Inhibition of expression of anti-apoptotic protein Bcl-2 and induction of cell death in radioresistant human prostate adenocarcinoma cell line (PC-3) by methyl jasmonate

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Abstract

Hormone refractory human prostate cancer cell lines are known to be radioresistant, a feature attributed to their ability to induce anti-apoptotic proteins of the Bcl-2 family when exposed to radiation. We investigated whether pro-apoptotic compounds such as methyl jasmonate, a plant stress hormone, can counteract the radiation-induced anti-apoptotic mechanism in a human prostate cancer cell line PC-3. Significant ($p < 0.05$) increase in cytotoxicity was observed in the combined treatment groups compared to single treatments with methyl jasmonate or radiation. Treatment of irradiated PC-3 cells with methyl jasmonate resulted in suppression of anti-apoptotic Bcl-2 protein and elevation of caspase-3 activity. Our results showed increased apoptosis in the combined treatment group as compared to the irradiated group or the untreated control. In summary, methyl jasmonate suppressed the radiation-induced Bcl-2 expression and enhanced the radiation sensitivity of human prostate cancer cells.

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Keywords: Methyl jasmonate; PC-3; Bcl-2; Radiation; Apoptosis; Caspase-3; Combined treatment

1. Introduction

Prostate cancer remains one of the most common causes of cancer death [1,2]. Apoptosis is the primary mechanism through which prostate cancer cells are killed when exposed to treatments such as chemotherapy or radiotherapy [3]. Most recent studies have shown that advanced stages of this disease exhibit a significant degree of resistance to certain therapies especially radiotherapy [2,4]. For instance, it has been shown that human prostate cancer cell lines (PC-3 and DU-145) express anti-apoptotic proteins, i.e., Bcl-2 and/or Bcl-xl, when exposed to radiation, thus rendering them resistant to treatment [4,5]. Palayoor et al. [6] showed that PC-3 cells exposed to 8 Gy $\gamma$-radiation recover from
the radiation-induced G2-M phase block and result in only 5–6% apoptotic cells by 48 h following treatment. Thus, hormone refractory prostate cancer cells can effectively be killed with either very high or repeated doses of radiation, both of which have clinical implications. Recently, certain Bcl-2 inhibitors including some phytochemicals have been shown to increase radiosensitivity in various cancers when combined with radiation therapy resulting in increased cytotoxic effects [7–10]. However, a majority of these inhibitors present an unselective cytotoxicity, and therefore, pose a great danger to normal cells.

Phytochemicals represent a new class of compounds with anti-carcinogenic properties that are gaining attention in the treatment of human cancers [11]. Among these plant-derived compounds are the jasmonates including cis-jasmone, jasmonic acid and methyl jasmonate (MJ) (Fig. 1). They serve as natural bioregulators and are involved in plant intracellular signaling and defense in response to injury [12]. Jasmonates are synthesized in plants from α-linolenic acid via the lipoxygenase pathway [13–15], a process involving the transformation of linolenic acid by lipoxygenase to 13-hydroperoxylinolenic acid, then to jasmonic acid which is finally converted to MJ [13]. It has been reported that jasmonate and its analogs inhibited proliferation and induced cell death in lymphoblastic leukemia (MOLT-4) and breast cancer cells cultured in vitro but did not affect normal human blood lymphocytes or erythrocytes, even when the latter were part of a mixed population of leukemic and normal cells [16,17]. In addition, MJ induced cell death in mutant-p53 expressing B-lymphoma and multi-drug resistant melanoma cells [18,19]. However, the mechanism(s) of action of jasmonates is still unclear, although some investigators have proposed three pathways: (1) the mechanism in which MJ causes mitochondrial perturbation leading to severe ATP depletion; (2) the mitogen-activated protein kinase-dependent mechanism, and (3) the reactive oxygen species-mediated mechanism [17]. Kim et al. [20] reported the induction of pro-apoptotic Bcl-2 proteins in human lung cancer cells exposed to MJ treatment. However, whether this unique property of MJ can be used to radio-sensitize cancer cells, especially those that have been shown to be radioresistant, to the best of our knowledge has not been reported. Thus, in the present study, we demonstrated that MJ can counteract the radiation-induced anti-apoptotic mechanism in PC-3 cells.

2. Materials and methods

2.1. Reagents and cell lines

Methyl jasmonate (methyl 3-oxo-2 (2-pentenyl) cyclopentaneacetic acid), fetal bovine serum (FBS), penicillin/streptomycin (P/S), Annexin V-FITC, propidium iodide (PI) and caspase-3 colorimetric assay kits (Catalog No. CASP3C) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Mouse monoclonal anti-Bcl-2 antibody (mouse clone Bcl-2 100) was purchased from GeneTex, Inc. (TX, USA); Goat anti-mouse IgG–HRP conjugate was purchased from Bio-Rad Laboratories (Hercules, CA, USA). Cells were irradiated using a Caesium irradiator (Gamma cell 40; Atomic Energy of Canada, Ltd., Ottawa, Ont., Canada). Stock solutions (1.0 M) of the methyl jasmonate (MJ) were prepared by dissolving in dimethyl sulfoxide (DMSO) and the aliquots stored at −20°C prior to use. All other reagents were obtained from Sigma unless otherwise specified. Human prostate adenocarcinoma cell line (PC-3) was purchased from ATCC (Manassas, VA, USA). Cells were maintained in RPMI-1640 supplemented with 10% FBS and 1% penicillin–streptomycin (100 U/ml penicillin; 100 µg/ml streptomycin).

2.2. Dye exclusion assay (DEA)

Dye exclusion assay was performed as described elsewhere [21]. Briefly, PC-3 cells (2 × 10⁶ cells) were seeded in 60-mm tissue culture plates and allowed to adhere overnight at 37°C in a humidified, 5% CO₂ atmosphere. Cells were then exposed to either single or combined treatments as shown in Table 1. Briefly, for the single treatment groups, cells were treated with MJ or γ-radiation whereas
in the combined treatment groups, cells where exposed to γ-radiation at 0.76 Gy/min for 10 min and immediately replenished with medium containing MJ and then incubated for 24 h. At the end of incubation, the detached and attached cells from both the single and combined treatment groups were harvested and counted using hemacytometer. Control group received drug free medium with 0.1% v/v DMSO. Total live and dead cells were counted separately using trypan blue and cytotoxicity in each group was determined relative to the untreated control groups as shown below:

\[
\text{Percent viability} = \frac{\text{Number of live cells in the treatment group}}{\text{Number of live cells in the untreated control}} \times 100
\]

\[
\text{Percent cytotoxicity} = 100 - \text{calculated percent viability}
\]

### 2.3. Colony formation assay (CFA)

The long-term effect of MJ on irradiated PC-3 cells was studied using CFA as described [21]. Briefly, cells (10^4/plate) were seeded in 60 mm tissue culture plates and incubated overnight to allow for cell attachment. Then, cells were exposed to either single or combined treatment as described earlier (Table 1) with the control group receiving only 0.1% v/v DMSO. After 24 h, cells from each treatment group were trypsinized, counted and plated into new 60 mm tissue culture plates (500 cells/plate); sufficient to yield a minimum of 100 colonies in the untreated control groups. Cells were then incubated in complete culture medium for up to 14 days undisturbed. On day 14, colonies were washed gently with 1× PBS, stained with crystal violet (0.5 g/100 ml in 95% ethanol) and surviving colonies were counted. Colonies containing at least 50 cells were scored and considered to have survived the treatment. The surviving fraction at each dose was calculated relative to the colony number in the unirradiated control groups. Data obtained represent at least two separate experiments.

\[
\text{Surviving fraction} = \frac{\text{Plating efficiency of the treated group}}{\text{Plating efficiency of the untreated control}}
\]

### 2.4. Western blot analysis of Bcl-2 protein expression in γ-irradiated PC-3 cells

Analysis of Bcl-2 expression using Western blotting was carried out as described [23] with slight modifications. Briefly, PC-3 cells (5 × 10^6 cells) were cultured and allowed to adhere overnight before exposure to either MJ (2.0 mM MJ) or gamma radiation (7.6 Gy) or both 2.0 mM MJ and 7.6 Gy as described in Table 1. The control group received drug free medium with 0.1% v/v DMSO. Following treatment, the adherent cells were lysed in a lysis buffer (50 mM Tris pH 7.5, 320 mg NaCl, 200 mM sodium orthovanadate, 0.5 M EDTA (pH 8.0), 10 μg/ml leupeptin, 1.7 μg/ml aprotinin, 0.1% Triton X-100, and 1 mM PMSF). Protein estimations in diluted samples were performed using the eppendorf Biophotometer and appropriate blank was employed. Equal amount of protein (~44 μg) from the various groups were mixed with 25 μL of sample buffer, subjected to 12% SDS-PAGE and then transferred onto nitrocellulose membrane and blocked overnight at 4 °C using 5% nonfat dry milk and 0.1% Tween 20 in PBS (wash buffer). The membrane was incubated with mouse anti-human Bcl-2 antibody (mouse clone Bcl-2 100) for 1 h at room temperature and subsequently with HRP-conjugated goat anti-mouse IgG antibody for 1 h. The membrane was then washed 3× with 0.1% wash buffer, and Bcl-2 was detected using enhanced chemiluminescence HRP substrate. Furthermore, the membrane was stripped using appropriate buffer and subjected to immunoblotting using a mouse anti-human β-actin antibody for one hour at room temperature followed by a HRP-conjugated goat anti-mouse IgG as secondary antibody. Using enhanced chemiluminescence HRP substrate, β-actin was detected and served as loading control.

### 2.5. Detection of apoptosis by flow cytometry

The transversion of phosphatidyl serine from the inner to outer plasma membrane leaflet, an initial event in the apoptotic pathway [24,25] was assessed by dual dye staining using Annexin V-FITC/PI. Briefly, PC-3 cells (2 × 10^6) were cultured in 100 mm dishes in culture medium and allowed to adhere overnight. Cells were exposed to either single (2.0 mM MJ or 7.6 Gy γ-radiation) or combined (2.0 mM + 7.6 Gy) treatments as described in Table 1. The control group received drug free medium with 0.1% v/v DMSO. Jurkat cells served as internal con-

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Table 1

<table>
<thead>
<tr>
<th>Treatment regimen on human prostate cancer cell line (PC-3) using MJ and/or γ-radiation</th>
<th>Combined treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mM MJ</td>
<td>0.5 mM MJ + 7.6 Gy γ-radiation</td>
</tr>
<tr>
<td>1.0 mM MJ</td>
<td>1.0 mM MJ + 7.6 Gy γ-radiation</td>
</tr>
<tr>
<td>2.0 mM MJ</td>
<td>2.0 mM MJ + 7.6 Gy γ-radiation</td>
</tr>
<tr>
<td>7.6 Gy γ-radiation</td>
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</table>

In the single treatments, cells were exposed to appropriate concentration of MJ or 7.6 Gy γ-radiation. In the radiation treated group, cells were immediately replenished with fresh medium following exposure to γ-radiation and then incubated for 24 h. For the combined treatment group, cells were treated with γ-radiation at a dose rate of 0.76 Gy/min for 10 min and then immediately replenished with the appropriate concentration of MJ containing medium and incubated for 24 h.
trol. Apoptosis was induced in Jurkat cells using staurosporine (1 μg/ml). At the end of treatment period, the control (untreated) and treated cells of both Jurkat cells and PC-3 cells were harvested and washed with cold PBS at 1200 rpm for 5 min. The cells were labeled with Annexin V-FITC according to the manufacturer’s instructions (Sigma–Aldrich Chemical Comp. St. Louis, MO, USA). Briefly, cells (1 × 10⁶) were washed with 2.0 ml Annexin-V binding buffer (Sigma–Aldrich Chemical Comp. St. Louis, MO). Cells were resuspended in 495 μL of 1 × Annexin-V binding buffer and then treated with 5 μl of Annexin-V-FITC and incubated in the dark for 15 min. At the end of incubation, cell–Annexin V-FITC conjugate was further stained with 10 μL PI (500 μg/mL) solution and then analyzed by flow cytometry to discriminate between live and apoptotic cells. All color assessments were logarithmically amplified and 10,000 events were acquired on a FACSCalibur flow cytometer (Becton–Dickinson, San Jose, CA, USA) and analyzed using the FlowJo 7.2.2 (Tree Star, Inc., Ashland, OR, USA).

2.6. Quantification of caspase-3 activity

The activity of caspase-3 was assessed using the caspase-3 Colorimetric Assay Kits (Sigma–Aldrich) as described earlier [22]. This assay is based on detection of the colored p-nitroaniline (pNA) molecule following cleavage from the labeled substrate (DEVD-pNA). Briefly, PC-3 cells were exposed to either the single or combined treatment as described in the dye exclusion assay section. Following 24 h treatment, cells were washed in 1× PBS and equal number of cells (10⁶) from each treatment group was suspended in 100 μl of lysis buffer (50 mM HEPES pH 7.4, 5 mM CHAPS, 5 mM DTT) for 20 min. Lysed cells were centrifuged at 16,000g for 15 min and then equal amount of protein (~15 μg) from each sample was used to determine caspase-3 activity according to manufacturer’s instruction. The optical density was measured at 405 nm using a microplate reader (Spectra MAX 340, Molecular Devices Co.). The caspase-3 activity for each treatment was calculated as shown below:

Activity, μmol pNA/min/ml = \( \frac{OD \times D}{\epsilon \times M \times T \times V} \)

Where: OD = optical density or absorbance at 405 nm, \( V = \) volume of sample in ml, \( D = \) dilution factor, \( T = \) reaction time in minutes, \( \epsilon_{mM} = \) molar absorptivity of p-nitroaniline = 10.5 at 405 nm.

2.7. Statistical analysis

Results were expressed as means ± SD of replicate analyses. Data analyses were performed (where appropriate) using ANOVA for a single factor, factorial treatment model with one way blocking to examine the effects of two factors and the differences at the many levels within each factor. Differences with p-values less than 0.05 (\( p < 0.05 \)) were considered statistically significant.

3. Results

3.1. Cytotoxicity of methyl jasmonate on PC-3 cells exposed to γ-irradiation

To evaluate the effect of MJ on irradiated PC-3 cells, we exposed PC-3 cells to either single or combined treatments as described in Table 1 and then determined the cell survivability. Our result (Fig. 2) shows that the short-term effect of γ-radiation (7.6 Gy) on PC-3 cells is negligible compared to the effect observed following a long-term exposure (Fig. 3a). Thus, such a single radiation dose may not be enough to cause significant short-term cytotoxicity. Although, PC-3 cells treated with 2.0 mM MJ showed about 44% cytotoxicity, significant (\( p < 0.05 \)) increase in cytotoxicity was observed in cells treated with both γ-radiation and 2.0 mM MJ (60%). However, at lower concentrations (0.5 and 1.0 mM) of MJ, we observed less than 1.2-fold increases in cytotoxicity when the combined treatment groups and their respective MJ treated groups were compared. As shown in Fig. 2, cells treated with 0.5 and 1.0 mM MJ resulted in 7% and 16% cytotoxicity, whereas combination with γ-radiation resulted in increase to only 8% and 20% cytotoxicity, respectively. These results suggest that lower concentrations (0.5 and 1.0 mM) of MJ may not be enough to counteract the short-term effect of anti-apoptotic mechanism in these irradiated cancer cells.

![Fig. 2. Short-term effects of methyl jasmonate and/or γ-irradiation on PC-3 cells following 24 h treatments. Cells were exposed to either single treatment with various concentrations (0.5, 1.0 or 2.0 mM) of MJ or combined treatment. Cytotoxicity was measured using dye exclusion assay method and plotted (means ± SD; n = 3) against various treatments. * represents p < 0.05. Figure is a representative of three separate experiments.](image-url)
3.2. Effect of MJ on the clonogenic survival of irradiated PC-3 cells

Next, to determine the effects of MJ on tumor cell radiosensitivity, clonogenic survival analysis was performed using human prostate cancer cell line (PC-3). Our result (Fig. 3a) shows significant ($p < 0.05$) decrease in surviving fractions of irradiated (0.081 ± 0.01) PC-3 clones compared to the untreated control (1.0). On the other hand, cells exposed to single treatments with MJ showed a dose-dependent effect on the surviving fractions of PC-3 clones (Fig. 3b). For example, the surviving fractions of PC-3 cells exposed to 0.5, 1.0 and 2.0 mM MJ were 0.113 ± 0.03, 0.081 ± 0.01 and 0.043 ± 0.01, respectively. However, combined treatment of PC-3 cells with radiation and MJ resulted in further reduction in the surviving fractions to 0.059 ± 0.04, 0.013 ± 0.02, and 0.005 ± 0.02 for 0.5, 1.0 and 2.0 mM, respectively (Fig. 3a). Thus, the specific effects of the various concentrations of MJ on radiosensitivity (as calculated by: surviving fractions of combined treatment/surviving fractions of MJ treated groups alone) were found to be 0.52, 0.16 and 0.12 for 0.5, 1.0 and 2.0 mM MJ, respectively. Furthermore, we have also observed a decrease in both the number and size of colonies in the MJ treated groups (data not shown).

3.3. Methyl jasmonate down-regulates Bcl-2 expression in irradiated human adenocarcinoma cells

Radioresistance of PC-3 cells to radiotherapy have been attributed to their ability to express anti-apoptotic proteins such as Bcl-2 [4,5]. Earlier report has shown that treatments with Bcl-2 inhibitors could increase the radio-sensitivity of PC-3[7–10,26,27]. Our results (Figs. 2 and 3) indicate increased cytotoxicity when MJ is combined with radiation treatment. Thus, we hypothesized that the observed increase in cytotoxicity may be related to changes in the levels of Bcl-2 expression. To test this hypothesis, we treated human prostate adenocarcinoma cells (PC-3) with gamma radiation and then exposed them to 2.0 mM MJ. Our result (Fig. 4) shows that expression of the anti-apoptotic protein Bcl-2 is stimulated following exposure to radiation (Fig. 4, lane 2) which may explain the observed low apoptotic cell death following radiation treatment of these cancer cells. However, treatment of these irradiated cells with 2.0 mM MJ resulted in suppression of Bcl-2 protein (Fig. 4, lane 3). Treatment of PC-3 cells with MJ alone resulted in no Bcl-2 expression (Fig. 4, lane 4). Thus, MJ treatment inhibits radiation-induced Bcl-2 protein expression in irradiated human radioresistant prostate cancer cells.

3.4. Apoptotic cell death in PC-3 cells exposed to methyl jasmonate and radiation

The mechanism(s) of cell death was analyzed via a dual-model approach using (a) Annexin V-FITC/PI dual staining, and (b) caspase-3 activity assay

3.4.1. Annexin V-FITC/PI dual staining

One of the early physiological changes in a cell undergoing apoptosis is the redistribution of the phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane [24]. The binding of PS to Annexin V conjugated to fluorescein isothiocyanate (Annexin
Section 2. Over expression of Bcl-2 protein is seen with radiation treated cells only (lane 2), whereas the signal detected at /C24 in vitro as shown in the respective quadrants (Fig. 5). Herein, Jurkat cells were used as internal control (Fig. 5A and B) and showed that 60% of cells treated with 2.0 mM MJ and 7.6 Gy γ-radiation detached from plate after 24 h, whereas groups treated with either 2.0 mM MJ or 7.6 Gy γ-radiation showed 44% and 2% cytotoxicity, respectively. These results are in agreement with a previous report by Palayoor et al. [6] which showed that only a small percentage (10–12%) of PC-3 exposed to 8 Gy radiation detached after 48 h. In the present study, the mechanism of cell death was determined using a model in which monolayer cells were exposed to MJ and/or ionizing radiation and the induction of apoptosis was investigated by Annexin binding as well as caspase-3 activity assay. As observed, the cytotoxic effect of radiation on these cancer cells was minimal at the dose and exposure time tested, resulting in little or no caspase-3 activity. However, combined treatment with 2.0 mM MJ following irradiation resulted in a 5-fold increase in caspase-3 activity compared to the

3.4.2. MJ stimulated caspase-3 activity in irradiated PC-3 cells

Caspase-3 activation is known to play a pivotal role in the apoptotic signaling cascade, ultimately resulting in internucleosomal fragmentation of DNA. Our result (Fig. 6) indicates that treatment of PC-3 cells with various concentrations (0.5, 1.0 and 2.0 mM) of MJ stimulated an increase of caspase-3 activity in a dose-dependent fashion. In addition, combined treatment of these cells with MJ and γ-radiation resulted in further increase in caspase-3 activity. However, increases in caspase-3 activity in the combined treatment groups were more pronounced at 2.0 mM MJ with about 5- and 1.5-fold increases in caspase-3 activities compared to radiation alone or MJ treated groups, respectively. In contrast, the untreated control and the radiated groups maintained a basal caspase-3 activity (Fig. 6). The effect of MJ or combined treatment on caspase-3 activity was inhibited in the presence of a caspase-3 specific inhibitor (Ac-DEVD-CHO), thus, suggesting the involvement of caspase-3 in the MJ-induced apoptosis in irradiated PC-3 cells.

4. Discussion

We reported earlier that MJ is highly cytotoxic to prostate cancer cells [28]; our data presented here demonstrate that MJ also enhances the cytotoxic effect of ionizing radiation and counteracts the anti-apoptotic mechanism induced by radiation in these cancer cells. We observed a more than additive effect with the combined treatment (MJ and γ-radiation) at 2 mM concentration of MJ, compared to single treatment. For instance, cytotoxicity as measured by the detachment of cells using DEA (Fig. 2), showed that 60% of cells treated with 2.0 mM MJ and 7.6 Gy γ-radiation detached from plate after 24 h, whereas groups treated with either 2.0 mM MJ or 7.6 Gy γ-radiation showed 44% and 2% cytotoxicity, respectively. These results are in agreement with a previous report by Palayoor et al. [6] which showed that only a small percentage (10–12%) of PC-3 exposed to 8 Gy radiation detached after 48 h. In the present study, the mechanism of cell death was determined using a model in which monolayer cells were exposed to MJ and/or ionizing radiation and the induction of apoptosis was investigated by Annexin binding as well as caspase-3 activity assay. As observed, the cytotoxic effect of radiation on these cancer cells was minimal at the dose and exposure time tested, resulting in little or no caspase-3 activity. However, combined treatment with 2.0 mM MJ following irradiation resulted in a 5-fold increase in caspase-3 activity compared to the
radiation treated group, and a 1.5-fold increase compared to MJ treated group (Fig. 5).

Earlier reports indicate that few prostate cancer cells die before the first post irradiation mitosis and that the average number of post irradiation growth divisions completed by cells that are destined to die is dose-dependent [29]. Elkind et al. [29] reported that Chinese Hamster V79 cells exposed to 10 Gy of radiation were able to complete approximately one cell division before they die. In addition, Palayoor et al. [6] showed that PC-3 cells recover from the radiation induced G2-M-phase block in 48 h following exposure to 8 Gy of gamma radiation. They also reported that following expo-

![Flow cytometric analysis of PC-3 cells stained with Annexin V-FITC/PI.](image)

Fig. 5. Flow cytometric analysis of PC-3 cells stained with Annexin V-FITC/PI. Cells were exposed for 24 h to either single (2.0 mM MJ or 7.6 Gy γ-radiation) or combined (2.0 mM MJ and 7.6 Gy γ-radiation) treatments. After treatments, both the adherent and non-adherent cells were harvested and used for the study. Jurkat cells were used to standardize the method and thus served as internal control. Untreated and treated cells were harvested and washed with ice-cold 1× PBS and stained following manufacturer’s instructions. Lower left quadrants of each point show the viable cells. The lower right and the upper right quadrants represent early apoptotic and necrotic or late apoptotic, respectively. (A) Jurkat cells; (B) Jurkat cells exposed to staurosporine (1 μg/ml); (C) untreated PC-3 cells; (D) γ-irradiated PC-3 cells; (E) PC-3 cells exposed to 2.0 mM MJ; (F) PC-3 cells treated with both MJ and γ-radiation. FL1-H and FL3-H represent Annexin V and propidium iodide, respectively.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Early apoptotic (%)</th>
<th>Necrotic + late apoptotic (%)</th>
<th>Total apoptotic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jurkat</td>
<td>Untreated</td>
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<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>3.92</td>
<td>75.28</td>
<td>79.20</td>
</tr>
<tr>
<td>PC-3</td>
<td>Control</td>
<td>7.99</td>
<td>2.08</td>
<td>10.07</td>
</tr>
<tr>
<td></td>
<td>γ-Irradiation</td>
<td>7.90</td>
<td>6.46</td>
<td>14.36</td>
</tr>
<tr>
<td></td>
<td>2 mM MJ</td>
<td>2.80</td>
<td>35.84</td>
<td>38.64</td>
</tr>
<tr>
<td></td>
<td>2 mM MJ + γ-Irradiation</td>
<td>2.06</td>
<td>77.88</td>
<td>79.94</td>
</tr>
</tbody>
</table>

Control or 7.6 Gy γ-irradiated PC-3 cells were treated for 24 h with MJ. Jurkat cells were used as internal control and were treated with staurosporine (1 μg/ml). Following treatments, control and treated cells were harvested and washed with ice-cold 1× PBS and stained as explained in Section 2. Calculated values are from Fig. 5 and Table 2 and represent two separate experiments.
sure to 8 Gy irradiation, only 10–12% of the cell population detached and approximately 5–6% was apoptotic after 48 h treatment. Our data showed 14.36% apoptotic cells (Annexin V/PI positive) following 24 h treatment with 7.6 Gy -radiation, thus, confirming earlier report of decreased apoptosis with radiation. However, the percentage of late apoptotic cells was greatly increased when irradiated cells were further exposed to MJ. Furthermore, our flow cytometry and DEA results on the quantification of cell death are comparable. Trypan blue positive cells have been shown to represent the necrotic plus late apoptotic cell populations [30]. PC-3 cells exposed to 2.0 mM MJ showed 40% trypan blue positive (necrotic + late apoptotic cells) when analyzed by DEA and 36% apoptosis (necrotic + late apoptotic populations) by flow cytometry. Immunoblotting assay indicates that MJ (at the concentration studied) is capable of counteracting the anti-apoptotic mechanism of irradiated PC-3 cells as evidenced in the suppression of anti-apoptotic protein-Bcl-2. Future studies in our laboratory will involve the analysis of cell cycle events following exposure of radioresistant prostate cancer cells to radiation and MJ treatments. This is important because previous reports have shown that the sensitivity of certain cancers is a function of the cell cycle phase. For instance, human prostate cancer cell line (DU-145) showed increased sensitivity to radiation treatment at G2 phase of the cell cycle [31]. Furthermore, the involvement of transcription factors such as NF-kB will be investigated since its activation by apoptotic stimuli such as ionizing radiation is known to offer protection against apoptosis. Thus, molecular understanding of the possible interaction between MJ and components of cell survival pathways may provide a clearer picture of the mechanism of action of this novel anticancer agent assay.

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