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**Heterogeneity in *c-jun* Gene Expression in Normal and Malignant Cells
Exposed to either Ionizing Radiation or Hydrogen Peroxide¹**

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³Abbreviations used are: PKA, cyclic AMP-dependent protein kinase; PKC, protein kinase C; NAC, *N*-acetyl-cysteine; ROI, reactive oxygen intermediate.

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ABSTRACT

We investigated the role of reactive oxygen intermediates and protein kinase C (PKC) in induction of *c-jun* gene expression in human ML-2 leukemic cells and normal human DET-551 fibroblasts by comparing the effects of either ionizing radiation or H_2O_2 exposure in the presence or absence of appropriate inhibitors. In these cell types, the radiation- and H_2O_2 -mediated increase in *c-jun* mRNA levels could be prevented by pretreatment of the cells with *N*-acetylcysteine, an antioxidant, or H7, an inhibitor of PKC and cAMP-dependent protein kinase (PKA), but not by HA1004, an inhibitor of PKA. These results suggest a role for PKC and reactive oxygen intermediates in the induction of *c-jun* gene expression in both normal and tumor cells. We also investigated potential differences in radiation- or H_2O_2 -induced *c-jun* gene expression in normal and tumor cells by examining steady-state *c-jun* mRNA levels in a number of human fibroblast, leukemia, melanoma, sarcoma, and carcinoma cell types. We observed heterogeneity in the steady-state level of *c-jun* mRNA in both the untreated normal and tumor cells and in such cells exposed to ionizing radiation or to H_2O_2 . Exposure to radiation produced a varied response which ranged from little or no induction to a more than two orders of magnitude increase in the steady-state level of the *c-jun* mRNA. Exposure to H_2O_2 gave a pattern similar to that of ionizing radiation. The basis for the differential induction in response to these agents may be attributable, in part, to either cell lineage or to genetic heterogeneity, since the cells examined are from different individuals, or a combination of these two parameters. In either case, our data indicated that although induction of *c-jun* gene expression may be a common response to ionizing radiation or H_2O_2 , this response was reduced or absent in some cell types.

INTRODUCTION

The *c-jun* proto-oncogene has been implicated in the regulation of cellular proliferation (1). This gene is a member of a family of early response genes that code for transcription factors (which includes *junB*, *junD*, *c-fos*, *fosB*, and *fra-1*) and its product is a component of the AP-1 transcription complex (2). The *c-jun* gene product binds to a specific DNA sequence motif, the phorbol ester responsive element, that regulates the transcription of genes responsive to phorbol esters (3). In addition to phorbol esters (4) and some growth factors (5-7), *c-jun* gene expression is also induced by ionizing radiation (8, 9), and this induction is believed to be mediated by a protein kinase C (PKC)-dependent mechanism (10).

Recently, we have characterized ionizing radiation-induced early response gene expression in human promyelocytic HL-60 leukemia cells (11). Using an HL-60 cell variant which is defective in PKC-mediated signal transduction (12, 13), we have shown that ionizing radiation induces early response gene expression through the formation of reactive oxygen intermediates (ROIs) and provided evidence that a PKC enzyme, perhaps an isoform distinct from PKC α and PKC β (11), may be involved in this induction. In these studies, the pattern of *c-jun* gene expression was representative of the other early response genes but was more sensitive to induction by ionizing radiation. In the present study, we extended our observations to another type of human leukemia, ML-2, and to a normal human fibroblast strain, DET-551, to ascertain if induction of *c-jun* gene expression by ionizing radiation in tumor and normal cells is mediated by a similar mechanism. We also investigated the universality of radiation-induced *c-jun* gene expression in a panel of normal human cell strains and human tumor cell lines. This panel of cells was also treated with H₂O₂ to assess if a similar pattern of *c-jun* gene expression was observed for ionizing radiation and for another agent which produces ROIs. Such a similarity would suggest that common signal transduction events mediate the induction of *c-jun* expression by these agents.

MATERIAL AND METHODS

Cell and Culture Conditions. The human HL-205 cells were a clonal isolate from the HL-60 cell line originally obtained from Dr. Robert C. Gallo, National Cancer Institute, Bethesda, MD. The HO melanoma cells were provided by Dr. Beppino C. Giovanella, Stehlin Foundation for Cancer Research, Houston, TX. The SK-MEL-131 cells were obtained from Dr. Alan Houghton, Memorial Sloan-Kettering Cancer Center, New York, NY. The MCF-7, MB-231, DU145, LNCaP, CEM, HT1080, Hs913t, HT-29, DET-551, IMR-90, WI-38 cells were obtained from the American Type Culture Collection, Rockville, MD. The Raji, MOLT-4, ML-2, and CCL-155 cells were provided by Dr. Jun Minowada, Fujisaki Cell Center, Okayama, Japan. The IMR70 fibroblast cells and P3 teratocarcinoma cells (14) were characterized at Argonne National Laboratory.

The cells were cultured in RPMI 1640 or EMEM media containing 12% fetal bovine serum with penicillin (100 units/ml) and streptomycin (100 µg/ml). The cultures were incubated at 37 °C in an atmosphere of 8% CO₂ in air in a humidified incubator.

Radiation and Chemical Treatment. Cells were plated in 100-mm tissue culture dishes or Petri plates in 10 ml of medium and irradiated at 37 °C with ⁶⁰Co γ rays using a Gammabeam-650 irradiator (Nordion International Inc., Ontario, Canada). All irradiations were performed on subconfluent cells in log phase growth at dose rates of 0.5–1.5 Gy/min. Control cells were taken to the radiation chamber but not exposed to radiation. Plates of cells were then incubated at 37 °C for the indicated time after radiation exposure prior to harvest of the RNA. When indicated, cells were treated with *N*-acetyl-L-cysteine (Sigma Chemical Co., St. Louis, MO), H7 (Seikagaku America, St. Petersburg, FL), HA1004 (Seikagaku), or H₂O₂ (Sigma). The antioxidant and protein kinase inhibitors were added 30 min prior to irradiation or treatment with H₂O₂ and remained in the medium until the isolation of the RNA. The maximum concentrations of the protein kinase inhibitors, H7 and

HA1004, and the antioxidant, NAC, were selected such that at least 90% of the cells were viable (as determined by incubation with trypan blue and hemacytometer counting) at the time RNA was harvested. The experimental concentrations were selected so as not to exceed the maximum nontoxic concentration but sufficient to produce an observable effect (e.g., prevention of *c-jun* induction).

RNA isolation and Analysis. RNA was purified by centrifugation through a CsCl cushion as described by Chirgwin *et al.* (15). Northern blot analysis was performed as described previously (16). Ethidium bromide staining intensity and hybridization with an 18S rRNA oligonucleotide probe were used for assessment of RNA quantities in each lane. The *c-jun* probe was a 40-mer oligonucleotide complementary to the coding region of the human *c-jun* proto-oncogene: 5'TAGAACAGTCCGTCACCTTCACGTGAGGTTACTTTGGCGTG^{3'}. The 18S rRNA probe was a 45-mer oligonucleotide complementary to the human sequence: 5'GGAGAGGGGCTGACCGGGTTGGTTTTGATCTGATAAATGCACGCA^{3'}. Hybridizations were performed for 18–24 h at 50 °C in 0.5 M sodium phosphate, pH 7.2, 2 mM ethylenediaminetetraacetic acid (EDTA), 1% bovine serum albumin, and 7% sodium dodecyl sulfate (SDS). The filters were washed in 5 times SSPE (15 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 1 mM EDTA, and 0.1% SDS) at room temperature and twice in 5 times SSPE at 50 °C. The band intensity of the autoradiographs was quantitated by densitometric scanning and normalized to that for the rRNA control.

RESULTS

We used the ML-2 cell line to examine time and dose dependence for the induction of *c-jun* gene expression by ionizing radiation. This cell line has a low constitutive level of *c-jun* gene expression which is highly induced when exposed to ionizing radiation. At 15 Gy of ionizing radiation, a rapid increase in the steady-state level of the *c-jun* mRNA was observed with a maximum level 2 h after irradiation (Fig. 1A). The time for maximal induction of *c-jun* gene expression was dependent on the radiation dose with higher doses resulting in a more rapid induction of *c-jun* gene expression (data not shown). For ML-2 cells irradiated with up to 20 Gy of γ radiation, the induction of *c-jun* gene expression was dose-dependent, with a detectable increase in the steady-state message level observed 3 h after treatment with a dose as low as 0.6 Gy of γ radiation (Fig. 1B).

To study the involvement of PKC and ROIs in the induction of *c-jun* gene expression in ML-2 cells exposed to ionizing radiation, we examined the effect of *N*-acetylcysteine (NAC), an antioxidant which diminishes oxidative stress by scavenging reactive oxygen (17, 18), H7, an inhibitor of PKC and PKA (19), and HA1004, a specific PKA inhibitor (20) (Fig. 2). Pretreatment of ML-2 cells for 30 min with NAC or H7 either reduced or prevented the induction of *c-jun* gene expression in the irradiated cells. No such effect was observed after pretreatment with HA1004. No toxic effects of the kinase inhibitors or NAC were detected in the unexposed or γ -irradiated cells. These results suggest a role for PKC and ROIs in mediating radiation-induced *c-jun* gene expression. A similar response pattern was observed for H₂O₂-mediated induction of *c-jun* expression (Fig. 3). Peroxide treatment resulted in a time-dependent increase in the steady-state level of *c-jun* mRNA which could be prevented by pretreatment with NAC or H7 but only partially by pretreatment with HA1004. These results suggest that *c-jun* gene expression evoked by radiation and H₂O₂ involves the

production of ROIs and PKC activity.

To determine if PKC and ROIs play a similar role in normal cells, we examined the effects of HA1004, H7, and NAC on the induction of *c-jun* gene expression in normal human DET-551 fibroblasts exposed to ionizing radiation (Fig. 4). Compared to the ML-2 cells, these cells had a higher constitutive level of *c-jun* mRNA which increased 3-fold upon exposure to 50 Gy of γ radiation. No increase in *c-jun* gene expression was observed in DET-551 fibroblasts after exposure to 15 Gy of γ -radiation. DET-551 fibroblasts exhibited a similar response to the ML-2 cells in that the induction of *c-jun* gene expression was prevented by pretreatment with H7 and NAC but not by HA1004. In addition, treatment of DET-551 cells with H7 or NAC reduced the constitutive level of *c-jun* mRNA in unirradiated cells with H7 being the most effective in this respect. No effect on *c-jun* steady-state mRNA levels was observed in the unirradiated cells treated with HA1004. These results suggest that PKC and ROIs play a role in the induction of *c-jun* gene expression in both normal and tumor cells exposed to ionizing radiation. Furthermore, our results suggest a role for PKC and ROIs in the constitutive expression of *c-jun* in normal human fibroblasts.

The radiation-induced increase in *c-jun* gene expression was higher by at least an order of magnitude in ML-2 cells than in DET-551 cells, raising the possibility of a heterogeneity in the response of different cells to this inducer. To investigate such a heterogeneity, we used Northern blot analysis to examine the steady-state level of *c-jun* mRNA in a series of human cell lines and strains exposed to γ radiation (Fig. 5). These included normal fibroblasts as well as a number of leukemia, melanoma, sarcoma, and carcinoma cell lines. We observed heterogeneity in the steady-state level of *c-jun* mRNA in both the untreated cells and the cells exposed to γ radiation. In the untreated cells, the steady-state level of *c-jun* mRNA varied from an almost undetectable level to constitutive levels which were several-fold higher. In the irradiated cells, *c-jun* mRNA levels were

examined 3 h after exposure to 15 Gy or 2 h after exposure to 50 Gy of ionizing radiation. These intervals represented the optimal times for maximal *c-jun* mRNA induction for a majority of the cells. Irradiation produced a varied response which ranged from a slight decrease to a pronounced increase in the steady-state level of *c-jun* mRNA. To facilitate a quantitative comparison of *c-jun* expression in the various cells, the level of its expression in unirradiated P3 cells was arbitrarily assigned a value of 1. The steady-state mRNA level in unirradiated and irradiated cells was then compared to that of unirradiated P3 cells after appropriate corrections for differences in RNA quantity. By this method, we observed a more than 10-fold variation in the steady-state level of *c-jun* mRNA in the unirradiated cell lines (Table 1).

High constitutive expression of this gene was observed in Hs913t and HT1080 fibrosarcoma, HT-29 colon carcinoma, and MCF-7 breast tumor cells in contrast to the almost undetectable level of constitutive expression observed in some leukemia, melanoma, and prostate tumor cells, and in some normal fibroblasts. In the γ irradiated cells, a similar heterogeneity of expression was observed. Cells from certain cell lines, myeloid and T-lymphoid leukemia in particular, showed a 10- to 160-fold induction of *c-jun* gene expression after exposure to γ rays. Yet, cells from other cell lines such as the DU145 prostate carcinoma and HT-29 colon tumor, showed little or no induction of *c-jun* expression while decreased *c-jun* expression was noted in the MCF-7 and HT1080 cell lines. The data demonstrate that, as a rule, low constitutive *c-jun* mRNA levels were not necessarily indicative of high inducibility after γ -irradiation. On the other hand, high constitutive *c-jun* expression was associated with poor inducibility after irradiation. In general, decreased steady-state levels of *c-jun* mRNA were observed in cells irradiated with 50 versus 15 Gy of γ -rays in highly induced cells. Other cell lines which exhibited low-multiple induction of *c-jun* gene expression at 15 Gy displayed a moderate increase in *c-jun* mRNA levels after 50

Gy of γ -irradiation. Our data indicate that although induction of *c-jun* gene expression may be a cellular response to ionizing radiation, this response was reduced or absent in some cell types.

Since ionizing radiation is known to induce the formation of ROIs (21), we compared the induction of *c-jun* gene expression by ionizing radiation with that of H_2O_2 , another producer of ROIs. The pattern of induction of *c-jun* gene expression by H_2O_2 was similar to that observed for ionizing radiation (Table 1), in that cell types which showed a pronounced increase in *c-jun* mRNA levels upon exposure to ionizing radiation also responded in a like manner to H_2O_2 treatment. Taken together, these results indicate that the level of induction of *c-jun* gene expression by agents which produce ROIs, such as H_2O_2 and ionizing radiation was defined by the nature of the cells.

DISCUSSION

The induction of *c-jun* gene expression by ionizing radiation suggests that this gene may be a component of the signal transduction pathway that mediates some aspects of the cellular response to ionizing radiation. Our results suggest a role for PKC in mediating this induction of *c-jun* gene expression in both normal and tumor cells. Previous studies which show that inhibition of protein kinase activity sensitizes cells to ionizing radiation (23) and delays the onset of radiation-induced G2 arrest (24) demonstrate a role for PKC in the biological response to radiation. Our results showing inhibition of γ -ray-induced *c-jun* expression in the leukemic ML-2 and normal fibroblast cells by H7, an inhibitor of PKA and PKC (19), but little or no inhibition by HA1004, a PKA inhibitor (20), implicate PKC in the radiation-mediated induction of *c-jun* gene expression. These results are in agreement with previous studies employing human tumor cells which suggest a role for PKC in the induction of *c-jun* as well as other radiation-responsive genes such as *TNF* and *EGR1* (10, 11). We have now extended these studies to demonstrate that PKC mediates ionizing radiation-induced *c-jun* gene expression in normal human fibroblasts. Furthermore, our demonstration of the ability of H7 to modulate H_2O_2 -induced expression of the *c-jun* gene suggests an additional role of PKC in the biological response mediated by ROIs.

The induction of *c-jun* expression was also inhibited by the addition of the antioxidant, *N*-acetylcysteine. This agent acts as a scavenger of reactive oxygen (17) and increases cellular glutathione (18). Treatment of cells with ionizing radiation results in the formation of ROIs (21) which have been implicated in the activation of other transcription factors, such as NF- κ B (24). The ability of NAC to inhibit ionizing radiation-induced *c-jun* expression supports a role for ROIs in the biological response to radiation.

These results suggest a scheme whereby multiple pathways mediate induction of *c-jun*

gene expression in response to H_2O_2 or ionizing radiation. For both agents, the most likely pathway (Fig. 6, pathway a) involves the production of ROIs which, by a direct or indirect mechanism, activate PKC to mediate induction of *c-jun* gene expression. An alternative pathway, (Fig. 6, pathway b), is also possible which involves direct induction of *c-jun* gene expression by ROIs. Our present experiments which showed that H_2O_2 -induced *c-jun* gene expression was inhibited by H7, but only to a limited extent by HA1004 do not support such an alternative; however, a previous study characterized the induction of an ionizing radiation responsive gene, *GADD45*, by a pathway which was independent of PKC (25). Similar alternative pathways are also probable for induction of *c-jun* gene expression by ionizing radiation. The previous observation of the inability of NAC to inhibit ara-C-mediated induction of *c-jun* gene expression (11) suggests a pathway which does not involve ROIs (Fig. 6, pathway c). In this case, the induction may be mediated by PKC or by an alternate process moderating expression. It is also interesting that both NAC and H7, but not HA1004, reduced the constitutive level of *c-jun* mRNA in unirradiated normal fibroblasts. This result suggests a role for PKC and the signal transduction pathways involved in the response to ionizing radiation in the maintenance of constitutive *c-jun* gene expression.

We investigated the generality of the radiation- and H_2O_2 -mediated *c-jun* response by examination of *c-jun* gene expression in a number of different human normal and malignant cell types. Exposure to radiation produced a varied response which ranged from inhibition to little or no induction to a more than two orders of magnitude increase in the steady-state level of *c-jun* mRNA. Exposure to H_2O_2 gave a similar pattern, but the magnitude of the response was lower. The basis for the difference across cell types was not clear but may be attributable in part to lineage-specific differences since the response to γ irradiation and H_2O_2 was similar for cells of the same lineage (e.g. myeloid and T-lymphoid leukemia, melanoma, or prostate tumor). A lineage-specific response may be attributable to differences in reactive

oxygen intermediate metabolism or PKC isozyme activities in the various cell types. Other studies have demonstrated a specific role for PKC isozymes in myeloid cell differentiation (13) and differential expression of PKC isozymes following ionizing radiation exposure with PKC β being rapidly and transiently activated (26). In the response of *c-jun* to ionizing radiation, genetic heterogeneity may also contribute to the differential response since the cells examined are from different individuals. In either case, our data indicate that although induction of *c-jun* gene expression may be a common response to ionizing radiation or H₂O₂, this response is reduced or absent in some cell types.

REFERENCES

1. Vogt, P. K., Bos, T. J., and Doolittle, R. F. Homology between the DNA-binding domain of the GCN4 regulatory protein of yeast and the carboxy-terminal region of a protein coded for by the oncogene *jun*. *Proc. Natl. Acad. Sci. USA*, 84: 3316-3319, 1987.
2. Angel, P., Allegretto, E. A., Okino, S., Hattori, K., Boyle, W. J., Hunter, T., and Karin, M. Oncogene *jun* encodes a sequence specific trans-activator similar to AP-1. *Nature (London)*, 332: 166-171, 1988.
3. Mitchell, P. J., and Tijian, R. Transcription regulation in mammalian cells by sequence-specific DNA binding proteins. *Science*, 245: 371-378, 1989.
4. Lamph, W. W., Wamsley, P., Sassone-Corsi P., and Verma, I. M. Induction of proto-oncogene JUN/AP-1 by serum and TPA. *Nature*, 334: 629-631, 1988.
5. Ryder, K. and Nathans, D. Induction of protooncogene *c-jun* by serum growth factors. *Proc. Natl. Acad. Sci. USA*, 85: 8464-8467, 1988.
6. Quantin, B. and Breathnach, R. Epidermal growth factor stimulates transcription of *c-jun* proto-oncogene in rat fibroblasts. *Nature (London)*, 334: 538-539, 1988.
7. Petrovaara, L., Sistonen, L., Bos, T. J., Vogt, P. K., Keski-Oja, J., and Alitalo, K. Enhanced *jun* gene expression is an early genomic response to transforming growth factor β stimulation. *Mol. Cell. Biol.*, 9: 1255-1262, 1989.
8. Sherman, M. L., Datta, R., Hallahan, D. E., Weichselbaum, R. R., and Kufe, D. W. Ionizing radiation regulates expression of the *c-jun* protooncogene. *Proc. Natl. Acad. Sci. USA*, 87: 5663-5666, 1990.
9. Devary, Y., Gottleib, R. A., Lau, L. F., and Karin, M. Rapid and preferential activation of the *c-jun* gene during the mammalian UV response. *Mol. Cell. Biol.*, 11: 2804-2811, 1991.
10. Hallahan, D. E., Sukhatme, V. P., Sherman, M. L., Virudachalam, S., Kufe, D., and Weichselbaum, R. R. Protein kinase C mediates x-ray inducibility of nuclear signal transducers ERG1 and JUN. *Proc. Natl. Acad. Sci. USA*, 88: 2156-2160, 1991.
11. Datta, R., Hallahan, D. E., Kharbanda, S. M., Rubin, E., Sherman, M. L., Huberman, E., Weichselbaum, R. R., and Kufe, D. W. Involvement of reactive oxygen intermediates in the induction of *c-jun* gene transcription by ionizing radiation. *Biochemistry*, 31: 8300-8306, 1992.
12. Homma, Y., Henning-Chubb, C. B., and Huberman, E. Translocation of protein kinase C in human leukemia cells susceptible or resistant to differentiation induced by phorbol 12-myristate 13-acetate. *Proc. Natl. Acad. Sci. USA*, 83: 7316-7319, 1986.
13. Tonetti, D. A., Horio, M., Collart, F. R., and Huberman, E. Protein kinase C β gene expression is associated with susceptibility of human promyelocytic leukemia cells to phorbol ester-induced differentiation. *Cell Growth & Differ.*, 3: 739-745, 1992.
14. Huberman, E., McKeowan, C. K., Jones, C. A., Hoffman, D. R., and Murao, S. Induction

of mutations by chemical agents at the hypoxanthine-guanine phosphoribosyl transferase locus in human epithelial teratoma cells. *Mutat. Res.*, 130: 127-137, 1984.

15. Chirgwin, J. M., Przybyla, A. E., MacDonald, A. E., and Rutter, W. J. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochem.*, 18: 5294-5299, 1979.

16. Glesne, D. A., Collart, F. R., and Huberman, E. Regulation of IMP dehydrogenase gene expression by its end products, guanine nucleotides. *Mol. Cell. Biol.*, 11: 5417-5425, 1991.

17. Aruoma, O. I., Halliwell, B., Hoey, B. M., and Butler, J. The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radical Biol. Med.*, 6: 593-597, 1989.

18. Burgunder, J. M., Varriale, A., and Lauterburg, B. H. Effect of N-acetylcysteine on plasma cysteine and glutathione following paracetamol administration. *Eur. J. Clin. Pharmacol.*, 36: 127-31, 1989.

19. Hidaka, H., Inagaki, M., Kawamoto, S., and Sasaki, Y. Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. *Biochemistry*, 23: 5036-5041, 1984.

20. Asano, T., and Hidaka, H. Vasodilatory action of HA1004 [N-(2-guanidinoethyl)-5-isoquinolinesulfonamide], a novel calcium antagonist with no effect on cardiac function. *J. Pharmacol. Exp. Ther.*, 231: 141-145, 1986.

21. Hall, E.J. In: E. J. Hall (ed.), *Radiobiology for the Radiologist*, 3rd ed., pp 17-38, Philadelphia, PA, J. B. Lippincott, 1988.

22. Hallahan, D. E., Virudachalam, S., Schwartz, J. L., Panje, N., Mustafi, R., and Weichselbaum, R. R. Inhibition of protein kinases sensitizes human tumor cells to ionizing radiation. *Radiat. Res.*, 129: 345-350, 1992.

23. Hallahan, D. E., Virudachalam, S., Grdina, D., and Weichselbaum, R. R. The isoquinoline sulfonamide H7 attenuates radiation-mediated protein kinase C activation and delays the onset of X-ray-induced G2 arrest. *Int. J. Radiat. Oncol. Biol. Phys.*, 24: 687-692, 1992.

24. Brach, M. A., Hass, R., Sherman, M. L., Gunji, H., Weichselbaum, R., and Kufe, D. Ionizing radiation induces expression and binding activity of the nuclear factor κ B. *J. Clin. Invest.*, 88: 691-695, 1991.

25. Papathanasiou, M. A., Kerr, N. C. K., Robbins, J. H., McBride, O. W., Alamo, I., Barrett, S. F., Hickson, I. D., and Fornace, A. J. Induction by ionizing radiation of the *gadd45* gene in cultured human cells: lack of mediation by protein kinase C. *Mol. Cell. Biol.*, 11: 1009-1016, 1991.

26. Woloschak, G. E., Chang-Liu, C. -M., Sherin-Jones, P. Regulation of protein kinase C by ionizing radiation. *Cancer Res.*, 50: 3963-3967, 1990.

Table 1. Expression of *c-jun* in γ -irradiated and H_2O_2 treated cells.

		<i>c-jun</i> expression (arbitrary units ¹)			
	Cell Type	Unirradiated ²	15 Gy	50 Gy	H_2O_2 (100 μ M)
ML-2	Myeloid leukemia	≤ 0.5	80.0	40.0	24.0
HL-205	Promyelocytic leukemia	≤ 0.5	1.0	5.6	4.0
CEM	T-lymphoblastoid leukemia	0.6	36.0	8.9	31.0
MOLT-4	T-lymphoblastoid leukemia	0.7	64.0	26.0	56.0
CCL-155	B-lymphoblastoid leukemia	≤ 0.5	3.5	2.8	ND ³
Raji	B-lymphoblastoid leukemia	≤ 0.5	≤ 0.5	0.8	ND
MCF-7	Breast carcinoma	2.3	1.1	2.5	2.0
MB231	Breast carcinoma	≤ 0.5	≤ 0.5	2.6	3.7
HO	Melanoma	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5
SK-MEL	Melanoma	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5
Hs913t	Fibrosarcoma	5.0	5.0	8.5	ND
HT1080	Fibrosarcoma	1.8	1.8	1.3	ND
IMR-90	Normal lung (fibroblasts)	≤ 0.5	≤ 0.5	0.8	≤ 0.5
WI-38	Normal lung (fibroblasts)	≤ 0.5	≤ 0.5	1.0	≤ 0.5
DET-551	Normal skin (fibroblasts)	0.9	0.9	2.8	≤ 0.5
LNCaP	Prostate carcinoma	≤ 0.5	1.2	1.0	1.8
DU145	Prostate carcinoma	≤ 0.5	0.8	0.8	1.2
P3	Teratocarcinoma	1.0	0.7	3.5	1.0
HT-29	Colon carcinoma	2.2	2.4	3.1	ND

¹ Amount of *c-jun* mRNA in untreated P3 cells was set to 1.0. All other values are expressed relative to that.

² RNA was isolated 3 h after exposure to 15 Gy or 2 h after exposure to 50 Gy or treatment with 100 μ M H_2O_2 .

³ ND - not determined

FIGURE LEGENDS

Figure 1. Time course (A) and dose response (B) for the induction of *c-jun* expression in irradiated ML-2 cells. A. ML-2 cells were irradiated with 15 Gy of γ rays at a dose rate of 1.5 Gy/min and total RNA isolated at the indicated times after exposure. B. ML-2 cells were irradiated at a dose rate of 0.5 to 1.5 Gy/min for indicated doses and RNA isolated 3 h after exposure.

Figure 2. Effects of *N*-acetylcysteine and protein kinase inhibitors on *c-jun* gene expression in γ -irradiated ML-2 cells. ML-2 cells were pretreated with the indicated agents 30 min prior to irradiation with 15 Gy of γ rays. RNA was harvested 3 h after exposure and *c-jun* mRNA levels analyzed as described in Materials and Methods.

Figure 3. Effects of *N*-acetylcysteine and protein kinase inhibitors on *c-jun* gene expression in H_2O_2 -treated ML-2 cells. ML-2 cells were treated with 100 μ M H_2O_2 and RNA isolated at the indicated times. Several dishes were pretreated with 100 μ M H7 or HA1004, or with 8 mM NAC for 30 min prior to addition of 100 μ M H_2O_2 . For these samples, RNA was harvested 2 h after treatment with H_2O_2 and *c-jun* mRNA levels analyzed as described in Materials and Methods.

Figure 4. Effects of *N*-acetylcysteine and protein kinase inhibitors on *c-jun* gene expression in unirradiated and γ -irradiated DET-551 cells. In the irradiated samples, DET-551 cells were pretreated with the indicated agents for 30 min prior to irradiation with 50 Gy of γ rays. RNA was harvested 2 h after exposure and *c-jun* mRNA levels analyzed as described in Materials and Methods. In the unirradiated samples, DET-551 cells were exposed to 100 μ M H7 or HA1004, or 8 mM NAc for the same time intervals as the irradiated samples prior to isolation of total RNA.

Figure 5. Analysis of steady-state *c-jun* mRNA levels in human cell lines. Cells were irradiated with 50 Gy of γ radiation and total RNA isolated 2 h after irradiation as described in Materials and Methods. The same blot was used for hybridization to the *c-jun* and 18 s rRNA probes.

Figure 6. Induction of *c-jun* gene expression by H_2O_2 and ionizing radiation.

A

B