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 nornal tissue. We suggest that the concept of 'persistent oxidative stress in cancer' may open up a new research area, explaining part of the characteristic tumor biology of cancer such as activated transcription factors and proto-oncogenes, genomic instability, chemotherapy-resistance, invasion and metastasis.

Key words: Reactive oxygen species; Oxidative DNA damage; Tumor biology; Mutation; Transcription factor

Reactive oxygen species (ROS) are involved in a diversity of important phenomena in medicine, such as ischemia-reperfusion injury, pulmonary oxygen toxicity, atherosclerosis, radiation effects, chemotherapeutic effects, mutagenesis, carcinogenesis and aging [1]. In this paper, we hypothesize a role for ROS in the biology of malignant neoplasia, especially adenocarcinoma.

Among the targets of ROS, DNA appears most important in tumor biology since it is firmly established that cancer is a genetic disease [2]. ROS induce several kinds of DNA damage, including strand breakage, base modification and DNA–protein cross-linkage [3]. 8-Hydroxy-2′-deoxyguanosine (8-OH-dG, also known as 7,8-dihydro-8-oxo-2′-deoxyguanosine) [4] is one of the major oxidatively modified DNA base products in vivo [3,5], is mutation-prone (G:C to T:A transversion) [6,7] and is easily detected by high performance liquid chromatography combined with an electrochemical detector [3]. It is known that either the hydroxyl radical (•OH), singlet oxygen or photodynamic action is responsible for the formation of 8-OH-dG [3,8].

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-OHdG 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.

What kind of cells produce ROS in this situation? Cancer cells themselves, endothelial cells or macrophages are major candidates. Based on our renal cell carcinoma immunohistochemical study of lipid peroxidation products (4-hydroxy-2-nonenal-modified proteins), we believe that cancer cells themselves produce ROS, at least in human renal cell carcinoma. Lipid peroxidation products were demonstrated in the cytoplasm of renal cell carcinoma cells with a granular pattern [9]. 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 nucleoside oxidase may be involved in the mechanism of H_2O_2 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 H_2O_2 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 H_2O_2 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 nitrilotriacetate [5,16]. Some tumor cells are resistant to oxidative cytolysis [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-P) and glutathione. ADF is a human thioredoxin homologue with many functions, including interleukin-2 receptor induction, thiol-dependent reducing activity, radical scavenging activity [19] and NF-κB 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-P, also increased in cancers

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Table 1
Content of 8-hydroxy-2′-deoxyguanosine in human carcinoma (multiple case study)

Organ	Histology	Control	Tumor	*n*; statistics	Method	Reference
Breast	Invasive ductal carcinoma	4.13 ± 0.43	40.1 ± 11.1	(5; $P < 0.01$)	GC/MS	[33]
Lung	Squamous cell carcinoma	11.2 ± 2.3	25.5 ± 7.0	(5; $P < 0.05$)	GC/MS	[13]
Liver	Hepatocellular carcinoma	1.57 ± 0.21	2.29 ± 0.38	(11, 18; $P = 0.174$)	HPLC/ECD	[34]
Kidney	Renal cell carcinoma	3.60 ± 0.20	5.56 ± 0.41	(31; $P < 0.0005$)	HPLC/ECD	[9]

The amount of 8-hydroxy-2′-deoxyguanosine per 10^5 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.

[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 glutatione 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

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

Organ	Histology	Control	Tumor
Brain	Fibrillary astrocytoma	1.36	2.49
Lung	Squamous cell carcinoma	9.68	10.1
Lung	Adenocarcinoma	7.33	23.0
Colon	Non-primary carcinoma	2.71	4.43
Stomach	Mucinous carcinoma	0.94	5.08
Ovary	Serous cystadenocarcinoma	3.11	9.20

Data based on GC/MS method are from [12]. The amount of 8-hydroxy-2′-deoxyguanosine per 10^5 deoxyguanosine in DNA is shown.

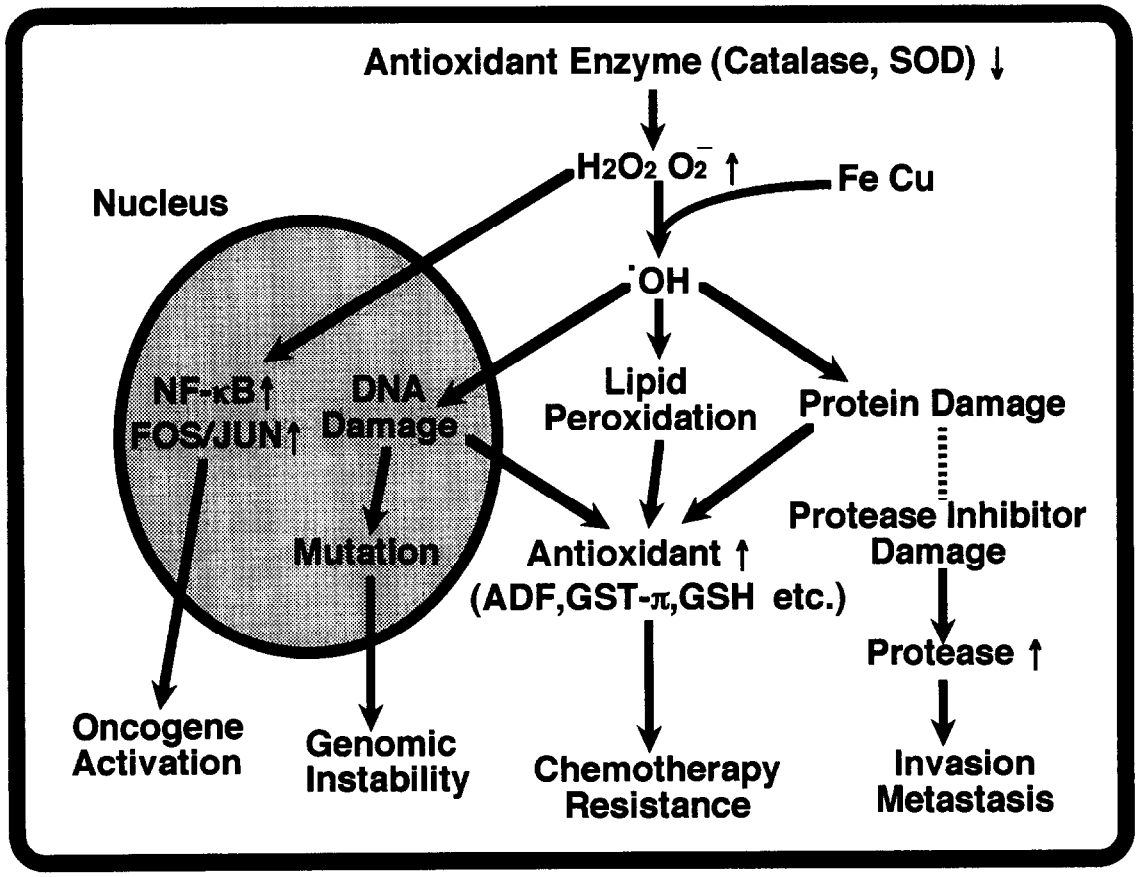

Fig. 1. Schematic outline of the role of reactive oxygen species in cancer. SOD, superoxide dismutase; ·OH, hydroxyl radical; ADF, adult T-cell leukemia-derived factor; GST, glutathione *S*-transferase; GSH, glutathione.

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 doxorubucin are known to function as antitumor agents by producing ROS in the target tissue [1]. Finally, ROS can specifically damage certain protease inhibitors, including α_1-proteinase inhibitor, α_2-macroglobulin, plasminogen activator inhibitor and α_2-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 [10].

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

[1] Halliwell, B. and Gutteridge, J.M.C. (1989) Free Radicals in Biology and Medicine, 2nd ed., Clarendon Press, Oxford.
[2] Cotran, R.S., Kumar, V. and Robbins, S.L. (1989) in: Robbins Pathologic Basis of Disease, pp. 239–305, Saunders, Philadelphia.
[3] Halliwell, B. and Aruoma, O.I. (1993) DNA and Free Radicals, Ellis Horwood, Chichester, England.
[4] Kasai, H. and Nishimura, S. (1984) Nucleic Acids Res. 12, 2137–2145.
[5] Toyokuni, S., Mori, T. and Dizdaroglu, M. (1994) Int. J. Cancer 57, 123–128.
[6] Kuchino, Y., Mori, F., Kasai, H., Inoue, H., Iwai, S., Miura, K., Ohtsuka, E. and Nishimura, S. (1987) Nature 327, 77–79.
[7] Shibutani, S., Takeshita, M. and Grollman, A.P. (1991) Nature 349, 431–434.
[8] Kasai, H., Yamaizumi, Z., Berger, M. and Cadet, J. (1992) J. Am. Chem. Soc. 114, 9692–9694.
[9] Okamoto, K., Toyokuni, S., Uchida, K., Ogawa, O., Takenawa, J., Kakehi, Y., Kinoshita, H., Hattori-Nakakuki, Y., Hiai, H. and Yoshida, O. (1994) Int. J. Cancer 58, 825–829.
[10] Szatrowski, T.P. and Nathan, C.F. (1991) Cancer Res. 51, 794–798.
[11] Gutteridge, J.M.C., Rowley, D.A. and Halliwell, B. (1982) Biochem. J. 206, 605–609.
[12] Olinski, R., Zastawny, T., Budzbon, J., Skokowski, J., Zegarski, W. and Dizdaroglu, M. (1992) FEBS Lett. 309, 193–198.
[13] Jaruga, P., Zastawny, T.H., Skokowski, J., Dizdaroglu, M. and Olinski, R. (1994) FEBS Lett. 341, 59–64.
[14] Sato, K., Ito, K., Kohara, H., Yamaguchi, Y., Adachi, K. and Endo, H. (1992) Mol. Cell Biol. 12, 2525–2533.
[15] Sun, Y. (1990) Free Radic. Biol. Med. 8, 583–599.
[16] Toyokuni, S., Uchida, K., Okamoto, K., Hattori-Nakakuki, Y., Hiai, H. and Stadtman, E.R. (1994) Proc. Natl. Acad. Sci. USA 91, 2616–2620.
[17] O'Donnell-Tormey, J., DeBoer, C.J. and Nathan, C. (1985) J. Clin. Invest. 76, 80–86.
[18] Yogoda, A. (1989) Semin. Urol. 7, 199–206.
[19] Mitsui, A., Hirakawa, T. and Yodoi, J. (1992) Biochem. Biophys. Res. Commun. 186, 1220–1226.
[20] Matthews, J.R., Wakasugi, N., Virelizer, J.-L., Yodoi, J. and Hay, R.T. (1992) Nucleic Acids Res. 20, 3821–3830.
[21] Fujii, S., Nanbu, Y., Nonogaki, H., Konishi, I., Mori, T., Masutani, H. and Yodoi, J. (1991) Cancer 68, 1583–1591.
[22] Nakamura, H., Masutani, H., Tagaya, Y., Yamauchi, A., Inamoto, T., Nanbu, Y., Fujii, S., Ozawa, K. and Yodoi, J. (1992) Cancer 69, 2091–2097.
[23] Shiratori, Y., Soma, Y., Maruyama, H., Sato, S., Takano, A. and Sato, K. (1987) Cancer Res. 47, 6806–6809.
[24] Tsuchida, S., Sekine, Y., Shineha ,R., Nishihira T. and Sato, K. (1989) Cancer Res. 49, 5225–5229.
[25] Batist, G., Tulpule, A., Sinha, B.K., Katki, A.G., Meyers, C.E. and Cowan, K.H. (1986) J. Biol. Chem. 261, 15544–15549.
[26] Nakagawa, K., Saijo, N., Tsuchida, S., Sakai, M., Tsunokawa, Y., Yokota, J., Muramatsu, M., Terada, M. and Tew, K.D. (1990) J. Biol. Chem. 265, 4296–4301.
[27] Perry, R.R., Mazetta, J., Levin, M. and Barranco, S.C. (1993) Cancer 72, 783–787.
[28] Schreck, R., Rieber ,P. and Baeuerle, P.A. (1991) EMBO J. 10, 2247–2258.
[29] Crawford, D., Zbinden, I., Amstad, P. and Cerutti, P. (1988) Oncogene 3, 27–32.
[30] Nose, K., Shibanuba, M., Kikuchi, K., Kageyama, H., Sakiyama, S. and Kuroki, T. (1991) Eur. J. Biochem. 201, 99–106.
[31] Swaim, M.W. and Pizzo, S.V. (1988) J. Leukocyte Biol. 43, 365–379.
[32] Johnson, D. and Travis, J. (1979) J. Biol. Chem. 254, 4022–4026.
[33] Malins, D.C. and Haimanot, R. (1991) Cancer Res. 51, 5430–5432.
[34] Shimoda, R., Nagashima, M., Sakamoto, M., Yamaguchi, N., Hirohashi, S., Yokota, J. and Kasai, H. (1994) Cancer Res. 54, 3171–3172.