Acta Med. Nagasaki 50: 63－68

A Paradigm Shift in the Understanding of Oxidative Stress and its Implications to Exposure of Low-level Ionizing Radiation

Brad L. UPHAM, James E. TROSKO

National Food Safety and Toxicology Center, Department of Pediatrics and Human Development, Michigan State University, Michigan, USA

For many years, research on oxidative stress focused primarily on determining how reactive oxygen species (ROS) damage cells by indiscriminate reactions with its macromolecular machinery, particularly lipids, proteins and DNA. However, many chronic diseases affiliated with oxidative stress are not always a consequence of tissue necrosis, DNA, or protein damage but rather to altered gene expression. Gene expression is highly regulated by the coordination of extra-, intra- and inter-cellular communication systems that typically maintain tissue homeostasis by sustaining a balance between proliferation, differentiation and apoptosis. Therefore, much research has shifted to the understanding of how ROS reversibly controls gene expression at noncytotoxic doses through cell signaling mechanisms. Cell proliferation typically involves a transient inhibition of gap junctional intercellular communication (GJIC) and the activation of mitogen activated protein kinase pathways (MAPK). We demonstrate that epidermal growth factor (EGF) inhibited GJIC in normal rat liver epithelial cells in addition to activating extracellular signal regulatory kinase, a MAPK. Inhibition of NADPH oxidase, which reduces oxygen to H_2O_2 with the very selective inhibitor diphenyleneiodonium, prevented EGF from inhibiting GJIC, suggesting that the generation of H_2O_2 is an essential component of the intracellular pathway controlling GJIC. We previously demonstrated that reduced-glutathione (GSH) was also a necessary cofactor of H_2O_2-induced inhibition of GJIC. These results demonstrate that ROS and GSH play essential roles in controlling EGF-dependent control of GJIC. Therefore, the overly simplistic approach of either preventing the generation of ROS or accelerate the removal by antioxidants could deleteriously alter normal signaling functions.

ACTA MEDICA NAGASAKIENSIA 50: 63－68, 2005

Keywords: Oxidative stress; Gap junctional intercellular communication; Epigenetic toxicity; Cancer; NADPH oxidase; Signal transduction

Introduction

Otto Warburg was the first to implicate oxygen in cancer as far back as the 1920s.[1] Although his theory of altered oxidative metabolism fell mostly on deaf ears, recently there has been a renewed interest in how cancer cells shift their energy production from oxidative phosphorylation to anaerobic glycolysis, the "Warburg Effect", and is being considered a fundamental property of cancer cells and not just a consequence of malignant cell transformation.[2-4] In contrast to the role of low oxygen tensions, the discovery of superoxide dismutase in 1968 by McCord and Fridovich[5] led to an explosion of research on the role of reactive oxygen, a product of high oxygen tensions, in the pathologies of biological organisms, and has been specifically connected with not only cancer but also many other human diseases.[6,7] For many years, research in oxidative stress focused primarily on determining how reactive oxygen species (ROS) damage cells by indiscriminate reactions with the macromolecular machinery of a cell, particularly lipids, proteins and DNA.

We know in great detail how ROS react with lipids leading to the peroxidation of biological membranes resulting in necrotic lesions[8], and how ROS reacts with the nucleotides of DNA leading to genetic instability.[8,9] However, many chronic diseases affiliated with oxidative stress, such as cancer, are not always a consequence of tissue necrosis, DNA damage and genetic instability, or protein damage but rather to altered gene expression through epigenetic mechanisms.[10-12] For example, organic and hydrogen peroxides act as tumor promoters and not as initiators[13-16], indicating that these oxidants are not mutagens but rather epigenetic effectors. Hydrogen peroxide has also been demonstrated to be a promoter but not an initiator using two stage *in vivo* carcinogenesis model systems and transformation in vitro systems.[14,16-18] These peroxide results were only several of many experiments indicating that ROS contributed to chronic diseases at

Address correspondence: Brad L. Upham, Ph.D., Michigan State University, 243 National Food Safety and Toxicology Center, East Lansing, Michigan 48824 USA

TEL: +1-517-432-3100 X186, FAX: +1-517-432-6340, E-mail: brad.upham@ht.msu.edu

Received May 22, 2005; Accepted June 20, 2005

non-genotoxic, non-damaging levels. Thus, in the past decade and a half, much research has shifted to the understanding of how ROS can reversibly control the expression of genes at noncytotoxic doses.[6] In this regard, at least 127 genes and signal transducing proteins have been reported to be sensitive to reductive and oxidative (redox) states in the cell.[6] This shift in thinking should start to shape both the design and the interpretation of experiments on low-level radiation effects.

Oxidative stress, a known mediator of cancer, can clearly interrupt reactive oxygen-dependent cell signaling pathways controlling gene expression that contribute to all stages of cancer, in particular tumor promotion. The source of reactive oxygen involved in signaling-induced events is under active investigation, particularly in the cardiovascular field in which non-phagocytic NADPH oxidase has been strongly implicated in growth factor-induced generation of reactive oxygen.[19-24] We report in this study that NADPH oxidase is also involved in epidermal growth factor (EGF)-induced inhibition of GJIC in a rat liver epithelial cell line.

Methods

Chemicals

Formaldehyde (37%) was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI); lithium salt of Lucifer Yellow, oxidized and reduced forms of glutathione, and buthionine sulfoximine from Sigma Chemical Co. (St. Louis, MO); acetonitrile and monohydrate sodium dihydrogen phosphate from EM Science (Gibbstown, NJ); H_2O_2 and methanol from JT Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ); sodium chloride from Columbus Chemical Industries (Columbus, WI); and sodium 1-octanesulfonate from ACROS (Pittsburgh, PA).

Cell culture

A diploid, non-tumorigenic WB-F344 rat liver epithelial cell line was obtained from Drs. J. W. Grisham and M. S. Tsao of the University of North Carolina (Chapel Hill, NC).[25] This cell line was used for the following reasons. Many *in vivo* tumor promotion assays were done in rat liver, specifically in the Fischer 344 rats. The WB-344 cell line was designed to complement *in vivo* studies performed in the same rat strain. The WB-cells are also diploid cells that are non-tumorigenic.[25] This cell line has been extensively characterized for its expressed gap junction genes and its ability to perform GJIC *via* all available techniques in the absence and presence of well-known tumor promoters, and tested for the ability of growth factors and oncogenes to modulate GJIC.[11] Intrahepatic transplantation of WB-cells into adult syngenic F344 rats results in the morphological differentiation of these cells into hepatocytes and incorporation into hepatic plates.[26] The pluripotency of these cells were further demonstrated by their differentiation into functional-contracting cardiomyocytes, when transplanted into the heart tissue of adult syngenic F344 rats.[27]

Cells were cultured in 2 ml of D-medium (Formula No. 78-5470EG, GIBCO Laboratories, Grand Island, NY) and supplemented with 5% fetal bovine serum (GIBCO Laboratories Grand Island, NY) and 50 μg/mL gentamicin (GIBCO Laboratories Grand Island, NY). The cells were grown in 35 mm diameter plastic culture dishes (Corning Glass Works, Corning, NY) and incubated at 37ºC in a humidified atmosphere containing 5% CO_2 and 95% air. Bioassays were conducted on confluent cells that were obtained after 2 days of growth.

GJIC

GJIC was determined by using the scrape loading/dye transfer (SL/DT) assay as adapted from previously described methods.[28] Following the chemical exposures, cells were rinsed 3 times with PBS, and then approximately 1 mL of 0.1% Lucifer yellow dye (LY), dissolved in PBS, was added to the cells. A surgical steel blade was used to make 3 scrapes through the monolayer of cells to inject the LY followed by a 3-minute incubation time at room temperature, and then the dye was discarded; the cells were rinsed 3 times with PBS, and then fixed with approximately 0.5 mL of 4% formalin. The SL/DT assay was done immediately following chemical exposure.

The migration of the dye in the cells was observed using a Nikon epifluorescence phase microscope, illuminated with an Osram HBO 200 W lamp, and equipped with a CCD camera (NucleoTech Corp, San Mateo, CA). The distance the dye spread from the scrape line was measured from the digitized images using 'Gel Expert' program (NucleoTech Corp, San Mateo, CA). Comparing the distance the dye traveled in the chemically treated cells to the distance the dye traveled in the vehicle controls assessed GJIC. GJIC was reported as a fraction of the control (FOC). A FOC value of approximately 1.0 indicates normal GJIC, and FOC values less than one indicates inhibition. All experiments were done at least in triplicate and the results were reported as an average ± the standard deviation at the 95% confidence interval.

GSH determination

Cells were lysed with 2 mL of 0.05 N perchloric acid, and the lysate was filtered through a 0.22 μm filter and used for the determination of GSH. The lysate was fractionated using an 150mm × 4.6 mm adsorbosphere C18 5μMF-Plus HPLC column (Alltech Assoc., Deerfield, IL) and a mobile phase that consisted of 50 mM sodium phosphate, 0.05 mM 1-octanesulfonic acid, 2% acetonitrile, pH = 2.70, and a flow rate of 1.0 mL/min. The HPLC system used was a 580 solvent delivery module from ESA (Chelmsford, MA). The GSH was detected with a Coulochem Model 5200 electrochemical detector (ESA, Chelmsford, MA) with applied potentials of +400, +900, and +950 mV set for the screening, analytical and guard cell electrodes, respectively.

Data analysis

All data were compared to, and expressed as, a FOC. Each value represents the average FOC of at least three measurements from three different culture plates ± the standard deviation at the 95% confidence interval. Curve fitting was done using SigmaPlot (Jandel Scientific Software, Jandel Corp., San Rafael, CA), which uses the Marquardt-Levenberg algorithm of a least squares fit. SigmaStat (V. 2.0, Jandel Scientific Software, Jandel Corp., San Rafael, CA) was used for paired *t*-tests, ANOVA and post hoc tests.

Results

We previously reported that hydrogen peroxide inhibited GJIC in a dose-dependent fashion.[29] This H_2O_2-induced inhibition of GJIC was reversible.[29] This inhibition of GJIC by H_2O_2 is not likely a consequence of downstream ROS products of H_2O_2, i.e. hydroxyl, alkoxyl or peroxyl radicals, considering that free radical scavengers such as propylgallate and Trolox®, which do not affect H_2O_2, did not reverse the inhibitory effect of H_2O_2 (Figure 1).[29]

The reduced form of glutathione (GSH) is the major reductant of H_2O_2, and the depletion of this antioxidant commonly results in greater oxidative damage of macromolecules. However, depletion of GSH has the opposite effect on the oxidative inhibition of GJIC (Figure 2).[29] Cells were depleted of GSH by either inhibiting γ-glutamylcysteine synthetase, which catalyzes the rate-limiting step of the biosynthesis of GSH, with buthionine sulfoximine (BSO) or the conjugation of GSH with diethylmaleate (DEM) (Figure 2). Treatment with BSO for 24 h results in a 72%, and with DEM for 1 h in a 95% reduction of GSH (Figure 2). Addition of H_2O_2 to these GSH-depleted cells did not inhibit GJIC (Figure 2). No GSH depletion was observed in cells treated with BSO for 1 h, and the addition of H_2O_2 to these GSH-sufficient cells containing BSO resulted in inhibition of GJIC, which indicates that GSH-depletion and not the BSO prevented H_2O_2 from inhibiting GJIC.

A transient production of H_2O_2 is a common response of a cell to growth factors and is essential in the activation of mitogen activated protein kinases (MAPKs).[30,31] Non-phagocytic NADPH-oxidase has been implicated in this transient production of H_2O_2 and diphenyleneiodonium (DPI) is a selective inhibitor of this enzyme.[31] Preincubation of DPI with the F344-WB cells prevented the inhibition of GJIC by EGF (Figures 3 and 4), thus implicating NADPH oxidase in EGF-induced inhibition of GJIC. Inhibition of GJIC by EGF was also prevented with the inhibition of Mek with PD98059 (Figure 4).

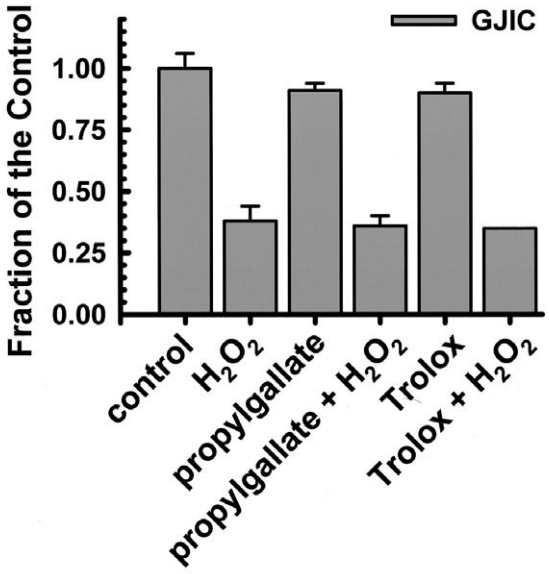

Figure 1. The effects of free radical scavengers on H_2O_2-induced inhibition of GJIC. The figure was graphed with data obtained from.[29] The concentrations of H_2O_2, propylgallate and Troloxa were 500, 750 and 750 μM, respectively. The data are reported as a fraction of the control, and GJIC assessed using the scrape load/dye transfer technique. The H_2O_2, H_2O_2 + Trolox® or propylgallate were significantly different from the control (ANOVA, $p \leq 0.001$, $F = 159$; Holm-Sidak post-hoc test, $p \leq 0.05$) but not with each other based on the same post-hoc analysis or based on a new ANOVA only comparing this peroxide-group (ANOVA, $p = 0.685$, $F = 0.404$) (ANOVA, $p = 0.685$, $F = 0.404$). Each value represents the average of three replicates±± one standard deviation.

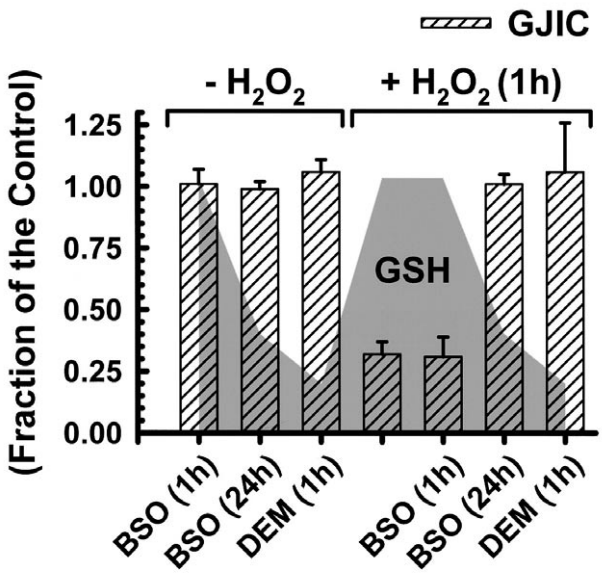

Figure 2. The effects of GSH levels on H_2O_2-induced inhibition of GJIC. The figure was graphed with data obtained from.[29] The GSH levels are indicated in the shaded grey area, and GJIC activity in the hatched bars. The concentrations of H_2O_2, buthionine sulfoximine (BSO), and diethylmaleate (DEM) were 500, 100 and 1000 μM, respectively. The data are reported as a fraction of the control, and GJIC assessed using the scrape load/dye transfer technique. GSH was assessed with HPLC-electrochemical detection. The H_2O_2, H_2O_2 + BSO (1h) treatments (GSH sufficient cells) were significantly different from the control, however BSO (24h) ± H_2O_2 or DEM (1h) ± H_2O_2 (GSH deficient cells) were not significantly different from the control (ANOVA, $p \leq 0.001$, $F = 43.1$; Holm-Sidak post-hoc test, $p \leq 0.05$). Each value represents the average of three replicates ± one standard deviation.

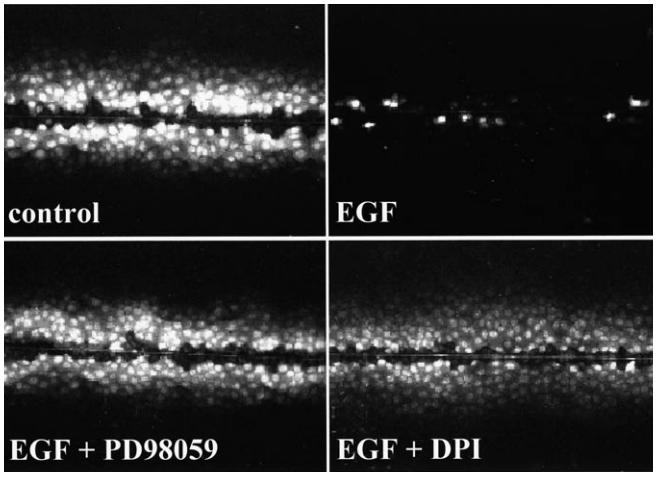

Figure 3. Scrape load/dye transfer images of cells treated with epidermal growth factor ± a MEK or NADPH oxidase inhibitor. Magnification was (×200), and the concentrations of epidermal growth factor (EGF), diphenyleneiodonium (DPI) and PD98059 were 10 μg/L, 5 μM, and 20 μM respectively. Cells were incubated for 15 min with EGF and preincubated with PD98059 and DPI for 15 min and 20 min, respectively. Quantitative results from triplicate sets of data of SL/DT images presented in Figure 4.

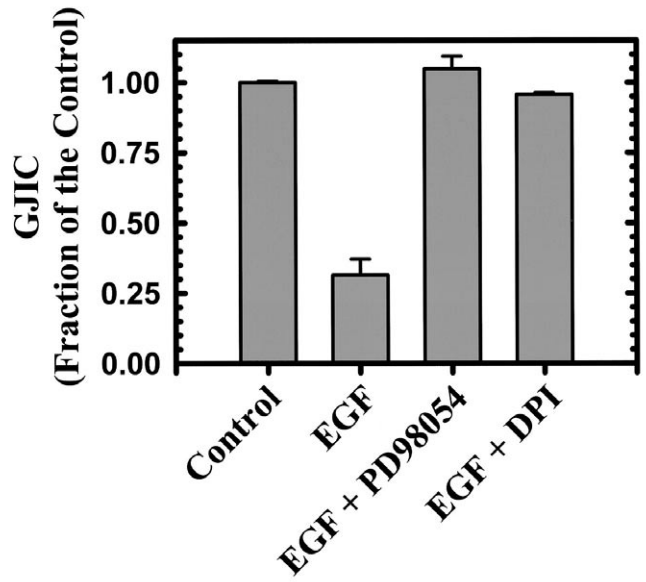

Figure 4. The reversal of EGF-induced inhibition of GJIC with MEK and NADPH Oxidase inhibitors. The concentrations and treatment times are the same as those in Figure 3. The concentration and pretreatment time for BSO was 100 μM and 24 h. Only the EGF and EGF + BSO treated cells were significant from the control (ANOVA, $p< 0.001$, $F= 304$; Tukey post-hoc $p< 0.05$). The same ANOVA/post-hoc test also indicated that EGF was significantly different from EGF + BSO treated cells.

Discussion

Oxidative stress has long been affiliated with acute and chronic human diseases, and was believed to be primarily a consequence of indiscriminate, cumulative damage to proteins, lipids and DNA from the vigorous production of ROS levels that overwhelm the cell's antioxidant defense system. However, pathological causes of many chronic diseases, particularly cancer, has also been linked with noncytotoxic, nongenotoxic events, and the role of ROS and antioxidants in human diseases must also address epigenetic events.[10]

Although many intracellular-signaling pathways are known to be redox-sensitive, the mitogen activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) signal transduction pathways have been examined far more than any other pathways.[6,32,33] These two pathways either directly or indirectly transduce most redox responses.[6] MAPK is not only activated by ROS[34] but actually requires the presence of H_2O_2.[35] Sundaresan et al.[35] were the first to demonstrate that a transient burst of H_2O_2 by an extracellular ligand, namely platelet derived growth factor (PDGF), using a vascular smooth muscle cell system is essential in the activation of ERK-MAPK. They demonstrated that the activation of ERK-MAPK by PDGF was prevented by the transfection of catalase, which dismutates H_2O_2 in these cells. Downstream events of MAPK activation, such as proliferation and increased cell motility, were also inhibited by catalase in their experiments. This was one of many studies that demonstrated endogenous growth factors (extracellular ligands) generate ROS, which are then required downstream in intracellular signaling to successfully transmit their signals to the nucleus.[31,36]

The successful transmission of an extracellular signal from the membrane to the nucleus via intracellular signaling pathways in solid tissue cell-types is also dependent upon intercellular signals through gap junctions.[10-12] Not surprisingly, H_2O_2 has also been demonstrated to reversibly inhibit gap junctional intercellular communication (GJIC) at noncytotoxic levels[29,37] (Figures 1 and 2). Similar to the results of the published MAPK experiments, inhibition of NADPH oxidase also prevented EGF-induced inhibition of GJIC (Figures 3 and 4). Inhibition of GJIC was also dependent on MEK-activity (Figures 3 and 4) and agrees with previously published results.[37,38] This is not surprising considering that the MEK-ERK pathways are redox regulated as cited above. One potentially major implication of these results is that if gap junctions were not closed, then the H_2O_2 generated by extracellular ligands could escape from the targeted cell through gap junction channels into neighboring cells, thereby potentially diluting H_2O_2 to a non-threshold level that would be insufficient for MAPK dependent activation of transcription factors. These examples demonstrate how extra-, intra-, and intercellular signaling pathways might interact to coordinate the epigenetic expression of genes in response to ROS (Figure 5).

In addition to oxidants serving normal roles as subcellular messengers in gene regulatory and signal transduction pathways, antioxidants have also been demonstrated to serve as subcellular messengers for normal cell function.[6] For example, a major H_2O_2-scavenging pathway is the two-electron reduction of H_2O_2 catalyzed by glutathione peroxidase, and clearly serves as a protective role against peroxide-dependent oxidative injury. However, depletion of intracellular pools of glutathione (GSH), by inhibiting the rate-limiting step of its biosynthesis, paradoxically reverses the biological effect of H_2O_2 in the

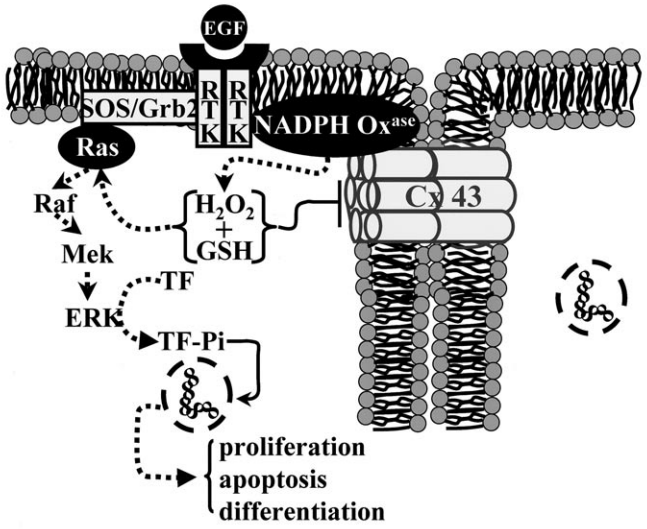

Figure 5. Gap junctions in cellular homeostasis. Extracellular signals, such as growth factors, interact with membrane receptors, which then activate intracellular signal transduction pathways that induce the transcription of genes *via* activated transcription factors. These intracellular pathways operate under cascade and scaffolding systems that cross communicates with each other in controlling the expression of genes that direct the proliferation, differentiation and apoptosis of cells within a tissue. These multiple checkpoints are further modulated by intercellular signals traversing gap junctions, thereby maintaining the homeostatic state of a tissue. Abnormal interruption of these integrated signaling pathways by food related and environmental toxicants results in diseased states, such as cancer.

inhibition of GJIC[29] (Figure 2). Other examples of this phenomenon where depletion of GSH prevents an oxidative chemical-induced cellular event is in: 1. lindane-dependent inhibition of GJIC in myometrial smooth muscle cells[39], 2. peroxide-induction of *c-jun*[40], and 3. the activation of NF-κB by H_2O_2 and $O_2^{\cdot -}$[41]. These results indicate that these signaling pathways not only required H_2O_2 but also GSH (Figure 5). Inhibition of GJIC and the induction of early-response genes are hallmarks of tumor promotion and in the results just described, a reduction in the natural antioxidant GSH, could also potentially protect a cell from proliferative responses to extracellular ligands.

In summary, the assessment of oxidative stress induced by low level, non-cytotoxic doses of radiation or chemicals will require not only understanding the indiscriminate reactions of ROS with the macromolecular machinery of a cell, but also the role they play in coordinating the expression of genes through the various cell signaling systems. Identification of redox regulated proteins and determining the underlying mechanisms by which they control gene expression through cell signaling will allow us to make a far more accurate assessment on the risk of low-level radiation-induced oxidative stress to human health. Understanding the redox machinery of a cell will also contribute to the development of more effective, and equally as important, safer chemopreventative and chemotherapeutic strategies. The overly simplistic approach of previous intervention studies with antioxidant supplementation to human populations proved either ineffective, the N-acetylcysteine EUROSCAN studies in Europe[42,43], or actually detrimental to human health, such as increased lung cancer of smokers in the B-carotene studies (CARET trials in USA and ABTC trials in Finland).[42,44] Thus, developing safe and effective intervention strategies for humans exposed to low-level ionizing radiation will depend, in part, on our mechanistic understanding of oxidative stress.

Acknowledgments

Supported by an NIEHS Superfund Basic Science Program grant to JET (PA42 ES04911).

References

1. Warburg O. On the origin of cancer cells. *Science* 123: 309-314, 1956
2. Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB. Alkylating DNA damage stimulates a regulated form of necrotic cell death. *Genes Dev* 18: 1272-1282, 2004
3. Anghileri LJ. Warburg's cancer theory revisited: a fundamentally new approach. *Arch Geschwulstforsch* 53: 1-8, 1983
4. Koike T, Kimura N, Miyazaki K et al. Hypoxia induces adhesion molecules on cancer cells: A missing link between Warburg effect and induction of selectin-ligand carbohydrates. *Proc Natl Acad Sci USA* 101: 8132-8137, 2004
5. McCord JM, Fridovich I. The reduction of cytochrome c by milk xanthine oxidase. *J Biol Chem* 243: 5753-5760, 1968
6. Allen RG, Tresini M. Oxidative stress and gene regulation. *Free Radic Biol Med* 28: 463-499, 2000
7. Hippeli, Susanne, Heiser, Ingrid, and Elstner, Erich F. Activated oxygen and free oxygen radicals in pathology: New insights and analogies between animals and plants. *Plant-Physiology-and-Biochemistry* 37: 167-178, 1999
8. Gille G, Sigler K. Oxidative stress and living cells. *Folia-Microbiologica* 40: 131-152, 1995
9. Cadet J, Douki T, Ravanat JL. Artifacts associated with the measurement of oxidized DNA bases. *Environ Health Perspect* 105: 1034-1039, 1997
10. Trosko JE, Upham BL. The emperor wears no clothes in the field of carcinogen risk assessment: ignored concepts in cancer risk assessment. *Mutagenesis* 20: 81-92, 2005
11. Trosko JE, Ruch RJ. Cell-cell communication in carcinogenesis. *Front Biosci* 3: 208-236, 1998
12. Upham BL, Weis LM, Trosko JE. Modulated gap junctional intercellular communication as a biomarker of PAH epigenetic toxicity: structure-function relationship. *Environ Health Perspect* 106: 975-981, 1998
13. Slaga TJ, Klein Szanto AJ, Triplett LL, Yotti LP, Trosko KE. Skin tumor-promoting activity of benzoyl peroxide, a widely used free radical-generating compound. *Science* 213: 1023-1025, 1981
14. Klein Szanto AJ, Slaga TJ. Effects of peroxides on rodent skin: epidermal hyperplasia and tumor promotion. *J Invest Dermatol* 79: 30-34, 1982
15. Gimenez-Conti IB, Binder RL, Johnston D, Slaga TJ. Comparison of the skin tumor-promoting potential of different organic peroxides in SENCAR mice. *Toxicol Appl Pharmacol* 149: 73-79, 1998
16. Mitchel RE, Morrison DP, Gragtmans NJ. Tumorigenesis and carcinogenesis in mouse skin treated with hyperthermia during stage I or stage II of tumor promotion. *Carcinogenesis* 8: 1875-1879, 1987
17. Radosevich CA, Weitzman SA. Hydrogen peroxide induces squamous metaplasia in a hamster tracheal organ explant culture model. *Carcinogenesis* 10: 1943-1946, 1989
18. Nakamura Y, Gindhart TD, Winterstein D, Tomita I, Seed JL, Colburn NH. Early superoxide dismutase-sensitive event promotes neoplastic transformation in mouse epidermal JB6 cells. *Carcinogenesis* 9: 203-207, 1988
19. Bae YS, Sung JY, Kim OS et al. Platelet-derived growth factor-induced H(2)O(2) production requires the activation of phosphatidylinositol 3-kinase. *J Biol Chem* 275: 10527-10531, 2000
20. Ammendola R, Ruocchio MR, Chirico G et al. Inhibition of NADH/NADPH oxidase affects signal transduction by growth factor receptors in normal fibroblasts. *Arch Biochem Biophys* 397: 253-257, 2002

21. Van HL, Meischl C, Stooker W, Meijer CJ, Niessen HW, Roos D. NADPH oxidase(s): new source(s) of reactive oxygen species in the vascular system? *J Clin Pathol* 55: 561-568, 2002
22. Brar SS, Kennedy TP, Sturrock AB et al. NADPH oxidase promotes NF-kappaB activation and proliferation in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 282: L782-L795, 2002
23. Li JM, Shah AM. Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology. *Am J Physiol Regul Integr Comp Physiol* 287: R1014-R1030, 2004
24. Saran M. To what end does nature produce superoxide? NADPH oxidase as an autocrine modifier of membrane phospholipids generating paracrine lipid messengers. *Free Radic Res* 37: 1045-1059, 2003
25. Tsao MS, Smith JD, Nelson KG, Grisham JW. A diploid epithelial cell line from normal adult rat liver with phenotypic properties of 'oval' cells. *Exp Cell Res* 154: 38-52, 1984
26. Coleman WB, Wennerberg AE, Smith GJ, Grisham JW. Regulation of the differentiation of diploid and some aneuploid rat liver epithelial (stemlike) cells by the hepatic microenvironment. *Am J Pathol* 142: 1373-1382, 1993
27. Malouf NN, Coleman WB, Grisham JW et al. Adult-derived stem cells from the liver become myocytes in the heart in vivo. *Am J Pathol* 158: 1929-1935, 2001
28. El-Fouly MH, Trosko JE, Chang CC. Scrape-loading and dye transfer. A rapid and simple technique to study gap junctional intercellular communication. *Exp Cell Res* 168: 422-430, 1987
29. Upham BL, Kang KS, Cho HY, Trosko JE. Hydrogen peroxide inhibits gap junctional intercellular communication in glutathione sufficient but not glutathione deficient cells. *Carcinogenesis* 18: 37-42, 1997
30. Esposito F, Ammendola R, Faraonio R, Russo T, Cimino F. Redox control of signal transduction, gene expression and cellular senescence. *Neurochem Res* 29: 617-628, 2004
31. Torres M, Forman HJ. Redox signaling and the MAP kinase pathways. *BioFactors* 17: 287-296, 2003
32. Gabbita SP, Robinson KA, Stewart CA, Floyd RA, Hensley K. Redox regulatory mechanisms of cellular signal transduction. *Arch Biochem Biophys* 376: 1-13, 2000
33. Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. Reactive oxygen species, cell signaling, and cell injury. *Free Radic Biol Med* 15: 1456-1462, 2000
34. Guyton KZ, Liu Y, Gorospe M, Xu Q, Holbrook NJ. Activation of mitogen-activated protein kinase by H2O2. Role in cell survival following oxidant injury. *J Biol Chem* 271: 4138-4142, 1996
35. Sundaresan M, Yu ZX, Ferrans VJ, Irani K, Finkel T. Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. *Science* 270: 296-299, 1995
36. Lander HM. An essential role for free radicals and derived species in signal transduction. *FASEB* J 11: 118-124, 1997
37. Huang RP, Peng A, Hossain MZ, Fan Y, Jagdale A, Boynton AL. Tumor promotion by hydrogen peroxide in rat liver epithelial cells. *Carcinogenesis* 20: 485-492, 1999
38. Cottrell GT, Lin R, Warn-Cramer BJ, Lau AF, Burt JM. Mechanism of v-Src- and mitogen-activated protein kinase-induced reduction of gap junction communication. *Am J Physiol Cell Physiol* 284: C511-C520, 2003
39. Loch-Caruso R, Upham BL, Harris C, Trosko JE. Divergent roles for glutathione in lindane-induced acute and delayed-onset inhibition of rat myometrial gap junctions. *Toxicol* Sci 85: 694-702, 2005
40. Kuo ML, Meng TC, Lin JK. Involvement of glutathione in induction of c-jun protooncogene by methylmethanesulfonate in NIH 3T3 cells. *Carcinogenesis* 17: 815-820, 1996
41. Ginn-Pease ME, Whisler RL. Optimal NF kappa B mediated transcriptional responses in Jurkat T cells exposed to oxidative stress are dependent on intracellular glutathione and costimulatory signals. *Biochem Biophys Res Commun* 226: 695-702, 1996
42. Wright GS, Gruidl ME. Early detection and prevention of lung cancer. *Curr Opin Oncol* 12: 143-148, 2000
43. van Zandwijk N, Dalesio O, Pastorino U, de Vries N, van Tinteren H. EUROSCAN, a randomized trial of vitamin A and N-acetylcysteine in patients with head and neck cancer or lung cancer. For the European Organization for Research and Treatment of Cancer Head and Neck and Lung Cancer Cooperative Groups. *J Natl Cancer Inst* 92: 977-986, 2000
44. Goodman GE. Prevention of lung cancer. *Crit Rev Oncol Hematol* 33: 187-197, 2000