Curcumin improves the antitumor effect of X-ray irradiation by blocking the NF-κB pathway: an in-vitro study of lymphoma

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Curcumin, a phenolic compound from the rhizomes of *Curcuma longa*, inhibits the growth of a variety of malignant cell types including lymphoma cells. We investigated the role of curcumin in modulating the response of Burkitt's lymphoma cells to ionizing radiation (IR) *in vitro* and explored the mechanisms that mediated this effect. We treated three Burkitt's lymphoma cell lines with vehicle, curcumin, IR, and curcumin in combination with IR. Cell viability, apoptosis, and cell cycle distribution were determined to ascertain the radiosensitization effect of curcumin. Nuclear factor-kappa B (NF-κB) activation was assessed by nuclear translocation of p65. Apoptosis-related proteins were monitored by western blot assay and real-time RT-PCR. Pretreatment of curcumin sensitized lymphoma cells to IR-induced apoptosis and increased G_2/M phase arrest in the cell cycle distribution. Accordingly, the antiapoptotic Bcl-xL protein, cell cycle modulating protein CDC2, and cyclin B1 were downregulated by the curcumin treatment. IR activated NF-κB as evidenced by an increased nuclear p65 translocation and cytoplasmic IκBα expression. However, pretreatment with curcumin significantly decreased the nuclear translocation of p65 and cytoplasmic IκBα degradation. Survivin and hexokinase II, downstream effectors of NF-κB that mediate the antiapoptotic effect of NF-κB, were suppressed by the pretreatment of curcumin. These observations suggest that the activated NF-κB pathway plays a prosurvival role in Burkitt's lymphoma in response to IR. Curcumin blocks this pathway and has therapeutic potential for improving the antitumor effects of radiotherapy.

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Keywords: apoptosis, curcumin, hexokinase, lymphoma, nuclear factor-kappa B, radiation, survivin

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Introduction

Increased nuclear transcription factor nuclear factor-kappa B (NF-κB) levels or enhanced NF-κB transcriptional activity is frequently observed in human tumor cells. NF-κB is activated in a variety of malignant cell types [1] including lymphomas [2]. Previous studies have shown that human lymphoid malignant cells have significantly enhanced levels of NF-κB activity compared with their normal counterparts, suggesting that the NF-κB family plays an important role in the pathogenesis of lymphoid malignancy [3,4]. In addition to intrinsic cellular mechanisms, numerous stimuli from the environment such as IR and chemotherapeutic agents can activate NF-κB. Activation of NF-κB induces overexpression of its downstream targets such as bcl-xL, bcl-2, and X-linked inhibitor of apoptosis protein and proliferative genes such as cyclin D1, which may in turn mediate resistance to apoptosis induced by radiation [5]. NF-κB has been reported to be activated by IR and may account for the development of radioresistance [6,7]. Therefore, inhibition of this transcription factor increases the sensitivity of cancer cells to the apoptotic action of radiation exposure.

Curcumin, a phenolic compound from the rhizomes of *Curcuma longa*, is found to have an inhibitory effect on a variety of human cancer cells. A recent study established that curcumin is a potent anticancer agent because of its multiple properties, including antiproliferative, antiangiogenesis, and apoptosis-inducing activities [8]. Curcumin inhibits proliferation and induces apoptosis in a wide array of cancer cell types *in vitro*, including cells from cancers of the bladder, breast, lung, pancreas, prostate, cervix, head and neck, ovary, kidney, brain, bone marrow, and skin [9]. It has been well documented that curcumin potentiates the effects of chemotherapeutic agents [10,11] and γ-radiation *in vitro* [12]. Transient inducible NF-kB activation induced by administration of curcumin provided a prosurvival response to radiation that may account for the development of radioresistance. Especially in lymphomas, curcumin has exhibited effective antitumor activity *in vitro* and *in vivo* [13]. The use of curcumin to improve the response rate to IR and to potentiate conventional radiotherapy is a promising area for research. Although the precise molecular pathways involved in the curcumin-induced apoptosis of cancer cells are not yet fully understood, inhibition of NF-κB activation has been suggested.

In the present study, we investigated the role of curcumin in modulating the response of Burkitt's lymphoma cells to IR *in vitro*. Curcumin enhanced the antitumor effects of

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radiation in a cell assay. Curcumin and radiation synergistically induced apoptosis in lymphoma cells. This effect is mediated at least in part through the suppression of NF-κB-activation induced by X-ray IR. Curcumin also downregulated the expression of NF-κB-targeted gene products.

Methods

Cell culture and reagents

Three human lymphoma cell lines, Raji, Ramos, and Namalwa, were purchased from the American Type Culture Collection. These cell clones were cultured in RPMI 1640 medium (Sigma-Aldrich, St Louis, Missouri, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, California, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin in a humidified incubator in the presence of 95% air/5% CO_2 at 37°C. For drug or IR experiments, cells at the late exponential growth phase were centrifuged at 1200 rpm, resuspended in fresh medium, and incubated.

Curcumin (98% purity; Sigma-Aldrich) was dissolved in dimethylsulfoxide (DMSO) to produce a 100 mmol/l stock solution and stored at −20°C. 6-Amino-4-(4-phenoxyphenylethylamino) quinazoline (QNZ) was purchased from Calbiochem (Darmstadt, Hessen, Germany). Ionizing radiation was delivered in a SIEMENS Primus accelerator (Hamburg, Germany) at 6 MV at room temperature (dose rate 200 cGy/min). Control cells were not irradiated; they were removed from the incubator and were then placed at the radiation site for the same period of radiation as the other cells. Cells were pretreated with curcumin for 4 h before IR exposure, after which they were cotreated with IR and curcumin [12].

Cell viability assay

Cell viability was determined with the tetrazolium salt water-soluble tetrazolium salt assay (Roche Diagnostics GmbH, Penzberg, Germany) according to the manufacturer's instructions. The cells were seeded in 96-well plates and incubated with water-soluble tetrazolium salt solution at 37°C for 3 h. The optical density of each well was read at 430 nm using a microplate reader (Bio-Tek, Winooski, Vermont, USA). The results were expressed as percent cell viability for each concentration of curcumin with respect to the controls.

Assessment of apoptosis

After treatment of the cells by curcumin, IR, or both, the cells were harvested, washed once in cold PBS, and resuspended in 100 µl PBS. The percentage of apoptotic cells was measured by flow cytometry after staining with fluorescein-conjugated annexin V and propidium iodide as described previously [14].

Cell cycle analysis

The cell cycle distribution was determined by staining with propidium iodide as described previously [15]. Briefly, 1×10^6 cells were washed once in ice-cold PBS and treated with RNase for 1 h, and the DNA was stained with propidium iodide solution (50 µg/ml; Sigma-Aldrich). The cell cycle distribution was determined using FACSAria2 flow cytometer (Becton Dickinson, San Diego, California, USA) with Cell Quest software.

Subcellular fractionation

Raji, Ramos, and Namalwa cells were incubated with various stimuli for the indicated times, harvested, washed twice in ice-cold PBS, and lysed in 1% Triton X-100 buffer. The cells were then lysed with 10% NP-40 and incubated for 20 min on ice. The homogenate was centrifuged for 5 min at 200*g* at 4°C. The supernatant containing the cytoplasmic extracts was collected and stored frozen at −80°C. The nuclear pellet was resuspended in 25 µl ice-cold nuclear extraction buffer. After intermittent mixing for 30 min, the extract was centrifuged, and supernatants containing the nuclear extracts were obtained. The protein content was measured by the Bradford method (Bio-Rad Laboratories Ltd, Hercules, California, USA). Following analysis for protein content, 20 µg of both fractions were subjected to SDS-polyacrylamide gel electrophoresis and examined by western blotting.

Western blot analysis

Twenty micrograms of lysate protein were fractionated by size using SDS-polyacrylamide gel electrophoresis followed by electroblotting onto polyvinylidene difluoride membranes. After blocking for 1 h at room temperature, the membranes were incubated with the following primary antibodies: anti-p65, anticleaved caspase-3, anti-phospho-IκBα$^{Ser32/36}$, and anti-β-actin (Cell Signaling Technology, Massachusetts, USA); and antisurvivin, anti-Bcl-XL, anti-cyclin B1, anti-hexokinase II, and antitranscription factor II H (Santa Cruz Biotechnologies, Santa Cruz, California, USA). The antibodies were detected using a chemiluminescence detection kit (LumiGLO, Cell Signaling) according to the manufacturer's instructions.

RNA preparation and real-time RT-PCR

Total RNA was extracted from the cells using Trizol Reagent (Invitrogen) following the manufacturer's instructions. First-strand cDNA was synthesized from 2 µg of RNA in 20 µl of reaction solution using an RT-PCR kit (Invitrogen) according to the manufacturer's instructions. Quantitative analysis of survivin mRNA was performed on an ABI 7500 sequence detection system (Applied Biosystems, Foster City, California, USA) using the SYBR Green PCR Master mix (Applied Biosystems, Warrington, UK).

Statistical analysis

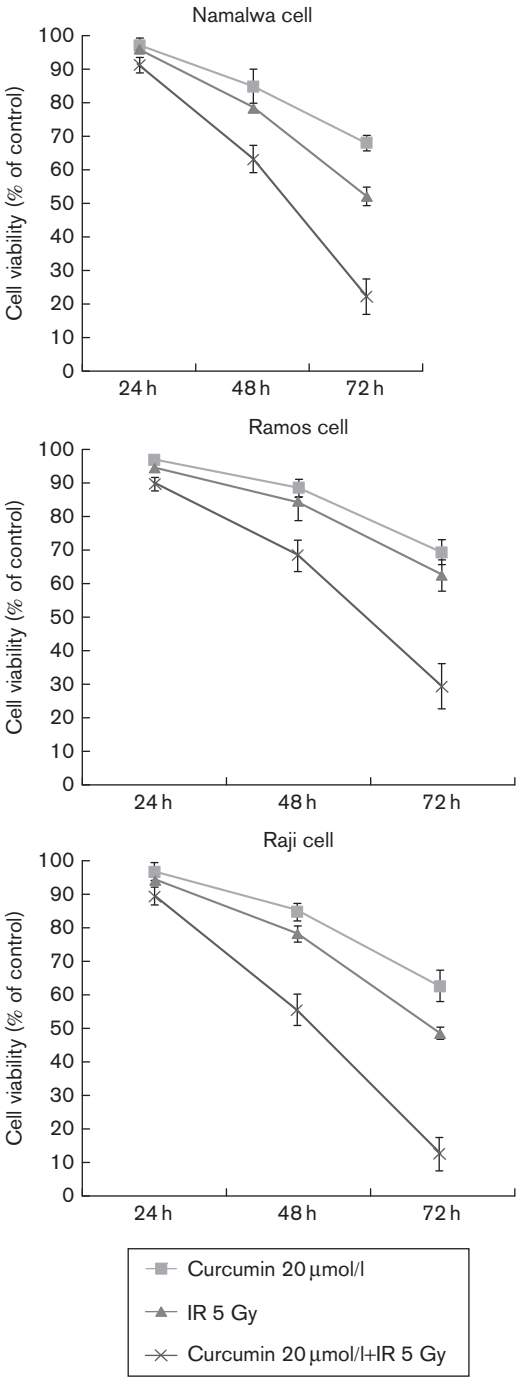
Data were presented as means ± SEM. Statistical analysis was performed by the analysis of variance method, and the level of statistical significance was set at $P < 0.05$.

Results

Curcumin enhanced the antiproliferative effect of ionizing radiation in human lymphoma cell lines

Incubation with 20 μmol/l curcumin for 72 h resulted in a decline in cell viability in all three lymphoma cell lines (68.18 ± 2.51% in Namalwa cells, 70.20 ± 5.22% in Ramos cells, and 62.28 ± 3.53% in Raji cells). Treatment with 5 Gy IR resulted in growth inhibition in a time-dependent manner, with the following percentage of cells that survived at 72 h: 52.33 ± 3.24% in Namalwa cells, 63.24 ± 4.94% in Ramos cells, and 48.22 ± 1.82% in Raji cells. Furthermore, pretreatment with curcumin followed by IR decreased cell viabilities of all the three cell lines significantly. The combination of curcumin and IR resulted in 22.28 ± 5.35% cell viability for Namalwa cells, 30.24 ± 7.02% for Ramos cells, and 12.23 ± 4.84% for Raji cells (Fig. 1).

Fig. 1



Curcumin enhanced the antiproliferative effect of ionizing radiation (IR) in human lymphoma cell lines. Lymphoma cells were treated with curcumin (20 μmol/l), IR (5 Gy), or a combination of curcumin (20 μmol/l) and IR (5 Gy). Cell viability was evaluated 72 h after treatment.

Curcumin sensitized lymphoma cells to ionizing radiation-induced apoptosis

Flow cytometric analysis of cells stained by fluorescein-conjugated annexin V and propidium iodide is a well-accepted method for the quantitative assessment of apoptosis. As shown in Fig. 2a and b, exposure of Namalwa, Ramos, and Raji cells to 20 μmol/l curcumin led to a moderate increase in apoptosis compared with the control cells ($P < 0.05$). Treatment with 5 Gy of IR resulted in the following percentages of apoptotic cells in the three cell lines, respectively: 18.37 ± 3.24, 24.07 ± 4.10, and 23.19 ± 3.83%. However, pretreatment with 20 μmol/l curcumin followed by IR resulted in remarkable increases in the number of apoptotic cells in all three cell lines than by either treatment alone ($P < 0.01$).

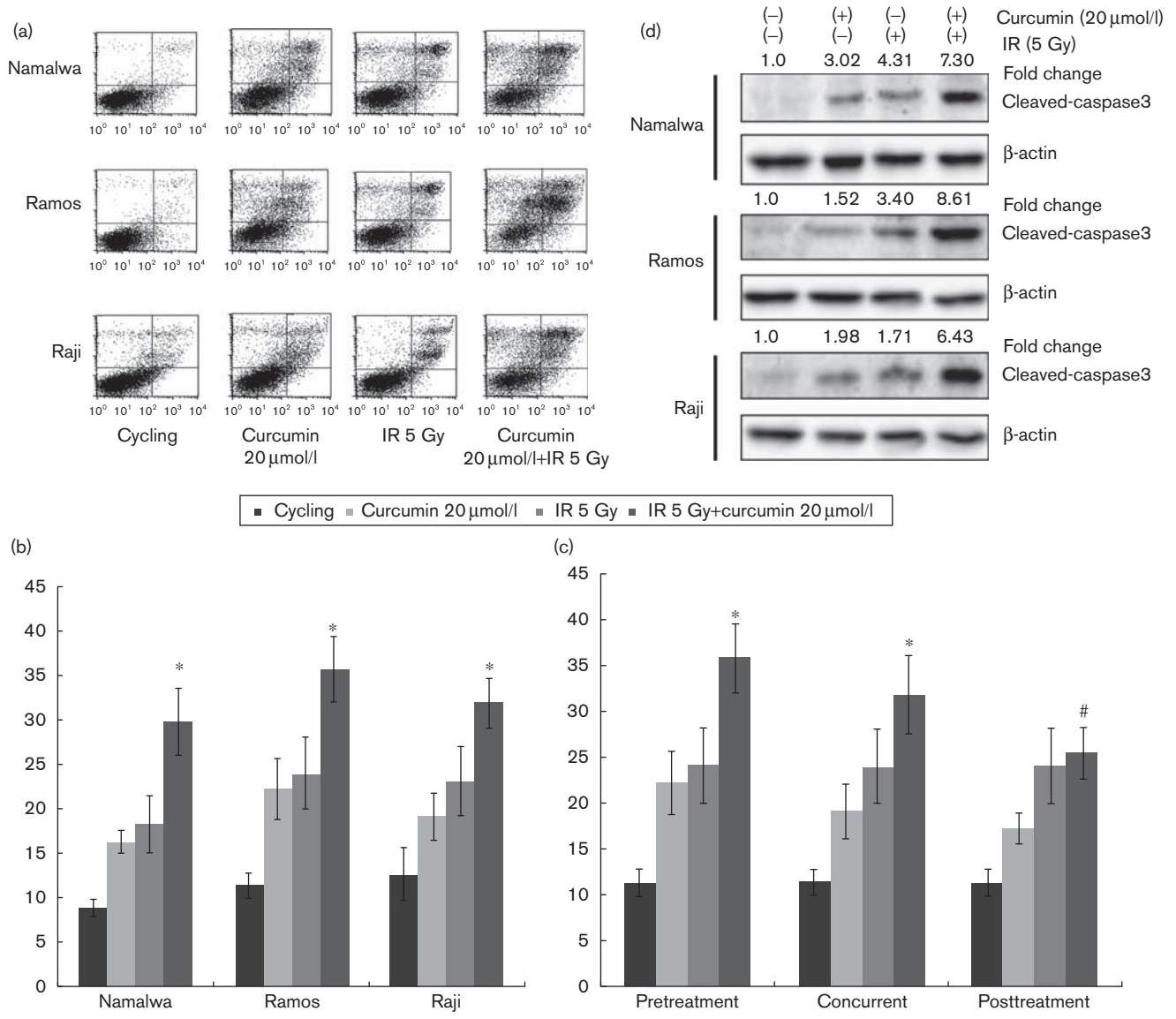
To determine the most effective combined treatment sequence, we either pretreated Ramos cells with 20 μmol/l curcumin for 4 h followed by 5 Gy radiation, concurrently treated Ramos cells with 20 μmol/l curcumin and 5 Gy radiation, or pretreated cells with radiation followed by curcumin treatment 1 h later. As shown in Fig. 2c, the sequence of curcumin followed by radiation showed the most prominent apoptosis-inducing effect (35.80%) compared with concurrent treatment (31.94%) or radiation pretreatment followed by curcumin (25.53%).

As Fig. 2d shows, cleaved caspase-3 expression increased either by incubation with 20 μmol/l curcumin for 4 h or by a single dose of 5 Gy of IR. Pretreatment of curcumin followed by IR caused an additive effect in inducing cleaved caspase-3 expression ($P < 0.01$).

Curcumin enhanced ionizing radiation-induced lymphoma cell G_2/M arrest

To investigate the molecular mechanism involved in the sensitization of IR-induced apoptosis by curcumin, we analyzed the cell cycle distribution and expression of molecules involved in the regulation of the cell cycle. As shown in Fig. 3a and b, after 72 h of IR, considerable changes in the cell cycle distribution were observed in lymphoma cells treated by curcumin, IR, or both combined. Cell arrest in G_2/M was more prominent by IR and incubation with 20 μmol/l curcumin compared with that in the control cells. Pretreatment of curcumin

Fig. 2

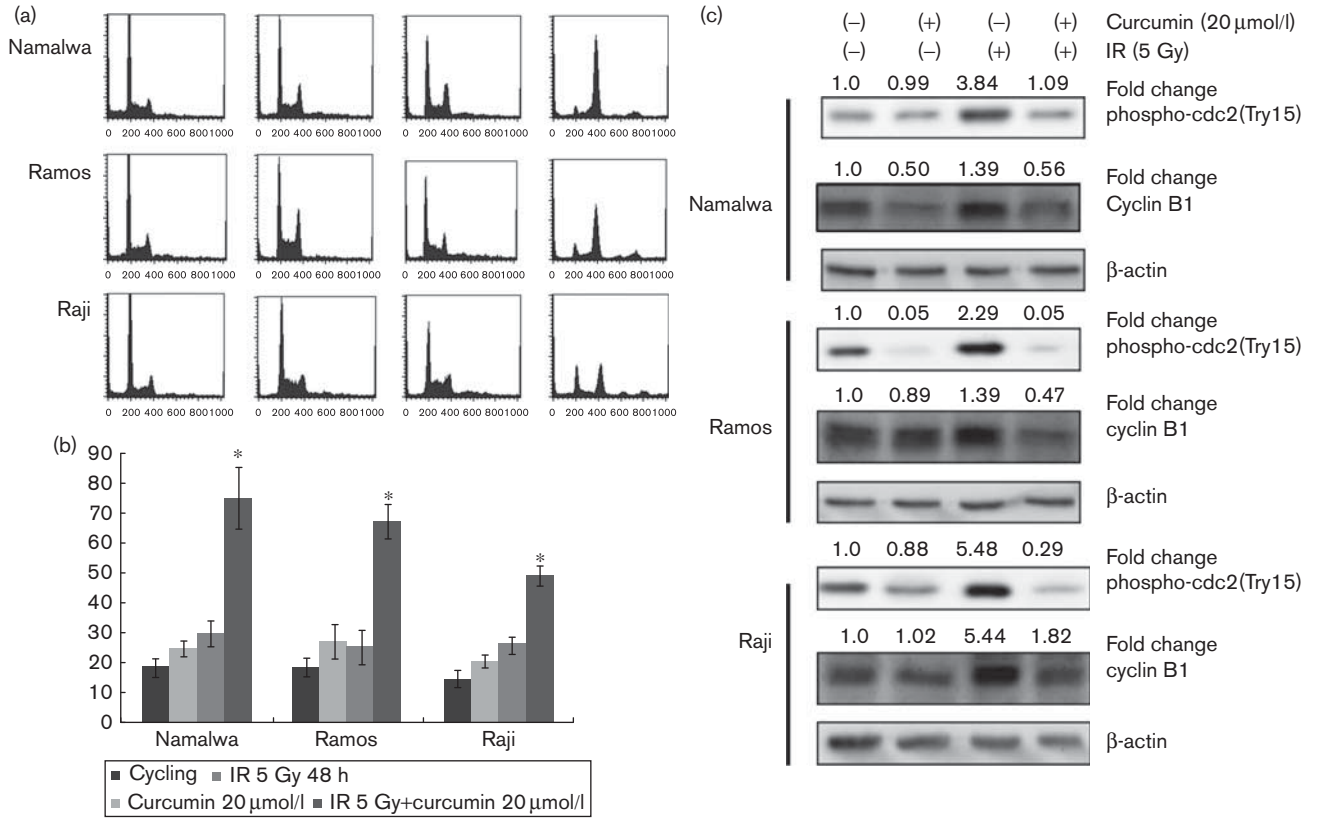


Curcumin sensitized lymphoma cells to ionizing radiation (IR)-induced apoptosis. (a) Raji, Ramos, and Namalwa cells were treated with 20 μmol/l curcumin for 4 h and were then exposed to a 5 Gy X-ray radiation. Twenty-four hours after radiation, the cells were harvested and analyzed for apoptosis by annexin V binding and propidium iodide uptake using flow cytometry. The results shown are representative of three independent experiments. (b) The percentage of apoptotic cells in each cell line was calculated. Each column represents the average and standard error of three independent experiments. Data are presented as the mean ± SE. *Significant difference between curcumin and IR alone; $P<0.01$. (c) Determination of the most effective combined treatment sequence in Ramos cells. Ramos cells were analyzed by flow cytometry to determine the most effective combined curcumin + radiation sequence. (i) Ramos cells were treated with 20 μmol/l curcumin for 4 h and then exposed to a 5 Gy X-ray radiation. (ii) Ramos cells were treated concurrently with 20 μmol/l curcumin and radiation. (iii) Ramos cells were exposed to 20 μmol/l curcumin 4 h after radiation. Each column represents the average and standard error of three independent experiments. Data are presented as the mean ± SE. *Significant difference with curcumin and IR alone; $P<0.01$. #Significant difference between curcumin and IR alone; $P<0.05$. (d) Curcumin synergistically increased the IR-induced expression of cleaved caspase-3 in lymphoma cells. Cells were treated with dimethylsulfoxide, 20 μmol/l curcumin, IR(5 Gy IR), or pretreated with 20 μmol/l curcumin for 4 h followed by a 5 Gy IR, and then harvested 24 h later for western blot analysis.

followed by IR led to a further increase in G_2/M arrest. Meanwhile, the proportion of cells in the G_0/G_1 phase was concomitantly decreased.

On the basis of these data, we determined the expression levels of phosphorylated CDC2 and cyclin B1, which are both involved in cell cycle progression, by western blot assays. Treatment of 20 μmol/l alone showed a decrease in the expression of CDC2 and cyclin B1 compared with DMSO-treated control cells. Pretreatment of 20 μmol/l curcumin for 4 h significantly suppressed the IR-induced overexpression of CDC2 and cyclin B1 in all three cell

Fig. 3



Curcumin enhanced ionizing radiation (IR)-induced lymphoma cell G_2/M arrest. (a) Raji, Ramos, and Namalwa cells were treated with 20 μmol/l curcumin for 4 h and then exposed to an X-ray radiation of 5 Gy. Twenty-four hours after radiation, cells were harvested and analyzed for the cell cycle distribution by propidium iodide staining using flow cytometry. (b) The proportion of G_2/M phase in each cell line is shown. Each column represents the average and standard error of three independent experiments. Data are presented as the mean ± SE. *Significant difference between control, curcumin, and IR alone; $P<0.001$. (c) Curcumin enhanced G_2/M cell cycle arrest through suppressing IR-induced upregulation of CDC2 and cyclin B1 expression. Cells were treated with dimethylsulfoxide, 20 μmol/l curcumin or 5 Gy IR or were pretreated with 20 μmol/l curcumin for 4 h followed by 5 Gy IR and then harvested 48 h later for western blot analysis.

lines (Fig. 3c). These results provided evidence for the downregulation of CDC2 and cyclin B1 in the curcumin-mediated enhancement of the G_2/M cell cycle arrest.

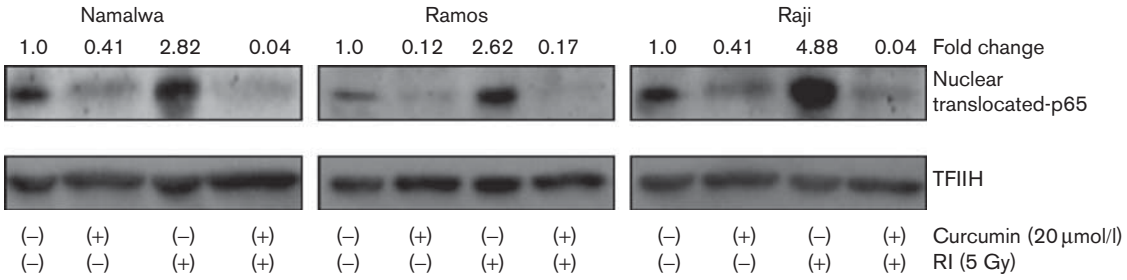
Curcumin inhibited ionizing radiation-induced nuclear translocation of NF-κB

As p65 is the primary subunit of the NF-κB dimer that translocates into and accumulates in the nucleus when NF-κB is activated, we examined the expression level of p65 in the nuclear fraction as an index for NF-κB activation. We previously observed that nuclear translocation of p65 significantly increased in cells harvested 1 h after IR [16]. Here, we examined the role of NF-κB in curcumin-mediated sensitization to apoptosis in Ramos, Raji, and Namalwa cells. Cells treated with DMSO or 20 μmol/l curcumin for 4 h were exposed to single doses of radiation (5 Gy) and harvested at 4 h after radiation for assessing NF-κB activation. As mentioned above, IR induced increases in nuclear p65 translocation; however, treatment with curcumin effectively reduced the IR-induced nuclear translocation of p65. These results suggest that curcumin inhibited the IR-induced activation of NF-κB (Fig. 4).

Curcumin inhibited IκBα protein degradation induced by ionizing radiation

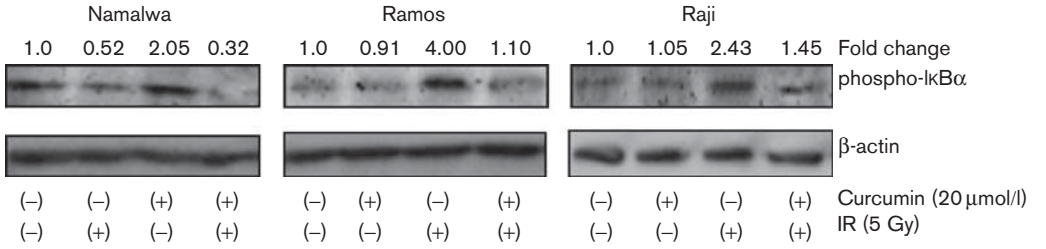
In the canonical pathway, NF-κB activation is mediated by the phosphorylation of IκBα, which in turn leads to the release of cytoplasmic NF-κB for nuclear translocation, accumulation, and DNA binding. To investigate the mechanism of IR-induced NF-κB nuclear translocation and the effect of curcumin along this pathway, western blot analysis was used to examine phosphorylated IκBα protein expression in the cytoplasm either after IR exposure and curcumin treatment, or both. Although phosphorylated IκBα proteins were upregulated by IR exposure (5 Gy), treatment of the lymphoma cells with 20 μmol/l of curcumin decreased this effect. However, pretreatment of cells with 20 μmol/l of curcumin significantly inhibited the IR-induced increase in the expression of phosphorylated IκBα protein (Fig. 5).

Fig. 4



Curcumin inhibited ionizing radiation (IR)-induced nuclear translocation of nuclear factor-kappa B (NF-κB). Lymphoma cells treated with dimethylsulfoxide or 20 μmol/l curcumin for 4 h were exposed to single doses of radiation (5 Gy) and harvested for assessing NF-κB activation at 4 h after radiation, when the increased expression of nuclear NF-κB was most prominent. Suppression of radiation-induced NF-κB activity was noted in all of the cell lines. The results shown are representative of three independent experiments.

Fig. 5



Curcumin inhibited IκBα protein degradation induced by ionizing radiation (IR). Raji, Ramos, and Namalwa cells were treated with dimethylsulfoxide or 20 μmol/l curcumin for 4 h, exposed to single doses of radiation (5 Gy) and harvested for assessing the IκBα expression level 1 h after radiation. Inhibition of IκBα protein degradation induced by IR was observed in all three cell lines.

Curcumin suppressed the expression of downstream targets enhanced by ionizing radiation

Finally, we determined the downstream targets of NF-κB that are involved in protecting cells from apoptosis. To this end, we first treated Burkitt's lymphoma cells with the NF-κB inhibitor QNZ for 24 h and examined the expression of survivin and hexokinase II by western blot analysis. The results showed that QNZ significantly suppressed the expression of survivin and hexokinase II, indicating that these two molecules are downstream targets of NF-κB (Fig. 6a). Next, the lymphoma cells were treated with curcumin, IR, or both. By real-time RT-PCR and western blot analysis, curcumin alone suppressed constitutive survivin and hexokinase II expression levels in all three lymphoma cells, whereas IR exposure statistically increased the expression levels of survivin and hexokinase II compared with the untreated controls. Furthermore, curcumin pretreatment significantly suppressed IR-induced survivin and hexokinase II expression (Fig. 6b and c). Taken together, it is reasonable to deduce that the curcumin sensitization of lymphoma cells to IR-induced apoptosis is at least partly mediated through the inhibition of the NF-κB–survivin and the NF-κB–hexokinase II pathways.

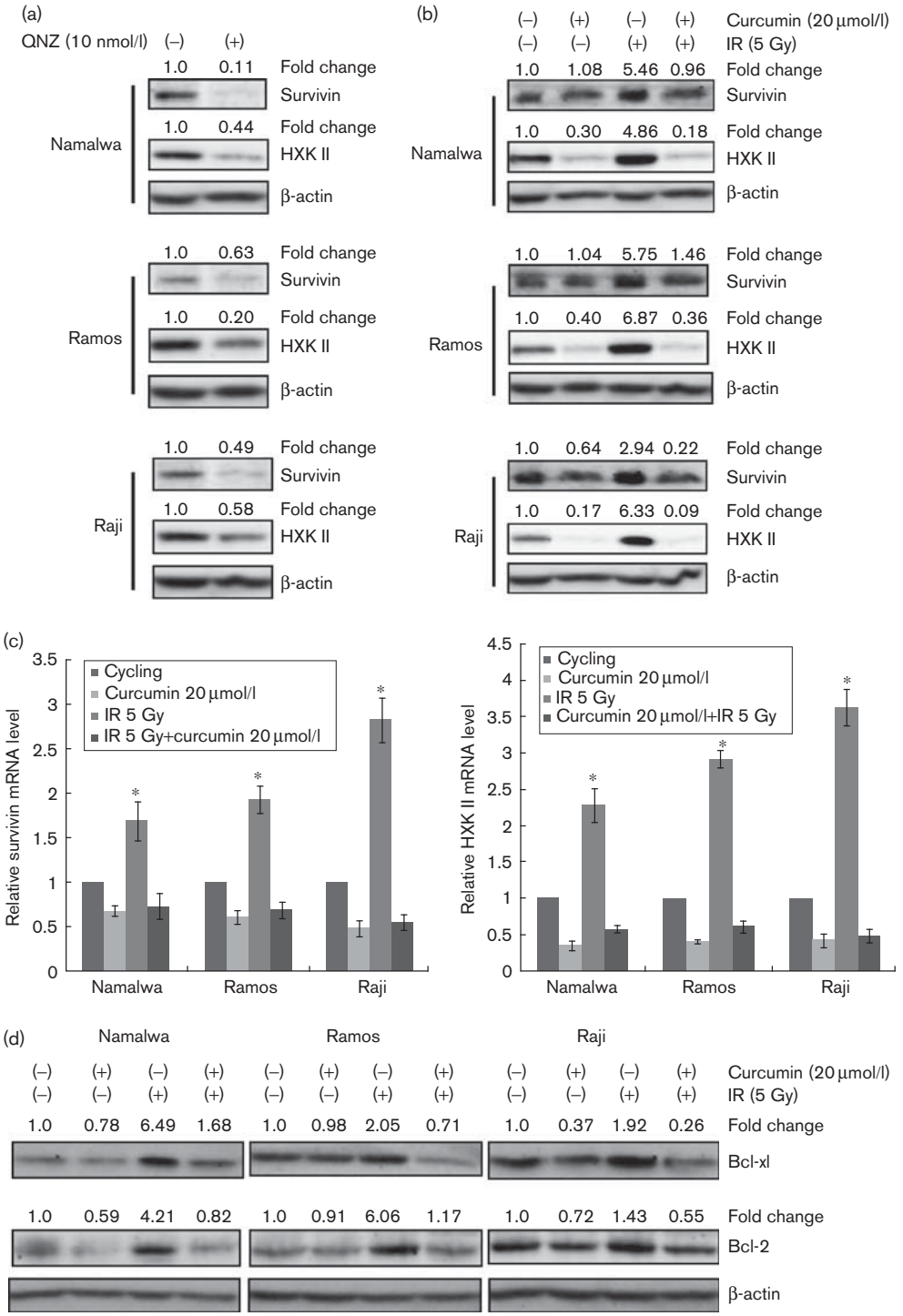
An immunoblot analysis showed that radiation induced expression of the antiapoptotic proteins Bcl-xL and Bcl-2 and that curcumin suppressed the expression of these proteins (Fig. 6d).

Discussion

The primary purpose of this study was to determine whether curcumin could sensitize lymphoma cells to IR-induced apoptosis and to explore the possible mechanism of this action. We demonstrated that pretreatment of curcumin could sensitize lymphoma cells to IR-induced apoptosis. Pretreatment with curcumin enhanced IR-induced lymphoma cell arrest in the G_2/M phase. The radiosensitization effect of pretreatment with curcumin was mediated by the suppression of radiation-induced NF-κB activation. Curcumin suppressed the expression of NF-κB-regulated antiapoptotic proteins including Bcl-2, Bcl-xL, survivin, and hexokinase II. It is reasonable to suggest that inhibition of NF-κB may be involved in the process of curcumin-induced radiosensitization.

In a variety of tumor cells, curcumin has been reported to have antineoplastic activity. Various mechanisms, including the inhibition of proliferation, invasion, angiogenesis,

Fig. 6



Curcumin suppressed the expression of downstream targets of nuclear factor-kappa B enhanced by ionizing radiation (IR). (a) Lymphoma cells were cultured with 10 nmol/l QNZ. Survivin and hexokinase II protein expressions were analyzed by western blot analysis 24 h later. The results shown are representative of three independent experiments. (b) Each lymphoma cell line was pretreated with 20 μmol/l curcumin and then treated with 5 Gy of radiation. Forty-eight hours after irradiation, whole cell extracts were prepared, and survivin and hexokinase II levels were analyzed by western blotting. Bands were quantified and normalized to a loading control. Data are representative of three independent experiments. (c) Each lymphoma cell line was pretreated with 20 μmol/l curcumin and then treated with 5 Gy of radiation. Twenty-four hours after irradiation, total RNA was prepared. Survivin and hexokinase II mRNA expressions were analyzed by quantitative RT-PCR. Results were normalized to an internal control. Each column represents the average and standard error of three independent experiments. *$P < 0.001$. (d) Each lymphoma cell line was pretreated with 20 μmol/l curcumin and then treated with 5 Gy of radiation. Forty-eight hours after radiation, whole cell extracts were prepared, and Bcl-XL and Bcl-2 protein expressions were analyzed by western blotting. Bands were quantified and normalized to a loading control. Data are representative of three independent experiments. HXK, hexokinase; QNZ, 6-amino-4-(4-phenoxyphenylethylamino) quinazoline.

and induction of apoptosis and cell cycle arrest have been proposed to explain this activity [8]. As reported previously, curcumin possesses potent proapoptotic properties and is known to have induced apoptosis in numerous cell types, including those of hematopoietic origin [17], but not in normal cells [18]. Further studies revealed that curcumin acted as a radio-sensitizer, potentiated the effect of gamma radiation on and potentially prevented radioresistance to radiation [19]. Consistent with our study, Khafif *et al.* [19] reported that although low concentrations of curcumin showed no inhibitory effect on clonogenic survival of squamous cell carcinoma cells, pretreatment with the same concentrations of curcumin significantly decreased the ability of cancer cells to form colonies compared with IR alone. Similarly, in a series of colorectal cancer cell lines, curcumin significantly enhanced the intrinsic tumor radiosensitivity through the inhibition of postradiation clonogenic survival [12]. When curcumin was administered to tumor-bearing nude mice, it enhanced the antitumor effects of radiation [20].

Previous studies have shown that treatment with curcumin alone caused a cycle arrest in the G_2/M phase [21]. Cells respond to IR by activating cycle checkpoints. It is well known that cells in the G_2/M phase of the cell cycle are more sensitive to radiation than cells in other phases of the cell cycle [22,23]. In the present study, cell cycle analysis showed that either curcumin or radiation alone led to an increase in the percentage of cells in G_2/M and a concomitant decrease in the percentage of cells in G_0/G_1. In addition, pretreatment with curcumin followed by radiation induced a more significant G_2/M cell cycle arrest compared with each modality alone. Taken together, pretreatment of curcumin-induced G_2/M arrest in lymphoma cells and thus increased their radiosensitivity, resulting in increased cell apoptosis in addition to the direct cytotoxicity of curcumin and IR.

Antiapoptotic signaling pathways that prevent radiation-induced apoptosis may be involved in radioresistance that impairs the efficacy of radiotherapy markedly. Thus, it is of considerable interest to identify the NF-κB pathway that mediates the inducible radioresistance in lymphoma cells. The NF-κB pathway plays a vital role in physiological lymphocyte proliferation and survival [24] and promotes malignant behavior by suppressing apoptosis and stimulating the transcription of proteins involved in cell cycle progression [25]. It has been reported that aberrant activation of NF-κB is widely found in lymphoid malignancies [24]. As presented in the current study, p65, a subunit of the NF-κB dimer, was constitutively expressed in Burkitt's lymphoma cells.

IR is known to induce NF-κB activation in numerous cell types including B-cell lymphomas. Moreover, induction of NF-κB activity has been suggested to confer radioresistance [6]. In both in-vitro and in-vivo models, IR has been reported to activate NF-κB, which attenuates radiation-induced apoptosis [7,26]. It is known that NF-κB mediates an adaptive resistance to ionizing radiation in B-cell malignancies [27]. We found that the nuclear translocation of nuclear p65 in Burkitt's lymphoma cells increased significantly after irradiation by 5 Gy X-rays. Meanwhile, the cytoplasmic level of the primary inhibitor of NF-κB, IκBα, decreased markedly as a result of phosphorylation and degradation by IR. Therefore, blocking NF-κB may be a potential novel adjuvant treatment for cancer in combination with radiotherapy, especially for lymphomas, in which NF-κB activity blocks apoptotic pathways that are engaged by and are required for radiotherapy. Multiple strategies have been explored to inhibit NF-κB activation and have shown promising activity *in vivo* and *in vitro*. Curcumin, a plant polyphenol that can protect against therapy-associated toxicity, has also been reported to sensitize human neuroblastoma and colorectal cancer cells to the apoptotic effect of IR through the inhibition of NF-κB [12,28]. By suppression of the constitutive and inducible NF-κB activity, curcumin modulated the radiosensitivity of colorectal cancer cells [12]. Our study is the first to show that curcumin sensitized Burkitt's lymphoma cells to IR-induced apoptosis and that the mechanism may be mediated by suppression of the IR-induced NF-κB activation.

Survivin, a member of the inhibitor of apoptosis protein family of proteins, is frequently overexpressed in various malignant cells, including lymphoma cells. Overexpression of survivin has been suggested to inversely relate to radiosensitivity [29] and was correlated with a poor prognosis [30]. In the current study, we investigated the molecular mechanisms that induce overexpression of survivin in response to IR in malignant lymphoma cells. We confirmed that survivin is one of the target genes of NF-κB and demonstrated that curcumin effectively suppressed survivin expression induced by IR at the mRNA and the translational levels. Taken together, we concluded that curcumin sensitized Burkitt's lymphoma cells to IR-induced apoptosis at least partially through inhibition of the NF-κB-survivin pathway.

Finally, we investigated whether the radiosensitization effect of curcumin was mediated through hexokinase II suppression in human lymphoma cell lines. Hexokinase II is a key enzyme that catalyzes the first step in the glycolysis pathway required for maintaining cell survival. Increasing evidence suggests that hexokinase II is overexpressed in a variety of malignant tumors and plays a role in the suppression of mitochondria-initiated apoptotic cell death, providing the cell a growth advantage [31,32]. Ahn *et al.* [33] reported that hepatocellular carcinoma cells with overexpression of hexokinase II showed 1.5-fold to two-fold higher cell survival and resistance to an anticancer agent compared with a nontransfected cell

line. Fortunately, treatment with a hexokinase II inhibitor suppressed malignant carcinoma cell growth significantly by inducing cell apoptosis [31,32]. As in our study, hexokinase II was constitutively expressed in lymphoma cells and suppressed by treatment with the NF-κB inhibitor QNZ, indicating that hexokinase II is a downstream target of NF-κB. We further showed that in addition to survivin suppression, pretreatment with curcumin also suppressed IR-induced hexokinase II expression significantly. Thus, it is reasonable to conclude that the radiosensitization effect of curcumin on lymphoma cell apoptosis is mediated through the suppression of survivin and hexokinase II expression.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

References

1 Aggarwal BB. Nuclear factor-kappaB: the enemy within. *Cancer Cell* 2004; **6**:203–208.
2 Nagy ZS, LeBaron MJ, Ross JA, Mitra A, Rui H, Kirken RA. STAT5 regulation of BCL10 parallels constitutive NFkappaB activation in lymphoid tumor cells. *Mol Cancer* 2009; **8**:67.
3 Kimura N, Miyakawa Y, Kohmura K, Umezawa K, Ikeda Y, Kizaki M. Targeting NF-kappaB and induction of apoptosis by novel NF-kappaB inhibitor dehydroxymethylepoxyquinomicin (DHMEQ) in Burkitt lymphoma cells. *Leuk Res* 2007; **31**:1529–1535.
4 Pavan A, Spina M, Canzonieri V, Sansonno S, Toffoli G, De Re V. Recent prognostic factors in diffuse large B-cell lymphoma indicate NF-kappaB pathway as a target for new therapeutic strategies. *Leuk Lymphoma* 2008; **49**:2048–2058.
5 Baud V, Karin M. Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls. *Nat Rev Drug Discov* 2009; **8**:33–40.
6 Cao N, Li S, Wang Z, Ahmed KM, Degnan ME, Fan M, *et al*. NF-kappaB-mediated HER2 overexpression in radiation-adaptive resistance. *Radiat Res* 2009; **171**:9–21.
7 Veuger SJ, Hunter JE, Durkacz BW. Ionizing radiation-induced NF-kappaB activation requires PARP-1 function to confer radioresistance. *Oncogene* 2009; **28**:832–842.
8 Kunnumakkara AB, Anand P, Aggarwal BB. Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. *Cancer Lett* 2008; **269**:199–225.
9 Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res* 2003; **23**:363–398.
10 Kamat AM, Sethi G, Aggarwal BB. Curcumin potentiates the apoptotic effects of chemotherapeutic agents and cytokines through down-regulation of nuclear factor-kappaB and nuclear factor-kappaB-regulated gene products in IFN-alpha-sensitive and IFN-alpha-resistant human bladder cancer cells. *Mol Cancer Ther* 2007; **6**:1022–1030.
11 Kunnumakkara AB, Guha S, Krishnan S, Diagaradjane P, Gelovani J, Aggarwal BB. Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-kappaB-regulated gene products. *Cancer Res* 2007; **67**:3853–3861.
12 Sandur SK, Deorukhkar A, Pandey MK, Pabon AM, Shentu S, Guha S, *et al*. Curcumin modulates the radiosensitivity of colorectal cancer cells by suppressing constitutive and inducible NF-kappaB activity. *Int J Radiat Oncol Biol Phys* 2009; **75**:534–542.
13 Li ZX, Ouyang KQ, Jiang X, Wang D, Hu Y. Curcumin induces apoptosis and inhibits growth of human Burkitt's lymphoma in xenograft mouse model. *Mol Cells* 2009; **27**:283–289.
14 Uddin S, Hussain AR, Al-Hussein KA, Manogaran PS, Wickrema A, Gutierrez MI, *et al*. Inhibition of phosphatidylinositol 3′-kinase/AKT signaling promotes apoptosis of primary effusion lymphoma cells. *Clin Cancer Res* 2005; **11**:3102–3108.
15 Kumar R, Atlas I. Interferon alpha induces the expression of retinoblastoma gene product in human Burkitt lymphoma Daudi cells: role in growth regulation. *Proc Natl Acad Sci USA* 1992; **89**:6599–6603.
16 Qiao Q, Nozaki Y, Sakoe K, Komatsu N, Kirito K. NF-kappaB mediates aberrant activation of HIF-1 in malignant lymphoma. *Exp Hematol* 2010; **38**:1199–1208.
17 Hussain AR, Ahmed M, Al-Jomah NA, Khan AS, Manogaran P, Sultana M, *et al*. Curcumin suppresses constitutive activation of nuclear factor-kappa B and requires functional Bax to induce apoptosis in Burkitt's lymphoma cell lines. *Mol Cancer Ther* 2008; **7**:3318–3329.
18 Syng-Ai C, Kumari AL, Khar A. Effect of curcumin on normal and tumor cells: role of glutathione and bcl-2. *Mol Cancer Ther* 2004; **3**:1101–1108.
19 Khafif A, Hurst R, Kyker K, Fliss DM, Gil Z, Medina JE. Curcumin: a new radio-sensitizer of squamous cell carcinoma cells. *Otolaryngol Head Neck Surg* 2005; **132**:317–321.
20 Li M, Zhang Z, Hill DL, Wang H, Zhang R. Curcumin, a dietary component, has anticancer, chemosensitization, and radiosensitization effects by down-regulating the MDM2 oncogene through the PI3K/mTOR/ETS2 pathway. *Cancer Res* 2007; **67**:1988–1996.
21 Tian B, Wang Z, Zhao Y, Wang D, Li Y, Ma L, *et al*. Effects of curcumin on bladder cancer cells and development of urothelial tumors in a rat bladder carcinogenesis model. *Cancer Lett* 2008; **264**:299–308.
22 Geldof AA, Plaizier MA, Duivenvoorden I, Ringelberg M, Versteegh RT, Newling DW, *et al*. Cell cycle perturbations and radiosensitization effects in a human prostate cancer cell line. *J Cancer Res Clin Oncol* 2003; **129**:175–182.
23 Pawlik TM, Keyomarsi K. Role of cell cycle in mediating sensitivity to radiotherapy. *Int J Radiat Oncol Biol Phys* 2004; **59**:928–942.
24 Jost PJ, Ruland J. Aberrant NF-kappaB signaling in lymphoma: mechanisms, consequences, and therapeutic implications. *Blood* 2007; **109**:2700–2707.
25 Raffoul JJ, Wang Y, Kucuk O, Forman JD, Sarkar FH, Hillman GG. Genistein inhibits radiation-induced activation of NF-kappaB in prostate cancer cells promoting apoptosis and G2/M cell cycle arrest. *BMC Cancer* 2006; **6**:107.
26 Meng A, Yu T, Chen G, Brown SA, Wang Y, Thompson JS, *et al*. Cellular origin of ionizing radiation-induced NF-kappaB activation *in vivo* and role of NF-kappaB in ionizing radiation-induced lymphocyte apoptosis. *Int J Radiat Biol* 2003; **79**:849–861.
27 Weston VJ, Austen B, Wei W, Marston E, Alvi A, Lawson S, *et al*. Apoptotic resistance to ionizing radiation in pediatric B-precursor acute lymphoblastic leukemia frequently involves increased NF-kappaB survival pathway signaling. *Blood* 2004; **104**:1465–1473.
28 Aravindan N, Madhusoodhanan R, Ahmad S, Johnson D, Herman TS. Curcumin inhibits NFkappaB mediated radioprotection and modulate apoptosis related genes in human neuroblastoma cells. *Cancer Biol Ther* 2008; **7**:569–576.
29 Asanuma K, Moriai R, Yajima T, Yagihashi A, Yamada M, Kobayashi D, *et al*. Survivin as a radioresistance factor in pancreatic cancer. *Jpn J Cancer Res* 2000; **91**:1204–1209.
30 Schlette EJ, Medeiros LJ, Goy A, Lai R, Rassidakis GZ. Survivin expression predicts poorer prognosis in anaplastic large-cell lymphoma. *J Clin Oncol* 2004; **22**:1682–1688.
31 Chen Z, Zhang H, Lu W, Huang P. Role of mitochondria-associated hexokinase II in cancer cell death induced by 3-bromopyruvate. *Biochim Biophys Acta* 2009; **1787**:553–560.
32 Gwak GY, Yoon JH, Kim KM, Lee HS, Chung JW, Gores GJ. Hypoxia stimulates proliferation of human hepatoma cells through the induction of hexokinase II expression. *J Hepatol* 2005; **42**:358–364.
33 Ahn KJ, Hwang HS, Park JH, Bang SH, Kang WJ, Yun M, *et al*. Evaluation of the role of hexokinase type II in cellular proliferation and apoptosis using human hepatocellular carcinoma cell lines. *J Nucl Med* 2009; **50**:1525–1532.