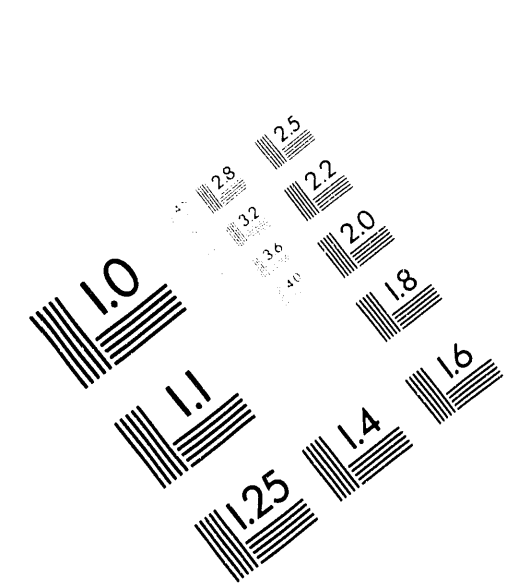

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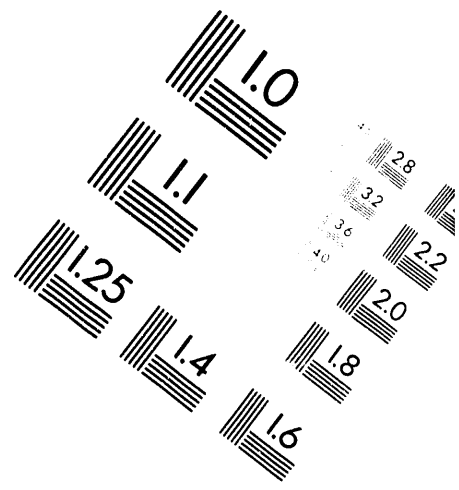

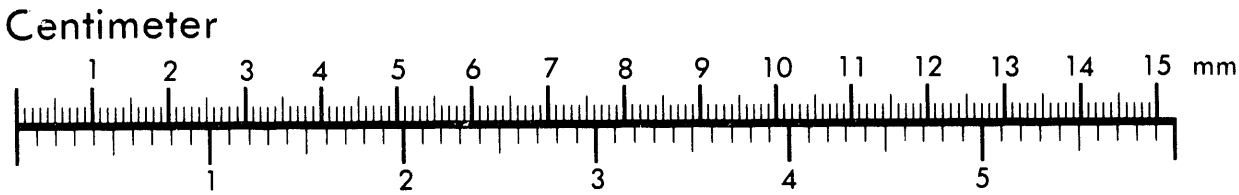

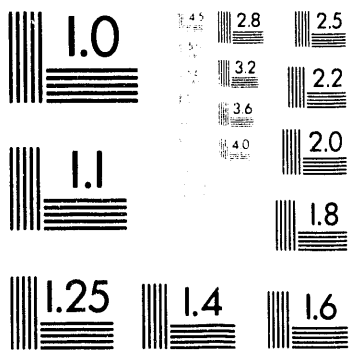

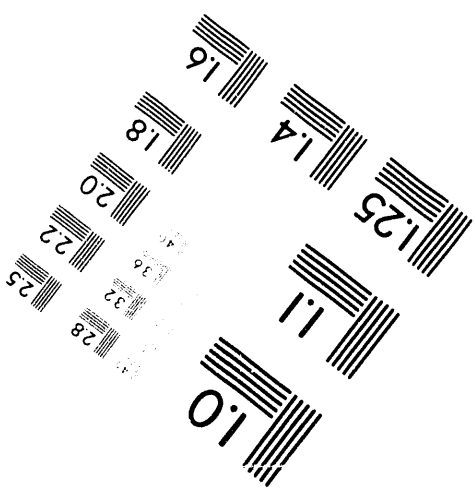

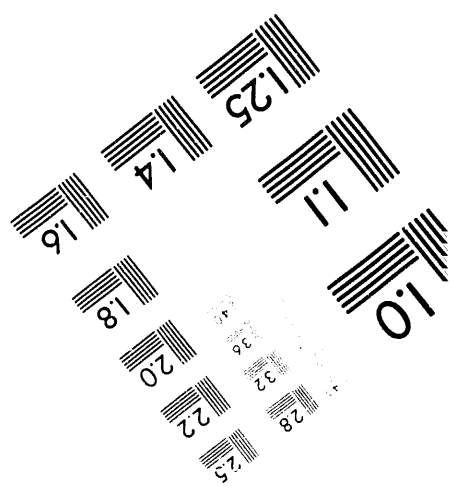

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c-*jun* Gene Expression in Human Cells Exposed to Either Ionizing Radiation or Hydrogen Peroxide

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June 1993

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ABSTRACT: We investigated the role of reactive oxygen intermediates (ROIs) and protein kinase C (PKC) in radiation- and H_2O_2-evoked c-*jun* gene expression in human HL-205 cells. This induction of c-*jun* gene expression could be prevented by pretreatment of the cells with *N*-acetylcysteine (an antioxidant) or H7 (a PKC and PKA inhibitor) but not by HA1004, a PKA inhibitor, suggesting a role for ROIs and PKC in mediating c-*jun* gene expression. We also investigated potential differences in c-*jun* gene expression in a panel of normal and tumor cells untreated or treated with ionizing radiation or H_2O_2. Treatment with radiation or H_2O_2 produced a varied response, from some reduction to an increase of more than an order of magnitude in the steady-state level of c-*jun* mRNA. These data indicate that although induction of c-*jun* may be a common response to ionizing radiation and H_2O_2, this response was reduced or absent in some cell types.

1. INTRODUCTION

The c-*jun* proto-oncogene has been implicated in the regulation of cellular proliferation (Vogt et al., 1987). This gene is a member of a family of early response genes that code for transcription factors which include *junB*, *junD*, c-*fos*, *fosB*, and *fra-1*, and its product is a component of the AP-1 transcription complex (Angel et al., 1988). 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 including c-*jun* (Mitchell and Tijian, 1989). In addition to phorbol esters, c-*jun* gene expression is also induced by ionizing radiation (Devary et al., 1991; Sherman et al., 1990), and this induction is believed to be mediated by a protein kinase C-dependent mechanism (Hallahan et al., 1991). We examined the role of reactive oxygen intermediates (ROIs) and protein kinase C (PKC) in the induction of c-*jun* in HL-205 leukemia cells by comparing the effects of ionizing radiation or H_2O_2 exposure in the presence or absence of appropriate inhibitors. We also investigated the universality of radiation-induced c-*jun* gene expression using a panel of normal human cell strains and human tumor cell lines. This panel of cells was also treated with H_2O_2 (which produces ROIs) to assess if a similar pattern of c-*jun* gene expression was observed. Such a similarity would suggest that common signal transduction events mediate the induction of c-*jun* gene expression by these agents.

2. METHODS

The human HL-205 cells were a clonal isolate from the HL-60 cell line obtained from Dr. Robert C. Gallo, National Cancer Institute, Bethesda, MD. The human HO melanoma cells were provided by Dr. Beppino C. Giovanella, Stehlin Foundation for Cancer Research, Houston, TX. Human SK-MEL-131 cells were obtained from Dr. Alan Houghton, Memorial Sloan-Kettering Cancer Center, New York, NY. The human Hs913t and HT1080 fibrosarcoma and the normal human IMR70, WI-38, and DET551 fibroblasts were obtained from the American Type Culture Collection, Rockville, MD. 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 8% CO_2 in air in a humidified incubator.

Cells were plated in 100-mm tissue culture dishes or Petri plates in 10 ml of medium and irradiated at 37 °C with ^{60}Co γ rays using a Gammabeam-650 irradiator (Nordion International Inc., Ontario, Canada). Irradiations were performed on subconfluent cells in log phase growth at dose rates of 1.5 Gy/min. Controls 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 the antioxidant *N*-acetylcysteine (NAC; Sigma Chemical Co., St. Louis, MO) with the protein kinase inhibitors H7 (Seikagaku America, St. Petersburg, FL) or HA1004 (Seikagaku), or with H_2O_2 (Sigma). The antioxidant and protein kinase inhibitors were added 30 min prior to irradiation or treatment with H_2O_2 and remained in the medium until the isolation of the RNA. The maximum concentrations of H7, HA1004, and the NAC were selected such that at least 90% of the cells were viable (as determined by incubation with trypan blue at the time RNA was harvested. The experimental concentrations did not exceed the maximum nontoxic concentration but were sufficient to produce an observable effect (i.e., prevention of c-*jun* induction).

RNA was purified by centrifugation through a CsCl cushion as described by Chirgwin et al., 1979. Northern blot analysis was performed as described previously (Glesne et al., 1991). Ethidium bromide staining intensity and hybridization with an 18S rRNA oligonucleotide probe were used to assess RNA quantities in each lane. The c-*jun* probe was a 40-nucleotide oligomer ($^{5'}$TAGAACAGTCCGTCACTTCACGTGAGGTTACTTTGGCGTG$^{3'}$) complementary to the coding region of the human c-*jun* proto-oncogene. The 18S rRNA probe was a 45-nucleotide oligomer ($^{5'}$GGAGAGGGGCTGACCGGGTTGGTTTTGATCTGATAAATGCACGCA$^{3'}$) complementary to the human sequence. 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 5x 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 5x SSPE at 50 °C. The band intensity of the autoradiographs was quantitated by densitometric scanning and normalized to that for the rRNA control.

3. RESULTS

To study the involvement of PKC and ROIs in the induction of c-*jun* in HL-205 cells exposed to ionizing radiation, we examined the effect of NAC (an antioxidant which diminishes oxidative stress by scavenging reactive oxygen; Aruoma et al., 1989; Burgunder et al., 1989), H7 (an inhibitor of PKC and PKA; Hidaka et al., 1984), and HA1004 (a specific PKA inhibitor; Asano and Hidaka, 1986). In the absence of these inhibitors, irradiation resulted in an order of magnitude increase in the steady-state level of c-*jun* mRNA (Fig. 1). Pretreatment of HL-205 cells for 30 min with NAC or H7 prevented this induction. For both of these inhibitors, the response was dose-dependent, with a partial restoration of c-*jun* gene expression at lower doses. No such effects were observed after pretreatment with HA1004. At the levels required for abrogation of c-*jun* induction, no toxic effects of the kinase inhibitors or NAC were detected in the unexposed or γ-irradiated cells. Peroxide treatment also resulted in a time-dependent increase in the steady-state level of c-*jun* mRNA that could be prevented by pretreatment with NAC or H7 but not by pretreatment with HA1004 (data not shown). These results suggest that c-*jun* gene expression evoked by either ionizing radiation or H_2O_2 involves the production of ROIs and PKC activity.

To study the universality of the radiation-mediated c-*jun* induction response, we examined the steady-state level of c-*jun* mRNA in a series of human cell lines and strains exposed to γ radiation (Fig. 2). We observed heterogeneity in the steady-state level of c-*jun* mRNA in both

Figure 1. Effects of *N*-acetylcysteine and protein kinase inhibitors on c-*jun* gene expression in γ-irradiated HL-205 cells. Cells were pretreated with the indicated agents 30 min prior to irradiation with 20 Gy of γ rays. RNA was harvested 3 h after exposure and c-*jun* mRNA levels analyzed as described in Methods.

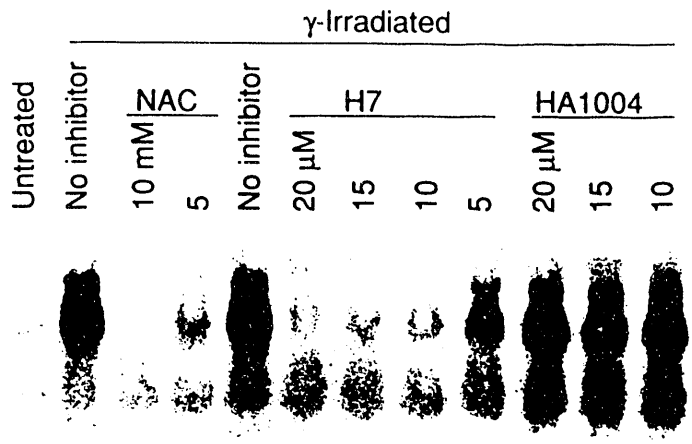
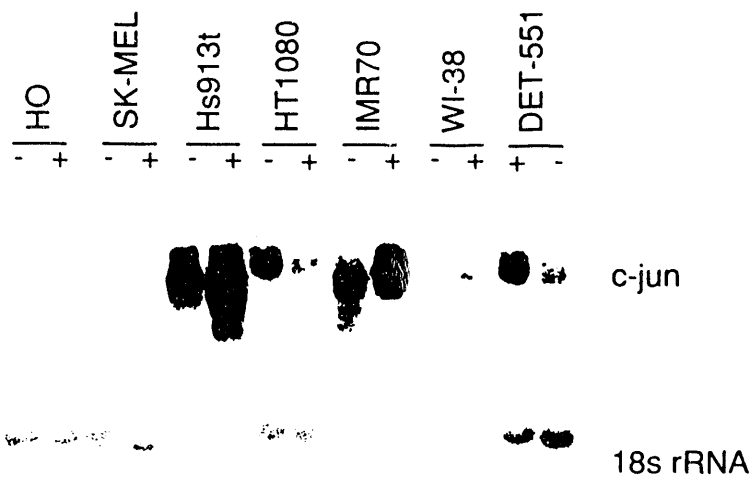


Figure 2. Analysis of steady-state c-*jun* mRNA levels in human cell lines. Cells were irradiated with 50 Gy of γ rays and total RNA was isolated 2 h after irradiation (see Methods). The same blot was used for hybridization to the c-*jun* and 18S rRNA probes.



untreated cells and in cells exposed to γ-radiation. High constitutive expression of the gene was observed in untreated Hs913t and HT1080 fibrosarcoma cells and in normal IMR70 fibroblasts, in contrast to the almost undetectable level of constitutive expression observed in untreated HO and SK-MEL melanoma cells and in normal WI-38 fibroblasts. In the γ-irradiated cells, a similar heterogeneity of expression was observed: c-*jun* steady-state mRNA levels were increased several-fold in Hs913t fibrosarcoma cells and in the fibroblast cell strains. Yet, irradiated HO and SK-MEL melanoma cells showed little or no induction of c-*jun*, and c-*jun* expression was decreased in the HT1080 cell line. Our data indicate that although induction of c-*jun* 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 (Hall, 1988), we compared the induction of c-*jun* by ionizing radiation with that by H_2O_2, another producer of ROIs. The pattern of induction of c-*jun* by H_2O_2 was similar to that observed for ionizing radiation (data not shown): cell types which showed either a pronounced increase or little or no increase in c-*jun* mRNA levels upon exposure to ionizing radiation responded in a like manner to H_2O_2 treatment. Taken together, these results indicate that the level of induction of c-*jun* by agents which produce ROIs, such as H_2O_2 and ionizing radiation, was defined by the nature of the cells.

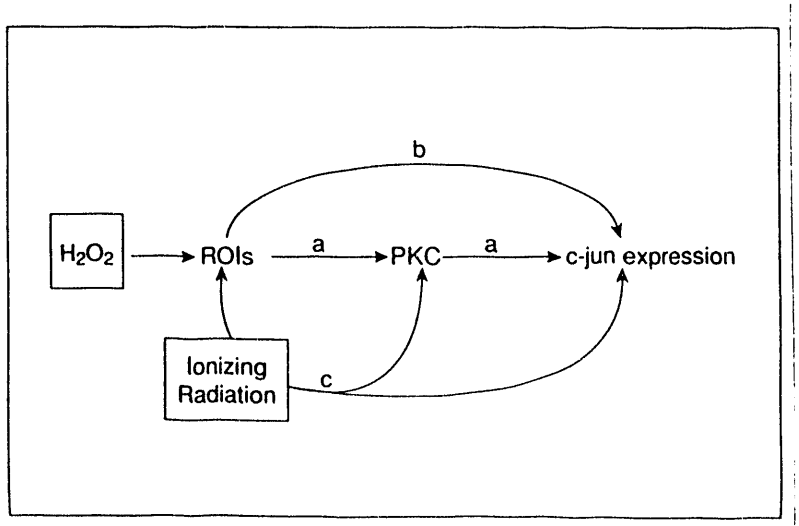
4. DISCUSSION

Our results show inhibition of γ-ray-induced c-*jun* gene expression in the leukemic HL-205 cells by H7, an inhibitor of PKA and PKC but little or no inhibition by HA1004, a PKA inhibitor and they implicate PKC in the radiation-mediated induction of c-*jun* gene expression. These results agree with previous studies using human tumor cells which suggest a role for PKC in the induction of c-*jun* and other radiation-responsive genes such as *TNF* and *EGR1* (Datta et al., 1992, Hallahan et al., 1991). Furthermore, 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, which are generated by both ionizing radiation and H_2O_2. Other studies have shown that inhibition of protein kinase activity sensitizes cells to ionizing radiation (Hallahan et al., 1992) and delays the onset of radiation-induced G2 arrest (Brach et al., 1991) demonstrating a role for protein kinases, most likely PKC, in the biological response to radiation.

The induction of c-*jun* expression was also inhibited by the addition of the antioxidant NAC. This agent acts as a scavenger of reactive oxygen (Aruoma et al., 1989) and increases cellular glutathione (Burgunder et al., 1989). Treatment of cells with ionizing radiation results in the formation of ROIs (Hall, 1988), which have been implicated in the activation of other transcription factors, such as NF-κB, (Brach et al., 1991). 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. 3, pathway a) involves the production of ROIs which, by a direct or indirect mechanism, activate PKC to mediate the induction of c-*jun*. An alternative pathway (Fig. 3, pathway b) is also possible, involving direct induction of c-*jun* 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 that was independent of PKC (Papathanasiou et al., 1991). Similar alternative pathways are also probable for the induction of c-*jun* by ionizing radiation. The previous observation of the inability of NAC to inhibit ara-C-mediated induction of c-*jun* gene expression (Datta et al., 1992), suggests a pathway which does not involve ROIs (Fig. 3, pathway c). In this case, the induction may be mediated by PKC or by an alternate process that moderates gene expression.

Figure 3. Induction of c-*jun* gene expression by H_2O_2 and ionizing radiation.



We investigated the generality of the radiation- and H_2O_2-mediated c-*jun* response by studies with a number of normal and malignant human cell types. Exposure to radiation produced a varied response, which ranged from some reduction to an increase of more than an order of magnitude in the steady-state level of c-*jun* mRNA. 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., melanoma). A lineage-specific response may be attributable to differences in ROI metabolism or PKC isozyme activities. Indeed, specific PKC isozymes have been implicated in myeloid cell differentiation (Tonetti et al., 1992) and differential expression of PKC isozymes following ionizing radiation exposure has been demonstrated in some cell types (Woloschak et al., 1990). 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 H_2O_2, this response is reduced or absent in some cell types.

This work was supported by the U.S. Department of Energy, Office of Health and Environmental Research, under contract W-31-109-Eng-38.

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