Pretreatment with Rituximab Enhances Radiosensitivity of Non-Hodgkin’s Lymphoma Cells

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The present study examines the effects of ionizing radiation in combination with rituximab (RTX), a chimeric human anti-CD20 monoclonal antibody, on proliferation, cell cycle distribution and apoptosis in B-lymphoma RL and Raji cells. Exposure to ionizing radiation (9 Gy) induced cell growth delay and apoptosis in RL cells, whereas Raji cells showed moderate radio-resistance. The simultaneous exposure of lymphoma cells to ionizing radiation and RTX (10 μg/mL) markedly enhanced apoptosis and cell growth delay in RL and Raji cells. Cooperative antiproliferative and apoptotic effects of RTX and radiation were achieved through the inhibition of c-myc and bcl-XL expression. Furthermore, RTX-modulated expression of cell cycle regulating proteins, such as p53, p21/WAF1, p27/KIP1, contributed to the development of radiation-induced cell killing and growth arrest. Each NHL cell line that underwent apoptosis induced by combination treatment revealed enhanced caspase-3 and poly (ADP-ribose) polymerase (PARP) cleavage as compared to only irradiated cells. These findings show that rituximab synergistically enhances radiation-induced apoptosis and cell growth delay through the expression of proteins involved in the programmed cell death and cell cycle regulation pathways.

INTRODUCTION

Non-Hodgkin’s lymphoma (NHL) represents heterogeneous lymphoproliferative malignancies with differing patterns of behavior and therapy response.1) Traditionally, radiation therapy (RT) plays an important role in the management of NHL. RT alone may be used as curative treatment for stages I and II in patients with indolent NHL. For more extensive and aggressive histologies RT is used in combination with chemotherapeutic substances. While indolent and aggressive NHLs are responsive to RT and chemotherapy, 50%–70% of patients relapse.2,3) As a result, there is a need for novel therapeutic strategies that may improve the outcome of NHL patients.

Various surface antigens expressed on the malignant B cells, such as CD5, CD10, CD19, CD20, CD52, and HLA-DR, are suggested as attractive candidates to be targets for NHL treatment.4,5) Rituximab (RTX) is a genetically engineered chimeric murine-human monoclonal antibody (mAb) to the CD20 antigen considered as pan-B cell marker and has demonstrated antitumor activity in various types of NHL.6,7) The efficacy of RTX appears to involve a number of mechanisms, such as inhibition of cell cycle progression and induction of apoptosis.8,9) Furthermore, binding of RTX to CD20 may induce immune effector mechanisms such as antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity.10,11) Recent clinical and in vitro data have shown that RTX synergizes with chemotherapy by sensitizing lymphoma cells to cytotoxic effects of chemotherapeutic compounds such as fludarabine, doxorubicin, cisplatin, glucocorticoids or retinoids.12–17) Despite such a large body of evidence concerning chemosensitization by RTX, no data are available on the mechanisms describing how RTX in combination with ionizing radiation acts on NHL cells. To demonstrate that RTX changes the cytotoxic potential of ionizing radiation, we irradiated primarily RTX-treated human B cell lines RL and Raji. We here show that ionizing radiation and RTX treatment cause synergistic growth inhibition, apoptosis and different protein expressions related to cytotoxic effects in radio-sensitive RL and radio-resistant Raji cells.
MATERIALS AND METHODS

Cell culture
CD20-positive follicular lymphoma cells (RL) carrying the t(14;18) and CD20-positive Burkitt EBV-infected lymphoma cells (Raji) were obtained from ATCC (Rockville, MD, USA) and cultured in RPMI 1640 medium (Gibco BRL, Grand Island, NY) at 37°C in 5% CO2. Culture medium was supplemented with heat-inactivated fetal calf serum (10%), antibiotics (100 U/mL penicillin/streptomycin) and 2 mM L-glutamine (all from Sigma-Aldrich, Vienna, Austria).

Chemicals and ionizing radiation treatment

RTX (MabThera®), a murine-human chimeric anti-CD20 IgG1 class mAb (provided by Roche Austria, Vienna, Austria), was used at a concentration of 10 µg/mL 24 hours prior to irradiation. Cells were irradiated at single doses of either 2 Gy or 9 Gy (16 MV x-rays) using an Elekta Precise Linear Accelerator (Elekta Oncology Systems, UK) at a dose rate of approximately 1.8 Gy/min. Irradiated cells were incubated at 37°C for 72 hours with and without RTX.

Cell viability assay

Cell viability was evaluated with the WST-1 Assay (Roche Diagnostics GmbH, Penzberg, Germany) according to the manufacturer’s instructions. WST-1 solution was added to the cells in 96-well plates, the cells were incubated at 37°C for 3 hours, and the optical density of each well was read at 430 nm using a microplate reader (SPECTRAFluor Plus, Tecan, Austria).

Apoptosis detection and cell cycle analysis using flow cytometry

To evaluate the induction of apoptosis, samples of cells were taken at relevant time points, resuspended in hypotonic fluorochrome solution (50 µg/mL propidium iodide (PI), 0.1% (w/v) sodium citrate, 0.1% (v/v) Triton X-100), stored in the dark for 30 minutes and kept at 4°C until FACS analysis. To determine DNA fragmentation PI fluorescence of individual nuclei was performed using flow cytometry (FACScan; Becton Dickinson, San Jose, USA) with an excitation wavelength of 488 nm and an emission wavelength of 670 nm. Results were shown as DNA histograms using CellQuest software (Becton Dickinson, San Jose, USA). The percentage of apoptotic cells was calculated by gating the sub-G1 region on the DNA content histogram. Distribution of cells in the G0 – G1, S, G2 – M phases of the cell cycle was determined using ModFit LT 3.0 (Verity Software House, Inc., ME, USA).

Western blot analysis

For evaluation of protein expression by Western blotting, cells were lysed for 15 minutes at 4°C in RIPA lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 µg/mL aprotinin, 1% NP40, 0.5% deoxycholic acid sodium salt, 0.1% sodium dodecyl sulfate [SDS] and sonified. After centrifugation at 12 000 × g for 10 minutes at 4°C, supernatant was collected and protein concentrations were determined using a commercial protein assay (BioRad Laboratories, Hercules, CA, USA); 40 µg of protein per lane was separated by 12% SDS-polyacrylamide gel electrophoresis and electrophobtacted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Protein loading was controlled by Ponceau red staining of membranes. After blocking for one hour in Tris-buffered saline (TBS) supplemented with 5% nonfat milk and 0.1% Tween 20 (Sigma-Aldrich Vienna, Austria) membranes were incubated for one hour at room temperature with antibody specific for either p21/WAF1, p53, p27/KIP1, p21/WAF1, bcl-2, bax, Dialkylaminomethane (Acrylamide)/ Bis-acrylamide, 25% (v/v), 0.1% (w/v) sodium citrate, 0.1% (v/v) Triton X-100), stored in the dark for 30 minutes and kept at 4°C until FACS analysis. To determine DNA fragmentation PI fluorescence of individual nuclei was performed using flow cytometry (FACScan; Becton Dickinson, San Jose, USA) with an excitation wavelength of 488 nm and an emission wavelength of 670 nm. Results were shown as DNA histograms using CellQuest software (Becton Dickinson, San Jose, USA). The percentage of apoptotic cells was calculated by gating the sub-G1 region on the DNA content histogram. Distribution of cells in the G0 – G1, S, G2 – M phases of the cell cycle was determined using ModFit LT 3.0 (Verity Software House, Inc., ME, USA).

Statistical analysis

All experiments were repeated at least 3 times. Results were expressed as the mean ± standard deviation of the mean. All laboratory data were evaluated according to standard statistical methods and using commercially available computer programs such as Microsoft Excel 2000 (Microsoft Inc.) and SigmaPlot 8.0 (SPSS Inc., Chicago, IL, USA). Statistical differences were studied using the Student t-test. In all tests, p values less than 0.05 were considered statistically significant.

RESULTS

RTX enhances antiproliferative effect of ionizing radiation in human NHL cell lines RL and Raji

Survival of RL and Raji lymphoma cells was evaluated after treatment with RTX at a concentration of 10 µg/mL, ionizing radiation at single doses of either 2 Gy or 9 Gy and the combination of RTX (10 µg/mL) and irradiation (2 and 9 Gy) (Figs. 1A and 1B). Cell incubation with RTX at a concentration of 10 µg/mL resulted in similar cell viability in each cell line (74.31% ± 12.08 cell viability in RL cells versus 79.4% ± 6.61 cell viability in Raji cells). When RL and...
Rituximab as Radiosensitizer


Raji cells were irradiated at single-doses of 2 or 9 Gy and continuously incubated for 72 hours following irradiation, each cell line exhibited a completely different growth pattern. While treatment with ionizing radiation resulted in time-dependent growth inhibition of RL cells with 68.80% ± 9.75 and 35.37% ± 11.6 of cells surviving after 2 or 9 Gy at 72 hours, respectively, growth of Raji cells was not inhibited at the same rate, revealing a cell viability of 97.8% ± 10.51 after 2 Gy and 87.8% ± 8.09 after 9 Gy. Furthermore, stimulation of Raji cell growth was observed at 24 hours in cells irradiated with both doses of 2 and 9 Gy. RL and Raji cell viabilities significantly declined after RTX pretreatment followed by irradiation. Thus, the combination of RTX and radiation at a clinically relevant dose of 2 Gy resulted in 43.7% ± 4.5 of cell viability for RL cells and 60.9% ± 7.11 for Raji cells. RTX pretreatment in combination with the higher radiation dose of 9 Gy led to a more pronounced cell growth delay in RL and Raji cells (14.9% ± 2.87 and 54.3% ± 9.75 cell viability, respectively). Since the combination of RTX and ionizing radiation at a dose of 9 Gy resulted in a more appreciable anti-proliferative effect in Raji cells, this dose was selected for further experiments.

Cell cycle regulation by ionizing radiation and/or RTX treatment in lymphoma cells

We analyzed cell cycle profiles of RL and Raji lymphoma cells treated with RTX, ionizing radiation or their combination. Compared with control untreated cells, treatment with RTX alone induced an increase in cells in the G0 – G1 phase (Table 1).

The more strongly pronounced final accumulation of cells in the G0 – G1 phase at 72 hours was observed in the Raji cell line (54.73% ± 2.16 versus 45.77% ± 2.41 in the RL cell line). Treatment of RL cells with ionizing radiation resulted in an increase in the G2 – M phase and a slight decline in the S phase within 48 hours. At 72 hours ionizing radiation arrested RL cells in the G2 – M and G0 – G1 phases with a significant decrease in the S phase population. In contrast, Raji cells treated with ionizing radiation alone arrested in the G2 – M phase over 72 hours. A more complex cell cycle perturbation was caused by combined treatment with ionizing radiation and RTX in both human lymphoma cell lines. Accumulation of cancer cells in both G0 – G1 and G2 – M phases and an accompanying reduction in the S phase was documented for RL and Raji cells. Cell cycle arrest in the G0 – G1 phase was more pronounced in RL than in Raji.
Table 1. Effects of ionizing radiation and RTX on cell cycle distribution.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RL follicular lymphoma cells</th>
<th>Raji Burkitt lymphoma cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G0 – G1, %</td>
<td>S, %</td>
</tr>
<tr>
<td>Control</td>
<td>38.95 ± 0.72</td>
<td>43.48 ± 0.82</td>
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<tr>
<td>Ionizing radiation (9 Gy)</td>
<td>39.16 ± 9.97</td>
<td>4.46 ± 8.41 ***</td>
</tr>
<tr>
<td>RTX (10 μg/mL)</td>
<td>45.77 ± 2.41 **</td>
<td>41.07 ± 3.21</td>
</tr>
<tr>
<td>RTX (10 μg/mL) + ionizing radiation (9 Gy)</td>
<td>42.51 ± 3.63</td>
<td>2.14 ± 1.25 ***</td>
</tr>
</tbody>
</table>

Lymphoma cells were treated with the indicated doses of ionizing radiation and/or RTX as described in Materials and Methods and then incubated over 72 hours. Mean values and standard deviations are representative of three independent experiments.

*Denotes marked difference when compared with control. *p < 0.05; **p < 0.01; ***p < 0.001

Fig. 2. Ionizing radiation, RTX or their combination modulate expression of proteins involved in cell cycle. (A) Exponentially growing RL and Raji cells were treated with RTX (10 μg/mL) and/or ionizing radiation (9 Gy). Untreated cells were used as control (C). Lane 1, RTX, 10 μg/mL (24 h); Lane 2, RTX 10 μg/mL (48 h); Lane 3, RTX 10 μg/mL (72 h); Lane 4, ionizing radiation, 9 Gy (24 h); Lane 5, ionizing radiation, 9 Gy (48 h); Lane 6, ionizing radiation, 9 Gy (72 h); Lane 7, RTX plus ionizing radiation (24 h); Lane 8, RTX plus ionizing radiation (48 h); Lane 9, RTX plus ionizing radiation (72 h). At the indicated times (in hours) total protein extracts were prepared from the cells. Protein extracts were processed for immunoblotting using antibodies to detect p53, p21/WAF1, p27/KIP1, c-myc as described in the Materials and Methods section. (B) Densitometric analysis and the integrated density values (IDV) of the modulated expression of c-myc in RL and Raji lymphoma cells after treatment with RTX (10 μg/mL) (▼), ionizing radiation (9 Gy) (●) or combination of RTX (10 μg/mL) and irradiation (9 Gy) (▲). IDV was obtained by integrating, after background correction, all of the pixel values in the area of each c-myc band corresponding to the level of α-tubulin expression. Data are given as mean and standard deviation obtained from three independent experiments. *p < 0.05; **p < 0.001; ***p < 0.001
Expression of cell cycle proteins

The basal and treatment-induced levels of cell cycle-related proteins, p53, p21/WAF1, p27/KIP1 and c-myc, in the human lymphoma cell lines RL and Raji were compared by Western blot analysis (Fig. 2A).

The constitutive level of p53 was similar in RL and Raji cells. Following RTX, radiation or combination treatment of RL cells an up-regulation of p53 was evident at 24 hours and more pronounced at 72 hours. Raji cells showed no evident changes in p53 expression on RTX treatment. Ionizing radiation slightly up-regulated p53 in Raji cells, whereas the combination of ionizing radiation and RTX caused weak down-regulation of p53. The expression levels of p21/WAF1 hardly amplified in RL cells during all forms of treatment. In contrast, p21/WAF1 revealed down-regulation after RTX treatment and up-regulation after irradiation or combination treatment in Raji cells. Since previous studies have shown that RTX displays its cell cycle inhibitory effects through p27/KIP1,18) RL and Raji cells were examined for p27/KIP1 expression under the influence of various kinds of treatment. Indeed, a clear up-regulation of cyclin-dependent kinase inhibitor (CDKI) p27/KIP1 was demonstrated in RL and Raji cells following RTX treatment. Ionizing radiation and combination therapy slightly decreased p27/KIP1 protein expression in RL cells and markedly suppressed it in Raji cells. These data are consistent with the time kinetics of the cell cycle disruption (Table 1) induced by RTX and ionizing radiation in NHL cells and confirm the role of both p21/WAF1 and p27/KIP1 CDKIs in this effect.

C-myc is constitutively absent in RL follicular lymphoma cells, whereas it is expressed in Raji Burkitt lymphoma cells (Fig. 2B). RTX or ionizing radiation alone significantly enhanced c-myc expression in RL cells within 24 hours after treatment (1.724 ± 0.296 IDV after RTX treatment and 3.956 ± 0.360 IDV after radiation exposure versus 0.002 ± 0.001 IDV in control RL cells). After this time, c-myc was repressed. The combination of ionizing radiation and RTX did not markedly induce c-myc in RL cells (0.130 ± 0.099 IDV). In Raji cells c-myc was significantly enhanced by ~3.4-fold after irradiation and remained overexpressed for 72 hours. In contrast, RTX treatment temporarily up-regulated c-myc by ~2.5-fold with subsequent down-regulation As shown, Raji cells reacted to the combination therapy with initial c-myc overexpression (~3.9-fold) at 24 hours followed by a significant c-myc decrease at 72 hours (2.080 ± 0.315 IDV versus 1.655 ± 0.231 IDV in control Raji cells).

RTX enhances radiation-induced apoptosis

We next determined whether ionizing radiation and RTX instead of rituximab influenced apoptotic response in RL and Raji lymphoma cells. We analyzed the level of apoptosis by flow cytometry as described in the Materials and Methods section. As shown in Fig. 3, irradiation alone induces apoptosis in both RL and Raji lymphoma cell lines (40.79% ± 3.44 and 25.93% ± 4.22, respectively). In contrast, no significant apoptosis induction was observed in the RL and Raji cells incubated with RTX alone (11.01% ± 2.46 versus 6.17% ± 3.61 in untreated cells; 9.35% ± 2.54 versus 6.77% ± 2.91 in untreated cells, respectively). The combination of ionizing radiation and RTX enhanced apoptosis response in each cell line. Of RL cells 55.95% ± 5.22 were seen to be apoptotic. The apoptosis rate for combined treatment in Raji cells (44.68% ± 5.44) was markedly higher than for cells treated with ionizing radiation only.
When RL cells were treated with ionizing radiation only, they exhibited caspase-3 activation at 48 hours with further enhancement at 72 hours. In the irradiated Raji cells caspase-3 cleavage was registered at 72 hours after radiation exposure. We also documented PARP cleavage accompanying caspase-3 activation. Caspase-3 and PARP cleavage were more pronounced in RL than in Raji cells. The combination of RTX and ionizing radiation in the RL cells induced enhanced cleavage of caspase-3 and PARP at the same times as did irradiation alone. Raji cells revealed accelerated caspase-3 and PARP activation in response to the combination of RTX and ionizing radiation. Cleaved forms of caspase-3 and PARP were observed at 48 hours after combination treatment.

The individual influences of bax, bcl-2 and bcl-XL expression levels on radiation and RTX sensitivity were also assessed for both lymphoma cell lines. Neither bax nor bcl-2 was seen to undergo changes in protein expression in RL or Raji cells after RTX and/or radiation treatment. Although both RTX and ionizing radiation transiently affected the initial expression of bcl-XL, we subsequently observed a slight bcl-XL up-regulation (~1.3-fold and ~1.5-fold, respectively) in Raji cells (Fig. 4B). In contrast, RL cells did not reveal RTX- or radiation-induced bcl-XL modulation. Combination treatment more rapidly inhibited expression of the anti-apoptotic bcl-XL protein in RL. Thus bcl-XL expression decreased by ~1.7-fold during 48 hours and ~1.3-fold at 72 hours after combination treatment. At that time Raji cells revealed reduction by ~0.01-fold for 48 hours and ~1.5-fold at 72 hours after exposure of RTX in combination with ionizing radiation.

DISCUSSION

As most studies focused on RTX in combination with chemotherapy, we investigated RTX in combination with ioniz-
ing radiation, another conventional approach in NHL treatment. The cellular mechanisms of growth arrest and apoptosis that follow ionizing radiation and RTX interaction in CD20-positive RL and Raji lymphoma cells were examined. To our knowledge, this is the first study investigating radio-modulatory activity of RTX.

Our results demonstrate that ionizing radiation caused time-dependent apoptosis in each NHL cell line. The level of apoptosis was ~1.57-fold higher in RL than in Raji cells. Concomitant measurements of apoptosis and cell viability showed that ionizing radiation was ~2.5-fold more active in RL cells than in Raji cells to initiate cell growth delay. Although we observed that 25.93% ± 4.22% of irradiated Raji cells underwent apoptosis, cell viability was not markedly reduced. One reason for this observation is that after treatment with ionizing radiation EBV-infected Raji cells repair themselves approximately 50% more efficiently than do other lymphoma cells. Up to 59% of DNA double-strand breaks in Raji cells induced by radiation at a dose of 50 Gy were reported to be repaired within 1 h after irradiation. 19,20 RTX alone caused a similar delay in cell proliferation in both NHL cell lines without apoptosis development. The combination of ionizing radiation and RTX significantly reduced the radioresistance of Raji cells. We here show that RTX synergistically enhanced the radio-induced apoptosis rate and cell growth arrest in Raji cells by approximately 1.7- and 1.6-fold, respectively. Similar results were observed for RL cells. Radio-dependent apoptosis in RL cells was increased by RTX by ~1.4-fold, and the antiproliferative effect by ~2.4-fold as compared to radiation alone. Each NHL cell line that underwent apoptosis induced by radiation and RTX revealed enhanced caspase-3 and PARP cleavage as compared to only irradiated cells.

In order to define proteins that might be regulated by RTX, thereby modulating radio-response of lymphoma cells, we investigated apoptosis- and cell cycle-related protein expression in RL and Raji cells. We first addressed the most important functional role of c-myc in cell cycle progression and apoptosis in lymphoma cells treated with ionizing radiation and RTX. We found that c-myc was continuously up-regulated in the irradiated Raji cells. At that time despite the constitutive absence of c-myc, RL cells revealed only a short-term protein increase following radiation. We interpreted these results to indicate that c-myc may contribute to the failure of cell growth delay in Raji cells. Maintenance of c-myc levels might indirectly allow cell cycle progression to continue. Some cells with deregulated c-myc activity have been reported to show attenuation of the DNA damage-induced G2 – M checkpoint. 21 Indeed, the time-dependent decrease in the radiation-induced G2 – M phase in Raji cells was accompanied by c-myc up-regulation. C-myc as a transcriptional repressor 22 prevented accumulation of CDKIs p27/KIP1 in the irradiated Raji cells, thus prompting further cycle progression. RTX rapidly down-regulated c-myc expression in the radiation-exposed Raji cells, whereas in RL cells anti-CD20 mAb abolished radiation-induced c-myc expression. We suggest that RTX-dependent reduction of c-myc expression in the irradiated lymphoma cells is sufficient to cause apoptosis and a strong anti-proliferative effect. This is in agreement with a study conducted with decreased c-myc expression in the human T-cell leukemia cell line MOLT-4 and showing radiation-induced apoptosis. 23 In view of our results, it appears that c-myc plays an important role in the regulation of apoptosis- and cell cycle-related proteins following RTX and radiation treatment. Thus, we observed that up-regulation of radiation-induced c-myc was accompanied by elevation of bcl-XL expression. At that time expression of bcl-2 and bax proteins in irradiated Raji cells did not change. While overexpressed bcl-2 in RL and Raji cells provides short-term enhancement of survival after irradiation, cells can die in the absence of additional survival signals. 24 Here we demonstrate that RTX successfully inhibited bcl-XL. We suggest that bcl-XL acts as such a survival signal. It is possible that the enhancement of radiation-induced apoptosis was mediated by RTX-triggered down-regulation of bcl-XL in lymphoma cells. Such speculation is derived from recent findings by Jazirehi et al. 25 who reported that RTX selectively modifies bcl-XL and sensitizes NHL cells to paclitaxel-induced apoptosis.

As was clearly shown, in immortal and tumor-derived cell lines overexpressed c-myc contributes to p53’s ability to activate p21/WAF1 following DNA damage. 26,27 Thus, we found that ionizing radiation just slightly up-regulated mutated p53 28 in Raji cells. Since p53 is mutated and was not markedly changed, we suggest that enhanced expression of p21/WAF1 after irradiation is p53-independent in Raji cells. However, ionizing radiation significantly up-regulated p53 and p21/WAF1 levels in RL cells, thereby causing apoptosis and cell growth arrest. RTX did not change radio-induced p53 or p21/WAF1 expression in RL cells. This observation is consistent with identical cell cycle distribution after irradiation and combination treatment. Exposure of Raji cells to ionizing radiation and combination treatment, followed by p21/WAF1 up-regulation, enlarged the number of cells in G1, thereby preventing cells from entering the S phase.

This report presents for the first time in vitro data on RTX in combination with ionizing radiation. RL indolent lymphoma cells are particularly sensitive to radiation damage, whereas Raji aggressive lymphoma cells are radio-resistant. RTX, namely anti-CD20 mAb, alone induced only antiproliferative activity in RL and Raji cell lines. Combined radiation and RTX treatment drastically enhanced the apoptosis rate and cell growth arrest in radio-sensitive RL cells. Prior exposure of Raji cells to RTX nearly abolished their radioresistance. RTX realizes its radio-sensitizing activity by modulating apoptosis- and cell cycle-related proteins (e.g. c-myc, bcl-2 family proteins, CDKIs p21/WAF1 and p27/KIP1).

KIP1, p53). The results of this study are of potential clinical interest. The data presented here suggest that it may be possible to enhance the anti-tumor activity of ionizing radiation by combining radiotherapy with RTX in NHL patients.

REFERENCES