Conf-920720--1

Effects of low-dose radiation on gene expression in Syrian hamster embryo cells: comparison of JANUS neutrons and gamma rays

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ANL/CP--75218

DE92 016176

INTRODUCTION

Past work by our group (1-4) and others (5-9) has shown the modulation of specific genes following exposure of cells to ionizing radiation. Many classes of genes have been found to be modulated in response to ionizing radiation, including those encoding cytoskeletal elements (2,3), cell growth arresting proteins (5), cytokines (1,6), and cellular oncogenes (7,8). The functions of this specific modulation of gene expression are currently being investigated by several groups; it has been suggested that gene modulation in response to radiation plays a role in cellular repair of DNA damage, cell survival, or cellular transformation (1-7). Several groups have examined induction of nuclear proto-oncogenes following exposure to DNA-damaging agents (4,7,8).

MATERIALS AND METHODS

Cells and Culture Conditions. In all experiments, we examined modulation of gene expression by ionizing radiations in Syrian hamster embryo (SHE) fibroblasts, which are normal diploid cells that can be neoplastically transformed by low doses of ionizing radiations (10).

Radiation Treatment. Cells plated in 100-mm Petri plates containing 10 ml of medium were irradiated with ^{60}Co γ-rays or fission-spectrum neutrons (0.85 MeV) from the JANUS reactor (11). All irradiations were performed at 37 °C on cycling cells; equitoxic doses of neutrons and γ-rays were selected on the basis of survival data (10).

Purification of RNA and Northern Blots. RNA preparations, RNA electrophoresis, and Northern blots were performed as previously described (15-18). Equal amounts of mRNA on the blot were confirmed by hybridization to *p53* or c-*myc*.

cDNA Clones. We thank the following people who provided us with cDNA clones: c-*jun* cDNA was obtained from Dr. W. Lamph (Salk Institute); *Rb* clone from Dr. Dryja; H4-histone clone from Drs. G. and J. Stein; c-*fos* and c-*myc* from American Type Culture Collection; and *p53* from Dr. A. Levine.

RESULTS AND DISCUSSION

Experiments were performed to determine the effects of radiation dose, dose rate, and quality on expression of genes encoding nuclear proteins (c-*jun*, *Rb*, H4-histone, *p53*, and c-*myc*). Cycling SHE cells were exposed to varying doses (0 to 200 cGy) of γ-rays

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administered at either high (14 cGy/min) or low (1 cGy/min) dose rates. One hour after exposure, RNA was harvested from the cells and analyzed by Northern blot hybridization for expression of genes encoding nuclear proteins. Microdensitometric analyses of a series of Northern blots are shown in Table 1. Results from similar experiments examining the effects of JANUS neutron exposure on expression of genes encoding nuclear proteins are depicted in the microdensitometric analyses presented in Table 2.

The experiments reported here were designed to examine the effects of radiation exposure on expression of genes encoding nuclear proteins. Our results confirm the work of Sherman *et al.* (7), documenting induction of c-*jun* following exposure to low-LET radiations, and similarly demonstrate a failure of high-LET radiations (neutrons) to induce c-*jun* in SHE cells. All of the experiments presented here provide further support to the

Table 1
Relative Expression of Transcripts Encoding Nuclear Proteins Following γ-Ray Exposure: Cycling Cells[a]

		Transcripts[b]				
Dose (cGy)	Dose Rate (cGy/m)	c-*jun*	*Rb*	H4-Histone	*p53*	c-*myc*
0	0	1.0 (.04)	1.0 (.01)	1.0 (.04)	1.0 (.11)	1.0 (.03)
6	1.0	1.6 (.04)[c]	0.9 (.08)	1.4 (.08)	1.1 (.09)	HND[d]
25	1.0	2.2 (.07)[c]	1.3 (.03)	1.4 (.03)	1.3 (.09)	HND
50	1.0	1.8 (.12)[c]	1.7 (.03)[c]	1.7 (.08)[c]	1.3 (.06)	HND
75	1.0	1.7 (.17)[c]	1.3 (.08)	2.0 (.07)[c]	1.4 (.02)	HND
25	14.0	1.5 (.11)[c]	0.9 (.09)	1.4 (.02)	1.1 (.02)	HND
50	14.0	0.8 (.21)	0.7 (.11)	1.6 (.04)[c]	1.3 (.07)	HND
75	14.0	1.1 (.12)	1.3 (.06)	1.1 (.05)	1.3 (.02)	HND
200	14.0	4.7 (.03)[c]	1.7 (.19)[c]	1.4 (.05)	0.9 (.11)	HND

[a]Cycling cells were irradiated with ^{60}Co γ-rays at the doses or dose rates indicated 1 h prior to RNA harvest.

[b]RNA levels were determined by Northern blot hybridization and quantitated by microdensity. Amount of gene-specific mRNA in untreated cells was set at 1.0. All other RNAs were expressed relative to that. Standard deviations are in parentheses.

[c]Significantly different from control at $P < 0.05$.

[d]HND, hybridizations not detected.

Table 2
Relative Expression of Transcripts Encoding Nuclear Proteins Following Neutron Exposure: Cycling Cells[a]

Dose (cGy)	Dose Rate (cGy/m)	Transcripts[b]				
		c-*jun*	*Rb*	H4-Histone	*p53*	c-*myc*
0	0	1.0 (.14)	1.0 (.05)	1.0 (.10)	1.0 (.01)	1.0 (.02)
6	0.5	0.7 (.16)	0.5 (.04)[c]	HND[d]	0.8 (.04)	1.1 (.04)
12	0.5	0.9 (.18)	0.7 (.05)	0.8 (.09)	0.7 (.02)	1.0 (.02)
24	0.5	0.9 (.13)	0.6 (.05)[c]	0.6 (.12)	0.4 (.11)[c]	1.3 (.01)
36	0.5	1.1 (.30)	1.0 (.10)	1.2 (.01)	1.1 (.07)	1.0 (.05)
12	12	0.8 (.05)	0.8 (.06)	0.4 (.03)	0.7 (.03)	1.2 (.05)
24	12	1.6 (.14)[c]	0.7 (.04)	0.6 (.01)	0.8 (.01)	0.9 (.05)
48	12	0.5 (.06)[c]	0.4 (.03)[c]	0.5 (.07)	0.8 (.12)	0.9 (.03)
96	12	1.2 (.10)	1.2 (.08)	1.3 (.01)	1.6 (.01)[c]	1.3 (.03)

[a]Cycling cells were irradiated with JANUS neutrons at the doses or dose rates indicated 1 h prior to RNA harvest

[b]RNA levels were determined by Northern blot hybridization and quantitated by microdensity. Amount of gene-specific mRNA in untreated cells was set at 1.0. All other RNAs were expressed relative to that. Standard deviations are in parentheses.

[c]Significantly different from control at $P < 0.05$.

[d]HND, hybridizations not detected.

hypothesis that high- and low-LET radiations produce different cellular responses to radiation-induced damage. Genes induced by low-LET γ-rays (c-*jun*, H4-histone, and, to a lesser extent, *Rb*) were unaffected following neutron exposure. The gene, *p53*, which was modestly induced following γ-ray exposure, was unaffected by neutron exposure; and c-*myc*, which was repressed following γ-ray exposure, was unaffected following neutron exposure. Taken together with previous work from our laboratory, this work suggests that the actual event (whether it be DNA damage, oxidative damage, protein denaturation, or some other intracellular event) which modulates the cellular response to ionizing radiations may be different for high- and low-LET radiations. In fact, a recent report from Karin's group (20) has shown that induction of c-*jun* and c-*fos* following exposure to DNA-damaging agents can be attributed to cellular oxidative damage. Failure of neutrons to elicit this response would implicate some alternate pathway for gene modulation following neutron exposure.

ACKNOWLEDGEMENTS

The authors thank Ms. K. Bexson for excellent secretarial expertise and Mr. G. Holmblad for assistance in irradiations. This work supported by the U.S. Department of Energy, Office of Health and Environmental Research, Contract No. W-31-109-ENG-38.

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DATE FILMED

8/26/92