes through membranes and can reach any cellular compartment [1]. Since 'OH is very reactive, essentially reacting at the site of its generation, it is likely that 'OH is produced from H2O2 in the presence of intracellular 'catalytic' iron or copper through the Fenton reaction [11] to induce 8-OH-dG formation and lipid peroxidation [1]. Indeed, 'OH generation in human cancers is confirmed by the fact that the spectrum of modified DNA bases induced was that of 'OH attack [12,13].

On the other hand, catalase gene expression is reportedly negatively regulated in human hepatoma cells [14]. Indeed, tumor cells are always low in manganese superoxide dismutase activity, usually low in copper and zinc superoxide dismutase activity, and almost always low in catalase activity. The activity of glutathione peroxidase and glutathione reductase is highly variable [15]. The amount of H2O2 or superoxide contained in tumor cells should be raised by this regulation. However, the antioxidant system is complicated. For example, glutathione peroxidase has catalase activity and may functionally compensate for lack of catalase.

In spite of evidence that cancer cells are oxidatively stressed (Tables 1 and 2), the stress is usually insufficient to cause cell death. A rapid and pronounced increase in 8-OH-dG content and 4-hydroxy-2-nonenal-modified proteins was associated with cell death in our experimental ROS-induced renal carcinogenesis model using ferric nitritroatocetate [5,16]. Some tumor cells are resistant to oxidative cytolyis [17], and adenocarcinomas in general, especially renal cell carcinomas, are highly chemotherapy resistant [18]. Accordingly, cancer cells should have mechanisms, other than the antioxidant enzymes mentioned above, to resist increased oxidative stress. Three possible candidates are adult T-cell leukemia-derived factor (ADF), glutathione-S-transferase π (GST-π) and glutathione. ADF is a human thiorerdoxin homologue with many functions, including interleukin-2 receptor induction, thiol-dependent reducing activity, radical scavenging activity [19] and NF-xB activation [20]. Recently, it was found that a variety of human cancers, including adult T-cell leukemia, squamous cell carcinoma of the uterine cervix [21] and hepatocellular carcinoma [22], show increased amounts of ADF. GST-π, also increased in cancers.
Table 1
Content of 8-hydroxy-2'-deoxyguanosine in human carcinoma (multiple case study)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Histology</th>
<th>Control n; statistics</th>
<th>Tumor n; statistics</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Invasive ductal carcinoma</td>
<td>4.13 ± 0.43</td>
<td>40.1 ± 11.1</td>
<td>(5; P &lt; 0.01)</td>
<td>GC/MS</td>
</tr>
<tr>
<td>Lung</td>
<td>Squamous cell carcinoma</td>
<td>11.2 ± 2.3</td>
<td>25.5 ± 7.0</td>
<td>(5; P &lt; 0.05)</td>
<td>GC/MS</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatocellular carcinoma</td>
<td>1.57 ± 0.21</td>
<td>2.29 ± 0.38</td>
<td>(11, 18; P = 0.174)</td>
<td>HPLC/ECD</td>
</tr>
<tr>
<td>Kidney</td>
<td>Renal cell carcinoma</td>
<td>3.60 ± 0.20</td>
<td>5.56 ± 0.41</td>
<td>(31; P &lt; 0.0005)</td>
<td>HPLC/ECD</td>
</tr>
</tbody>
</table>

The amount of 8-hydroxy-2'-deoxyguanosine per 10^6 deoxyguanosine in DNA is shown by means ± S.E.M. Some of the data were modified for unity. All the samples except those from [34] are paired samples from the same patient. The patients were all heavy smokers [13]. Control liver samples were from patients with metastatic liver tumors [34]. GC/MS, gas chromatography with mass spectrometry; HPLC/ECD, high performance liquid chromatography with electrochemical detector.

Table 2
Content of 8-hydroxy-2'-deoxyguanosine in human neoplasm (single case study)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Histology</th>
<th>Control</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Fibrillary astrocytoma</td>
<td>1.36</td>
<td>2.49</td>
</tr>
<tr>
<td>Lung</td>
<td>Squamous cell carcinoma</td>
<td>9.68</td>
<td>10.1</td>
</tr>
<tr>
<td>Lung</td>
<td>Adenocarcinoma</td>
<td>7.33</td>
<td>23.0</td>
</tr>
<tr>
<td>Colon</td>
<td>Non-primary carcinoma</td>
<td>2.71</td>
<td>4.43</td>
</tr>
<tr>
<td>Stomach</td>
<td>Mucinous carcinoma</td>
<td>0.94</td>
<td>5.08</td>
</tr>
<tr>
<td>Ovary</td>
<td>Serous cystadenocarcinoma</td>
<td>3.11</td>
<td>9.20</td>
</tr>
</tbody>
</table>

Data based on GC/MS method are from [12]. The amount of 8-hydroxy-2'-deoxyguanosine per 10^6 deoxyguanosine in DNA is shown.

[23] and in the serum of cancer patients [24], is induced upon oxidative stress and is associated with chemoresistance [25,26]. Glutathione, a tripeptide thiol present in virtually all animal cells, is also synthesized by tumors. For example, infiltrating ductal breast carcinoma has more than twice the levels of glutathione found in normal breast tissue [27].

What is the significance of persistent oxidative stress in cancer? First, it may constantly activate transcription factors, such as NF-κB, through the intracellular signal transduction system [28] and induce expression of proto-oncogenes such as c-fos, c-jun and c-myc [29,30]. Secondly, oxidative stress induces DNA damage such as modified base products and strand breaks that may lead to further mutation and chromosomal...
aberration of cancer (genomic instability) [13]. Thirdly, it can activate specific antioxidant systems in cancer cells that may make cancer cells resistant to chemotherapy. Indeed, bleomycin and doxorubicin are known to function as antitumor agents by producing ROS in the target tissue [1]. Finally, ROS can specifically damage certain protease inhibitors, including α1-protease inhibitor, α2-macroglobulin, plasminogen activator inhibitor and α2- plasmin inhibitor by oxidizing methionine residues of the active site [31,32]. Inactivation of these protein inhibitors could induce enhanced action of proteases, such as elastase, plasminogen activator and plasmin. This may facilitate tumor invasion and metastasis [lo].

Our hypothesis of a role for ROS in tumor biology is summarized in Fig. 1. We believe that persistent oxidative stress may partly explain the characteristics of cancer, such as activated proto-oncogenes and transcription factors, genomic instability, chemotherapy-resistance, invasion and metastasis. Further study is necessary to establish the role of ROS in tumor biology and to prove that this hypothesis can be applied to various kinds of malignant tumors.

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References