Molecular dissection of the roles of the SOD genes in mammalian response to low dose irradiation

Final Report

DE-AI02-04ER63707

Principal Investigator:
Eric Y. Chuang
Radiation Oncology Branch
Center for Cancer Research
National Cancer Institute
Bethesda, MD 20892

Report period: 9/1/03-8/31/06

Abstract

It has been long recognized that a significant fraction of the radiation-induced genetic damage to cells are caused by secondary oxidative species. Internal cellular defense systems against oxidative stress play significant roles in countering genetic damage induced by ionizing radiation. The role of the detoxifying enzymes may be even more prominent in the case of low-dose, low-LET irradiation, as the majority of genetic damage may be caused by secondary oxidative species.

In this study we have attempted to decipher the roles of the superoxide dismutase (SOD) genes, which are responsible for detoxifying the superoxide anions. We used adenovirus vectors to deliver RNA interference (RNAi or siRNA) technology to down-regulate the expression levels of the SOD genes. We have also over-expressed the SOD genes by use of recombinant adenovirus vectors. Cells infected with the vectors were then subjected to low dose γ-irradiation. Total RNA were extracted from the exposed cells and the expression of 9000 genes were profiled by use of cDNA microarrays.

The result showed that low dose radiation had clear effects on gene expression in HCT116 cells. Both over-expression and down-regulation of the SOD1 gene can change the expression profiles of sub-groups of genes. Close to 200 of the 9000 genes examined showed over two-fold difference in expression under various conditions. Genes with changed expression pattern belong to many categories that include: early growth response, DNA-repair, ion transport, apoptosis, and cytokine response.

RELEVANCE STATEMENT

This grant application is prepared specifically in response to the DOE low dose research program notice 03-07, which calls for studies aimed at developing a better scientific basis for understanding the risks of human exposure to low dose radiation. Our proposal addresses ***three of the four*** areas that are outlined in the grant solicitation: ***endogenous oxidative damage versus low dose radiation-induced damage***, ***radio-adaptive responses***, ***and bystander effects***. In addition, we will use state-of-the-art technologies such as RNAi, DNA microarrays in our studies. We will also use a novel three-dimensional system to culture the cells to be studied. Therefore, both our objectives and approaches closely match those stated in the program announcement. We believe our proposal is highly relevant to this program solicitation.

Introduction

It has been long recognized that a significant fraction of the radiation-induced genetic damage to cells are caused by secondary oxidative species. Internal cellular defense systems against oxidative stress play significant roles in countering genetic damage induced by ionizing radiation. The role of the detoxifying enzymes may be even more prominent in the case of low-dose, low-LET irradiation, as the majority of genetic damage may be caused by secondary oxidative species.

In this study we have attempted to decipher the roles of the superoxide dismutase (SOD) genes, which are responsible for detoxifying the superoxide anions. We used adenovirus vectors to deliver RNA interference (RNAi or siRNA) technology to down-regulate the expression levels of the SOD genes. We have also over-expressed the SOD genes by use of recombinant adenovirus vectors. Cells infected with the vectors were then subjected to low dose γ-irradiation. Total RNA were extracted from the exposed cells and the expression of 9000 genes were profiled by use of cDNA microarrays.

Background

B1. Low dose radiation may have significantly different biological responses than high-dose radiation.

The health effects of low dose radiation are of major concern to the public as well as scientists and regulatory agencies. The current principles and guidelines are based on the assumption that risks are linear with no thresholds in the low dose range. Therefore, risk estimates are mostly based on extrapolation from data generated in higher dose situations. This is because there is relatively abundant experimental and epidemiological data for higher doses and little information in the low dose range. In addition, recent advances in our understanding of the molecular and cellular biology of mammalian radiation response suggest that the assumption of linear dose response may be fundamentally flawed in the low dose range.

B2. The existence of radio-adaptive response casts doubts on the current linear, non-threshold approach for risk estimate.

At the cellular level, radiobiologists have long noticed the existence of the so-called ‘adaptive response’ when mammalian cells are irradiated at lower doses(1-4). The radioadaptive response refers to the development of resistance to a second, higher dose of radiation after exposure to an initial, smaller radiation dose. Adaptive response has been observed in a wide range of cell types with many different endpoints. For example, an initial radiation dose of as small as 1 cGy can attenuate subsequent radiation induced damage(3). These damages include gene mutations (5, 6), chromosomal aberrations (7), sister chromatid exchange(1), micronucleus formation(8). The exact molecular mechanisms involved in adaptive response are not clear. However, factors such as the adapting dose, dose rate (2, 9), pH (10), and stage of the cell cycle(11), are known to be involved. Most evidence has been collected in vitro. However, there is also evidence pointing to its existence in vivo in mouse (12-14) and in humans (15-18). The widespread existence of adaptive response points to potential molecular and cellular mechanisms that are able to significantly protect cellular damage generated by lower levels of radiation. Most importantly, the lack of such adaptive response in the higher dose range suggests the existence of thresholds beyond which cellular and molecular mechanisms cannot function properly. This would indeed cast doubt on the linear, non-threshold model.

B3. Radiation-induced bystander effects add another level of complexity to risk estimate from low dose radiation.

Another area of active investigation in radiation biology is the so-called 'bystander effects', which refers to genotoxic or cytotoxic effects in cells that are not traversed by radiation. This phenomenon is important because it tends to amplify the effects of radiation. This is in contrast to adaptive response, which tends to attenuate the radiation damage to the cells.

Radiation-induced bystander effects were observed decades ago in vivo (19-22) in the form of chromosome damage or increased tumor formation in non-irradiated cells. Later Emerit and colleagues provided evidence that oxidative stress may be involved in (23-25). Mothersill *et al* showed that a radiation-inducible, diffusible protein-like factor and/or oxidative stress may be responsible for the bystander effects(26-28). This is confirmed by other groups(29-31). Nagasawa and Little provided the first evidence that in cultures exposed to low fluence, high LET α-particles, sister chromatid exchange could be induced in cells whose nuclei were not traversed by the α-particles(32-35). The existence of bystander effects in radiationinduced oncogenic transformation has also been reported (36, 37).

The mechanisms underlying bystander effects remain unclear. However, there are two suggested pathways, both with supporting evidence. One pathway involves gap junctions. Connexins, which constitute some of the major components of gap junctions, appeared to be important in several studies (38, 39). The other pathway involves oxidative metabolism or ROS (reactive oxygen species). Some early studies have indicated the importance of ROS, especially superoxide anions, in bystander effects induced by γ-irradiation (40, 41). Several investigators have provided evidence that low-fluence α- particles can generate superoxide anions and hydrogen peroxides and these ROS are important in α- particle induced bystander effects (27, 42-44). In this project, we will investigate whether oxidative metabolism and especially deficiencies in SOD can influence the modulation of bystander effects by low dose radiation.

B4. Current understanding of mammalian cellular and molecular response to ionizing radiation points to the existence of various potential 'thresholds'.

At the molecular biology level, a great deal has been learned about the complexity of cellular response to ionizing radiation although we are still at the beginning stage of gaining a thorough understanding. Mammalian cells are equipped with a comprehensive system to cope with radiation and other cytotoxic agents. There are 'early warning' systems that sense damage in the DNA and effector systems that carry out various responses according to the extent of damage. If the damage is not severe, it is repaired and the cells remain healthy. If it is more severe, the cell undergoes arrest while it is being repaired. If it is too serious to be repaired, the apoptotic program is activated and the cell undergoes suicide (programmed cell death). Under this framework, which we are just beginning to understand, it is reasonable that risks may not be linear. For example, when the cells are exposed to low dose radiation, the most likely cellular response is cell cycle arrest and DNA repair. Apoptosis will only be invoked when certain threshold is surpassed. Therefore, in terms of apoptotic cytotoxicity, the threshold is already built-in in mammalian cells. The existence of 'shoulders' at the lower dose ranges of the survival curves of many cell lines is another proof of this. At the DNA level, it is now unclear as to the existence of thresholds. However, it is likely that the cellular repair machinery is built to handle

most endogenously generated damage (mostly from byproducts oxidative metabolism) as well as certain amount of external damage. When damage (most likely external) exceeds certain levels, the repair system may be overwhelmed and the damaged cells go through cell cycle arrest or apoptosis. Therefore, it is likely that thresholds may also exist for cellular DNA repair system.

B5. The roles of oxidative metabolism in cellular radio-adaptive response are still not clearly defined.

The goal of this project is to examine whether the SOD enzymes, which are the corner stones of cellular oxidative metabolism and cellular defense systems against internally and externally generated ROS, play any roles in cellular response to low dose irradiated cells. We will examine whether deficiency in SOD enzymes will alter cellular adaptive or bystander responses to radiation. The results obtained may advance our understanding of the relative contribution of oxidative metabolism and the ROS detoxification system in cellular response to low dose radiation. We are interested in the SOD enzymes because ROS has been implicated in cellular response to radiation(45). By use of free radical scavengers, it is estimated that about two-thirds of X-ray or γ- radiation induced damage to DNA is caused by free radicals. In radiation-induced bystander effects, there is ample evidence to indicate that ROS generation and the SOD enzymes play key roles (*section B3*). However, little is known about the specific roles of SOD enzymes and oxidative metabolism in cellular adaptive effects of low dose radiation. Bravard et al. provided evidence that SOD may play some roles in radio-adaptive response (46). However, a study by Wolff (4) suggested that elevated intracellular levels of SOD did not influence the adaptive response. Therefore, there is a need for additional studies of the roles of SOD enzymes in adaptive response to low dose radiation.

B6. Novel technological advances such as RNA interference and DNA microarray provide powerful tools to study the roles of oxidative stress and the functions of the SOD genes.

In the past few years, a new technology termed RNA interference (RNAi or siRNA) becomes popular tools to study the functions of various genes. The mechanism was initially described in *C.elegans(47),* where a few molecules of short dsRNA could completely abolish the function of a gene that is homologous to the dsRNA. This is caused by the degradation of the mRNA of target genes. The degradation is carried out by a endogenous enzyme Dicer(48). Later, it was discovered that similar mechanisms exist in many other organisms, including mammalian cells(49, 50). This opened up new possibilities to study the function of any genes by knocking down its expression levels. For example, a recent paper published by Peng *et a*l successfully targeted the DNA-PKcs gene by the siRNA approach(47). A further discovery of mammalian promoters (based on RNA polymerase III) makes it possible to create stable cell lines with the expression of target genes down-regulated(51-53). In this project, we will use the RNAi technology to down-regulate the levels of genes encoding the cytoplasmic SOD1(*CuZnSOD*) or mitochodrial SOD2(*MnSOD*) genes to facilitate the study of their potential involvement in cellular radiation response. The down-regulation of the SOD genes can potentially increase the intracellular levels of the superoxide anions and oxidative stress. This increase may influence the cellular response to low dose radiation. We will also study alternations in global gene expression patterns that may be caused by the deficiencies of the SOD genes.

Hypothesis

Superoxide dismutase (SOD) genes play significant roles in low LET, low dose radiation induced genetic instability in mammalian cells.

Brief summary of the goals of the project

The overall goal of this proposal is to carry out mechanistic studies of the roles of the anti-oxidant SOD genes in mammalian cellular response to low dose ionizing radiation. Specifically, we have the following objectives:

Specific aim 1. ***To study the roles of anti-oxidant SOD genes in modulating the levels of oxidative DNA damage caused by endogenous oxidative stress or exposure to low dose radiation.***
In this aim, we will establish human cell lines with their SOD genes disrupted by the siRNA technology. We will then evaluate low dose irradiation-induced oxidative stress and DNA damage in these cells in comparison with normal cells.

Specific aim 2. ***To examine the roles of the SOD genes and oxidative stress in radio-adaptive response and bystander effects in siRNA-transduced cells established in specific aim 1.***
We will use the cell lines established in specific aim 1 to examine whether the SOD genes play any roles in radio-adaptive response and bystander effects.

Specific aim 3. To generate gene expression profiles of SOD-deficient cells exposed to low dose radiation.
We will use SOD-deficient cells generated in specific aim 1 to profile genes involved in cellular response to low dose radiation and in radio-adaptive response by use of high-density cDNA arrays. We will conduct the experiments in this specific aim in collaboration with Dr. Eric Chuang, who is a co-investigator of this project. He directs the Microarray Research Laboratory of the Radiation Oncology Sciences Program at the National Cancer Institute (NCI). We will examine gene expression in the following cell populations by use of a 17,000 cDNA array:

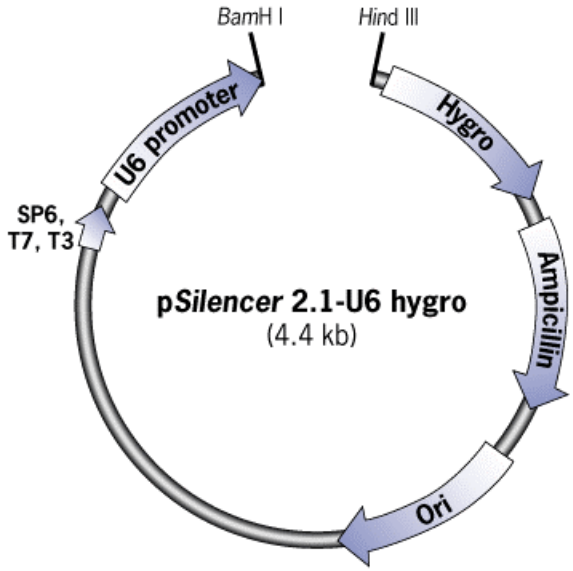
In summary, the proposed study will provide a comprehensive estimate of genetic instability induced by low-dose irradiation and the potential involvement of the SOD genes and reactive oxidative stress in this important process.

Experimental Methods

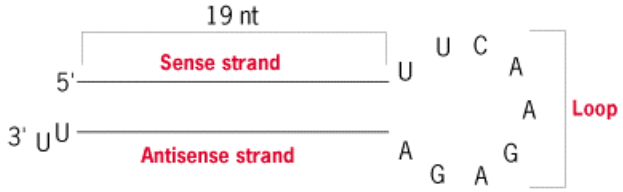
1. Construct adenovirus vectors encoding SOD gene or minigenes encoding siRNA to SOD genes (Figure 1 A-C).
2. Evaluating the efficacy of the vectors in mediating the expression and suppression of SOD genes.
3. Infection and irradiation (10 cGy) of target cells (p53+ and p53- HCT116 cells).
4. Extraction of total RNA and microarray analysis.
5. Identification and confirmation of a small subset of genes with most significant alterations.
6. Functional study of the roles of genes on maintaining genomic stability

Figure 1. A) A diagram of the U6 promoter-based short RNA-expressing plasmid pSilencer2.1-hygro. B) The structure of siRNA encoded by the plasmid. C) the structure of the minigene that encode the siRNA.

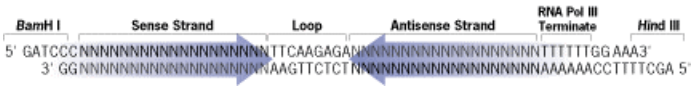
A



B



C



Result and Discussion

The goal of the experiment was to identify the genes that are involved in mammalian cellular response to low dose irradiation. We paid special attention to the roles of the SOD genes by use of the SOD-deficient cell lines constructed in specific aim 1.

Although there have been quite a few studies on the use of genomic approaches to profile gene expression after exposure to ionizing radiation(54-59), there have been no studies published that focus on the effects of low dose radiation induced radio-adaptive response, especially in the context of SOD deficiency. Because so little is known at the molecular level about the adaptive responses, a high-throughput screening approach such as cDNA microarrays will yield a wealth of potentially useful information.

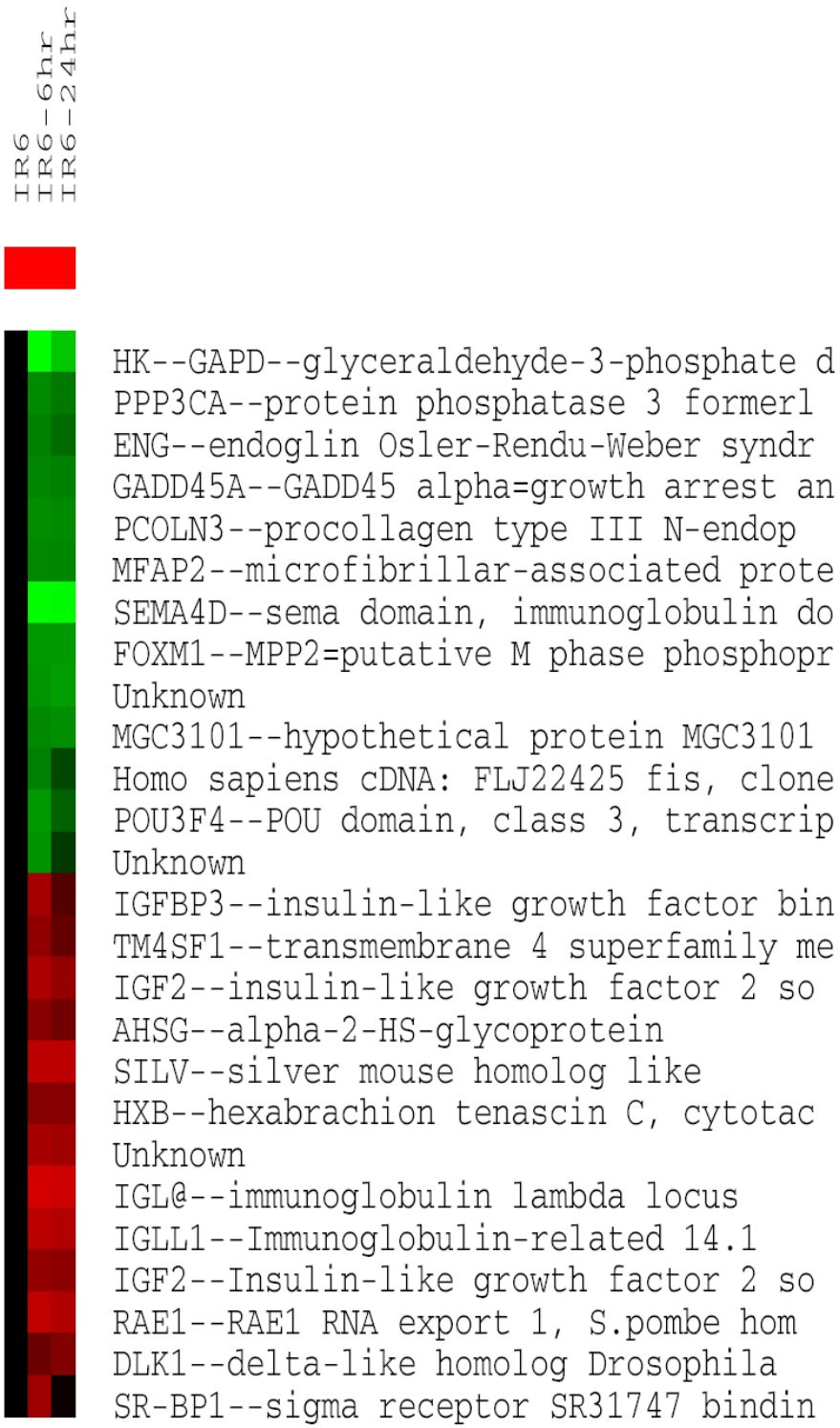
In this experiment, we used microarray analysis to examine SOD-deficient and SOD-proficient cells with otherwise identical genetic backgrounds. This allowed us to identify genes that are downstream of the SOD genes. We selected our treatment groups with goal of identifying the genes that are important in cellular response to low dose radiation and radio-adaptive response, with special emphasis on genes whose patterns are affected by the SOD gene deficiencies.

The result showed that low dose radiation had clear effects on gene expression in HCT116 cells. Both over-expression and down-regulation of the SOD1 gene can change the expression profiles of sub-groups of genes. Close to 200 of the 9000 genes examined showed over two-fold difference in expression under various conditions. Genes with changed expression pattern belong to many categories that include: early growth response, DNA-repair, ion transport, apoptosis, and cytokine response. (Table 1) The gene that had the most significant change in expression between over-expression and down-regulation was the EGR1-early growth response gene. When over-expressed, its expression level was 2.7-fold of the normal; when down-regulated, the expression level was 0.6-fold of the normal.

Figure 2. Cluster analysis of genes induced by 10-cGy radiation in HCT116 cells with over expression or reduced expression of the SOD1 gene.

HCT116-AdSOD1-10cGy

HCT116-AdsiSOD-10cGy



Tabe I. A partial list of genes that showed significant changes in expression

Group / Gene name	AdGFP			AdSOD1			AdsiSOD		
	0	6 hr	24 hr	0	6 hr	24 hr	0	6 hr	24 hr
ASS--argininosuccinate synthetase	0	0.6435	2.0607	0	0.1592	1.8584	0	0.9041	0.9595
VEGFB--vascular endothelial growth factor	0	1.1118	1.7875	0	0.463	2.3388	0	0.5808	0.4181
EGR1--early growth response	0	0.2753	2.0859	0	0.165	2.7285	0	0.8383	0.638
MSH2--MSH2=DNA mismatch repair mutS ho	0	0.7482	1.6663	0	0.9254	2.0694	0	0.5513	0.668
IGF2--Insulin-like growth factor 2	0	0.3866	1.305	0	0.0705	1.0622	0	2.2895	2.2038
CLNS1A--chloride channel, nucleotide	0	0.6303	2.1806	0	0.7582	1.3966	0	0.4279	0.6925
STAT1--STAT1=IFN alpha/beta-responsive	0	0.6513	2.4735	0	0.222	1.9837	0	-0.07	0.4005
CASP6--CASPASE-6=mch2 alpha	0	0.9937	2.1533	0	0.3248	2.2239	0	0.4889	0.9407
CDKN1B--cyclin-dependent kinase inhibi	0	0.905	2.2445	0	0.3006	2.3322	0	0.1886	0.5774
TSC22--transforming growth factor beta	0	0.5496	1.9555	0	0.4002	2.0879	0	0.4714	0.6824
FDX1--ferredoxin 1	0	0.2651	1.68	0	-0.2577	1.8959	0	0.2097	0.2459
WRN--Werner syndrome	0	0.7287	1.8741	0	0.2015	2.0753			
IGFBP-7	0	1.2312	3.1362	0	0.1911	2.9734	0	1.588	1.5924
NDUFA4--NADH dehydrogenase (ubiquinone	0	1.5328	2.8428	0	0.3729	3.1676	0	-0.0221	0.196
GADD45A--GADD45 alpha=growth arrest an	0	-0.877	-1.9496	0	-1.01	-0.7354	0	-2.1016	-2.0018
CLIC4--chloride intracellular channel	0	0	1.5823	0	-0.4113	1.8662	0	1.432	0.881
ATP6J--ATPase, H+ transporting, lysosome	0	0.4077	2.1015	0	0.1668	2.0271	0	0.5606	0.8488
S100A13--S100 calcium-binding protein	0	0.8509	2.5217	0	-0.0517	2.2472	0	0.675	0.7197
SERP1--stress-associated endoplasmic r	0	0.8714	2.062	0	0.4542	2.2235	0	0.3354	0.4905

Reference

1. Olivieri, G., Bodycote, J., and Wolff, S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine, Science. *223:* 594-7., 1984.
2. Shadley, J. D., Afzal, V., and Wolff, S. Characterization of the adaptive response to ionizing radiation induced by low doses of X rays to human lymphocytes, Radiat Res. *111:* 511-7., 1987.
3. Wolff, S. Failla Memorial Lecture. Is radiation all bad? The search for adaptation, Radiat Res. *131:* 117-23., 1992.
4. Wolff, S. Aspects of the adaptive response to very low doses of radiation and other agents, Mutat Res. *358:* 135-42., 1996.
5. Sanderson, B. J. and Morley, A. A. Exposure of human lymphocytes to ionizing radiation reduces mutagenesis by subsequent ionizing radiation, Mutat Res. *164:* 347-51., 1986.
6. Kelsey, K. T., Memisoglu, A., Frenkel, D., and Liber, H. L. Human lymphocytes exposed to low doses of X-rays are less susceptible to radiation-induced mutagenesis, Mutat Res. *263:* 197-201., 1991.
7. Khandogina, E. K., Mutovin, G. R., Zvereva, S. V., Antipov, A. V., Zverev, D. O., and Akifyev, A. P. Adaptive response in irradiated human lymphocytes: radiobiological and genetical aspects, Mutat Res. *251:* 181-6., 1991.
8. Azzam, E. I., Raaphorst, G. P., and Mitchel, R. E. Radiation-induced adaptive response for protection against micronucleus formation and neoplastic transformation in C3H 10T1/2 mouse embryo cells, Radiat Res. *138:* S28-31., 1994.
9. Shadley, J. D. and Wolff, S. Very low doses of X-rays can cause human lymphocytes to become less susceptible to ionizing radiation, Mutagenesis. *2:* 95-6., 1987.
10. Bosi, A., Micheli, A., Pietrosanti, S., and Olivieri, G. Effect of pH shifts on radiosensitivity of human lymphocytes irradiated in the G2 stage, Mutat Res. *250:* 325-9., 1991.
11. Shadley, J. D. Chromosomal adaptive response in human lymphocytes, Radiat Res. *138:* S9- 12, 1994.
12. Wojcik, A. and Tuschl, H. Indications of an adaptive response in C57BL mice pre-exposed in vivo to low doses of ionizing radiation, Mutat Res. *243:* 67-73., 1990.
13. Semenets, T. N., Semina, O. V., Poverennyi, A. M., Deigin, V. I., Korotkov, A. M., and Saenko, A. S. [Adaptive response in vivo: reasons for increase of radioresistance in CFU-S], Radiats Biol Radioecol. *33:* 812-5., 1993.
14. Yoshida, N., Imada, H., Kunugita, N., and Norimura, T. Low dose radiation-induced adaptive survival response in mouse spleen T- lymphocytes in vivo, J Radiat Res (Tokyo). *34:* 269-76., 1993.
15. Barquinero, J. F., Barrios, L., Caballin, M. R., Miro, R., Ribas, M., Subias, A., and Egozcue, J. Occupational exposure to radiation induces an adaptive response in human lymphocytes, Int J Radiat Biol. *67:* 187-91., 1995.
16. Tedeschi, B., Caporossi, D., Vernole, P., Padovani, L., Appolloni, M., Anzidei, P., and Mauro, F. Do human lymphocytes exposed to the fallout of the Chernobyl accident exhibit an adaptive response? 2. Challenge with bleomycin, Mutat Res. *332:* 39-44., 1995.
17. Monsieurs, M. A., Thierens, H. M., Vral, A. M., Van De Wiele, C., De Ridder, L. I., and Dierckx, R. A. Adaptive response in patients treated with 131I, J Nucl Med. *41:* 17-22., 2000.
18. Thierens, H., Vral, A., Barbe, M., Meijlaers, M., Baeyens, A., and Ridder, L. D.

Chromosomal radiosensitivity study of temporary nuclear workers and the support of the adaptive response induced by occupational exposure, Int J Radiat Biol. *78:* 1117-26., 2002.
19. Parsons, W. B., Watkins, C. H., Pease, G. L., and Cancer, D. S. C. Changes in sternal bone marrow following roentgen-ray therapy to the spleen in chronic granulocytic leukaemia, Cancer. *7:* 179-189, 1954.
20. Souto, J. Tumour development in the rat induced by the blood of irradiated animals., Nature. *195:* 1317-1318, 1962.
21. Hollowell, J. G., Jr. and Littlefield, L. G. Chromosome damage induced by plasma of x-rayed patients: an indirect effect of x-ray, Proc Soc Exp Biol Med. *129:* 240-4., 1968.
22. Littlefield, L. G., Hollowell, J. G., Jr., and Pool, W. H., Jr. Chromosomal aberrations induced by plasma from irradiated patients: an indirect effect of X radiation. Further observations and studies of a control population, Radiology. *93:* 879-86., 1969.
23. Emerit, J., Loeper, J., and Chomette, G. Superoxide dismutase in the treatment of postradiotherapeutic necrosis and of Crohn's disease, Bull Eur Physiopathol Respir. *17:* 287., 1981.
24. Emerit, I., Keck, M., Levy, A., Feingold, J., and Michelson, A. M. Activated oxygen species at the origin of chromosome breakage and sister-chromatid exchanges, Mutat Res. *103:* 165-72., 1982.
25. Emerit, I., Levy, A., Cernjavski, L., Arutyunyan, R., Oganesyan, N., Pogosian, A., Mejlumian, H., Sarkisian, T., Gulkandanian, M., Quastel, M., and et al. Transferable clastogenic activity in plasma from persons exposed as salvage personnel of the Chernobyl reactor, J Cancer Res Clin Oncol. *120:* 558-61, 1994.
26. Seymour, C. B. and Mothersill, C. Relative contribution of bystander and targeted cell killing to the low- dose region of the radiation dose-response curve, Radiat Res. *153:* 508-11., 2000.
27. Mothersill, C., Stamato, T. D., Perez, M. L., Cummins, R., Mooney, R., and Seymour, C. B. Involvement of energy metabolism in the production of 'bystander effects' by radiation, Br J Cancer. *82:* 1740-6., 2000.
28. Lyng, F. M., Seymour, C. B., and Mothersill, C. Initiation of apoptosis in cells exposed to medium from the progeny of irradiated cells: a possible mechanism for bystander-induced genomic instability?, Radiat Res. *157:* 365-70., 2002.
29. Barcellos-Hoff, M. H. and Brooks, A. L. Extracellular signaling through the microenvironment: a hypothesis relating carcinogenesis, bystander effects, and genomic instability, Radiat Res. *156:* 618-27., 2001.
30. Morgan, W. F., Hartmann, A., Limoli, C. L., Nagar, S., and Ponnaiya, B. Bystander effects in radiation-induced genomic instability, Mutat Res. *504:* 91-100., 2002.
31. Nagar, S., Smith, L. E., and Morgan, W. F. Characterization of a novel epigenetic effect of ionizing radiation: the death-inducing effect, Cancer Res. *63:* 324-8., 2003.
32. Nagasawa, H. and Little, J. B. Induction of sister chromatid exchanges by extremely low doses of alpha- particles, Cancer Res. *52:* 6394-6., 1992.
33. Nagasawa, H. and Little, J. B. Unexpected sensitivity to the induction of mutations by very low doses of alpha-particle radiation: evidence for a bystander effect, Radiat Res. *152:* 552-7, 1999.
34. Nagasawa, H. and Little, J. B. Bystander effect for chromosomal aberrations induced in wildtype and repair deficient CHO cells by low fluences of alpha particles, Mutat Res. *508:* 121-9., 2002.

35. Nagasawa, H., Huo, L., and Little, J. B. Increased bystander mutagenic effect in DNA doublestrand break repair- deficient mammalian cells, Int J Radiat Biol. *79:* 35-41., 2003.
36. Sawant, S. G., Randers-Pehrson, G., Geard, C. R., Brenner, D. J., and Hall, E. J. The bystander effect in radiation oncogenesis: I. Transformation in C3H 10T1/2 cells in vitro can be initiated in the unirradiated neighbors of irradiated cells, Radiat Res. *155:* 397-401., 2001.
37. Lewis, D. A., Mayhugh, B. M., Qin, Y., Trott, K., and Mendonca, M. S. Production of delayed death and neoplastic transformation in CGL1 cells by radiation-induced bystander effects, Radiat Res. *156:* 251-8, 2001.
38. Azzam, E. I., de Toledo, S. M., Gooding, T., and Little, J. B. Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluences of alpha particles, Radiat Res. *150:* 497-504., 1998.
39. Azzam, E. I., de Toledo, S. M., and Little, J. B. Direct evidence for the participation of gap junction-mediated intercellular communication in the transmission of damage signals from alpha -particle irradiated to nonirradiated cells, Proc Natl Acad Sci U S A, 2001.
40. Emerit, I., Arutyunyan, R., Oganesian, N., Levy, A., Cernjavsky, L., Sarkisian, T., Pogossian, A., and Asrian, K. Radiation-induced clastogenic factors: anticlastogenic effect of Ginkgo biloba extract, Free Radic Biol Med. *18:* 985-91., 1995.
41. Emerit, I., Garban, F., Vassy, J., Levy, A., Filipe, P., and Freitas, J. Superoxide-mediated clastogenesis and anticlastogenic effects of exogenous superoxide dismutase, Proc Natl Acad Sci U S A. *93:* 12799-804., 1996.
42. Iyer, R. and Lehnert, B. E. Factors underlying the cell growth-related bystander responses to alpha particles, Cancer Res. *60:* 1290-8., 2000.
43. Lorimore, S. A., Coates, P. J., Scobie, G. E., Milne, G., and Wright, E. G. Inflammatory-type responses after exposure to ionizing radiation in vivo: a mechanism for radiation-induced bystander effects?, Oncogene. *20:* 7085-95., 2001.
44. Azzam, E. I., De Toledo, S. M., Spitz, D. R., and Little, J. B. Oxidative Metabolism Modulates Signal Transduction and Micronucleus Formation in Bystander Cells from alpha-Particleirradiated Normal Human Fibroblast Cultures, Cancer Res. *62:* 5436-42., 2002.
45. St Clair, D. K., Wan, X. S., Oberley, T. D., Muse, K. E., and St Clair, W. H. Suppression of radiation-induced neoplastic transformation by overexpression of mitochondrial superoxide dismutase, Mol Carcinog. *6:* 238-42, 1992.
46. Bravard, A., Luccioni, C., Moustacchi, E., and Rigaud, O. Contribution of antioxidant enzymes to the adaptive response to ionizing radiation of human lymphoblasts, Int J Radiat Biol. *75:* 639-45., 1999.
47. Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., and Mello, C. C. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans, Nature. *391:* 806-11., 1998.
48. Ketting, R. F., Fischer, S. E., Bernstein, E., Sijen, T., Hannon, G. J., and Plasterk, R. H. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans, Genes Dev. *15:* 2654-9., 2001.
49. Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., and Tuschl, T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells, Nature. *411:* 494-8., 2001.
50. Elbashir, S. M., Lendeckel, W., and Tuschl, T. RNA interference is mediated by 21- and 22-

nucleotide RNAs, Genes Dev. *15:* 188-200., 2001.
51. Brummelkamp, T. R., Bernards, R., and Agami, R. A system for stable expression of short interfering RNAs in mammalian cells, Science. *296:* 550-3., 2002.
52. Yu, J. Y., DeRuiter, S. L., and Turner, D. L. RNA interference by expression of shortinterfering RNAs and hairpin RNAs in mammalian cells, Proc Natl Acad Sci U S A. *99:* 6047- 52., 2002.
53. Miyagishi, M. and Taira, K. U6 promoter-driven siRNAs with four uridine 3' overhangs efficiently suppress targeted gene expression in mammalian cells, Nat Biotechnol. *20:* 497-500., 2002.
54. Amundson, S. A., Bittner, M., Chen, Y., Trent, J., Meltzer, P., and Fornace, A. J., Jr. Fluorescent cDNA microarray hybridization reveals complexity and heterogeneity of cellular genotoxic stress responses, Oncogene. *18:* 3666-72., 1999.
55. Voehringer, D. W., Hirschberg, D. L., Xiao, J., Lu, Q., Roederer, M., Lock, C. B., Herzenberg, L. A., and Steinman, L. Gene microarray identification of redox and mitochondrial elements that control resistance or sensitivity to apoptosis, Proc Natl Acad Sci U S A. *97:* 2680-5., 2000.
56. Amundson, S. A., Bittner, M., Meltzer, P., Trent, J., and Fornace, A. J., Jr. Induction of gene expression as a monitor of exposure to ionizing radiation, Radiat Res. *156:* 657-61., 2001.
57. Sreekumar, A., Nyati, M. K., Varambally, S., Barrette, T. R., Ghosh, D., Lawrence, T. S., and Chinnaiyan, A. M. Profiling of cancer cells using protein microarrays: discovery of novel radiation-regulated proteins, Cancer Res. *61:* 7585-93., 2001.
58. Chen, X., Shen, B., Xia, L., Khaletzkiy, A., Chu, D., Wong, J. Y., and Li, J. J. Activation of nuclear factor kappaB in radioresistance of TP53-inactive human keratinocytes, Cancer Res. *62:* 1213-21., 2002.
59. Park, W. Y., Hwang, C. I., Im, C. N., Kang, M. J., Woo, J. H., Kim, J. H., Kim, Y. S., Kim, H.,