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

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ABSTRACT: We investigated the role of reactive oxygen intermediates (ROIs) and protein kinase C (PKC) in radiation- and H\(_2\)O\(_2\)-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 e-jun gene expression in a panel of normal and tumor cells untreated or treated with ionizing radiation or H\(_2\)O\(_2\). Treatment with radiation or H\(_2\)O\(_2\) 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\(_2\)O\(_2\), 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 Tjian, 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\(_2\)O\(_2\) 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\(_2\)O\(_2\) (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 \(\mu\)g/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 60Co 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 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 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'TAGAACAGTCGCCACAGGTGTACCTTGGCGTG3') complementary to the coding region of the human c-jun proto-oncogene. The 18S rRNA probe was a 45-nucleotide oligomer (5'GGAGAGGGGCTGACCGGTGTTTTGATCTGATAATGCACGCA3') 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 H2O2 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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Untreated</th>
<th>NAC 10 mM</th>
<th>H7 20 μM</th>
<th>HA1004 20 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inhibitor</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

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-ray 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 H₂O₂, another producer of ROIs. The pattern of induction of c-jun by H₂O₂ 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 H₂O₂ treatment. Taken together, these results indicate that the level of induction of c-jun by agents which produce ROIs, such as H₂O₂ 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 H2O2-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 H2O2. 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 H2O2 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 H2O2-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 H2O2 and ionizing radiation.
We investigated the generality of the radiation- and H$_2$O$_2$-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 $\gamma$ irradiation and H$_2$O$_2$ 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$_2$O$_2$, this response is reduced or absent in some cell types.

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5. REFERENCES
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