Rituximab-Induced Inhibition of YY1 and Bcl-xL Expression in Ramos Non-Hodgkin’s Lymphoma Cell Line via Inhibition of NF-κB Activity: Role of YY1 and Bcl-xL in Fas Resistance and Chemoresistance, Respectively

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Rituximab treatment of B non-Hodgkin’s lymphoma (NHL) cell lines inhibits the constitutive NF-κB activity and results in the sensitization of tumor cells to both chemotherapy and Fas-induced apoptosis. Cells expressing dominant active IκB or treated with NF-κB-specific inhibitors were sensitive to both drugs and Fas agonist mAb (CH-11)-induced apoptosis. Down-regulation of Bcl-xL expression via inhibition of NF-κB activity correlated with chemosensitivity. The direct role of Bcl-xL in chemoresistance was demonstrated by the use of Bcl-xL-overexpressing Ramos cells, Ramos hemagglutinin (HA)-Bcl-x, which were not sensitized by rituximab to drug-induced apoptosis. However, inhibition of Bcl-xL in Ramos HA-Bcl-x resulted in sensitization to drug-induced apoptosis. The role of Bcl-xL expression in the regulation of Fas resistance was not apparent; Ramos HA-Bcl-x cells were as sensitive as the wild type to CH-11-induced apoptosis. Several lines of evidence support the direct role of the transcription repressor yin-yang 1 (YY1) in the regulation of resistance to CH-11-induced apoptosis. Inhibition of YY1 activity by either rituximab or the NO donor DETANOONOate or after transfection with YY1 small interfering RNA resulted in up-regulation of Fas expression and sensitization to CH-11-induced apoptosis. These findings suggest two mechanisms underlying the chemosensitization and immunosensitization of B-NHL cells by rituximab via inhibition of NF-κB. The regulation of chemoresistance by NF-κB is mediated via Bcl-xL expression, whereas the regulation of Fas resistance by NF-κB is mediated via YY1 expression and activity. The potential clinical significance of these findings is discussed.

2) Does Bcl-xL expression regulate drug- and/or Fas-induced apoptosis, downstream of NF-κB expression and sensitize the cells to Fas-induced apoptosis? 3) Does overexpression of Bcl-xL result in resistance to chemotherapeutic drugs (16).

Western blot analysis for protein expression

Tumor cells (2 × 10^6) were incubated at 37°C for 6 h. The cells were lysed on ice with 200 μl of ice-cold RIPA buffer (1% Nonidet P-40, 0.1% SDS, 0.5% deoxycholic acid, and complete protease inhibitor mixture tablet (Roche)). Lysates were transferred to microcentrifuge tubes, sonicated in a sonicator (model W-220F; Heat-System Ultrasonic) for 10 s, and were centrifuged at 12,000 × g at 4°C for 5 min. Protein concentrations were quantified using the Bio-Rad protein assay. Gel loading buffer (Bio-Rad) was added to the cell lysates at a 1:1 ratio. Samples were boiled for 5 min, separated on 12% SDS-PAGE, and transferred to nitrocellulose membrane Hybrid ECL (Amersham Biosciences) in a Trans-Blot SD semidry transfer cell system (Bio-Rad). Primary Abs for Fas (Da-koCytomation), anti-phospho-IκB, anti-inhibitor of κB kinase (IKK), anti-phospho-IκB (Cell Signaling Technology), anti-IκB, anti-Bcl-xL (Santa Cruz Biotechnology), and anti-YY1 (Geneika) were used at predetermined optimal concentrations. The blots were developed by LumiGLO reagent and peroxide (Cell Signaling Technology).

Treatment of 2F7 cells with inhibitors

The specific NF-κB inhibitor Bay 11-7085, obtained from Calbiochem, was dissolved in DMSO (Sigma-Aldrich). DETANONOate was purchased from Alexis. The NF-κB inhibitor Bay 11-7085 (a specific inhibitor of IκB phosphorylation) was used at 6 μM and Bay 11-7085 (20) and was a gift from Dr. K. Umezawa (Keio University, Yokohama, Japan).

EMSA

Briefly, cells (10^6) were harvested after treatment and washed twice with ice-cold 1× PBS. After washing, the cells were lysed in 1 ml of Nonidet P-40 lysis buffer (10 mM Tris-HCl (pH 7.5), 10 mM NaCl, 3 mM MgCl2, and 0.5% Nonidet P-40) on ice for 5 min. Samples were centrifuged at 300 × g at 4°C for 5 min. The pellet was washed twice in Nonidet P-40 buffer. Nuclei were then lysed in nuclear extraction buffer (20 mM HEPES (pH 7.9), 25% glycerol, 0.42 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM PMSE, and 0.5 mM DTT) and sonicated for 10 s at 4°C. Both buffers contained the complete protease inhibitor mixture tablets from Roche. The protein concentration was determined using the Bio-Rad protein assay kit. The nuclear proteins were frozen at −80°C. Nuclear protein (5 μg) was mixed for 30 min at room temperature with biotin-labeled oligonucleotide NF-κB and YY1 probe using an EMSA kit (Panomics) according to the manufacturer’s instructions. Ten micromolars of nuclear extract was subjected to 5% PAGE for 90 min in Tris-glycine EDTA buffer. DNA-bound protein was transferred to a nylon membrane (Hybond-N’; Amersham Biosciences) using the Trans-Blot SD semidyey transfer cell system (Bio-Rad). The membranes were transferred to UV cross-linker (FB-UVXL-1000; Fisher Scientific) for 3 min. The detection was made according to the manufacturer’s instructions after the membranes were exposed on Hyperfilm ECL (Amersham Biosciences) as previously described (8–10).

Transfection with YY1 siRNA

The prostate carcinoma cell line (PC-3) was cultured in 1 ml of RPMI 1640 medium supplemented with 5% FBS. Transfections were performed using Lipofectamine 2000 CD reagent (Invitrogen Life Technologies) and the SureSilencing siRNA kit (SuperArray Bioscience) according to the manufacturer’s instructions. Transfected PC-3 cells were treated for 18 h with CH-11, then fixed and permeabilized for anti-active caspase-3–FITC Ab staining, and analyzed by flow cytometry as described above.
Isobologram analysis for synergy

To establish whether the cytotoxic effect of the combination of rituximab and CH-11 Ab was more than additive, isobolograms (21) were developed from a battery of combination at various concentrations of rituximab (10, 20, and 40 μg/ml) and anti-Fas Ab CH-11 (5, 10, and 20 ng/ml). Combinations yielding 25 ± 5% cytotoxicity were graphed as a percentage of the concentrations of single agent alone that produced the same amount of cytotoxicity as that used in combination (6–10).

Statistical analysis

All results were expressed as the mean ± SD of data obtained from three or four independent and separate experiments. The statistical significance of differences between group means was determined using one-way ANOVA to compare variance. Significant differences were considered for probabilities <5% (p < 0.05).

Results

Rituximab-mediated up-regulation of Fas expression and sensitization to CH-11-induced apoptosis

Treatment of Ramos, 2F7, and Raji NHL-B cell lines with rituximab sensitized the tumor cells to CH-11 (Fas agonist Ab)-induced apoptosis. The sensitization was a function of the concentration of CH-11 used (Fig. 1A, top). The sensitization achieved by rituximab was found to be synergistic, as determined by isobologram analysis (Fig. 1A, bottom). Because B cell lines also express FcRs, it was possible that rituximab-mediated sensitization to CH-11 might have resulted from cross-linking of rituximab with FcRs on the tumor cells. This possibility was shown to be unlikely by demonstrating that an engineered rituximab molecule lacking the CH2 domain (rituximab CH2–) sensitized B-NHL cells to CH-11-mediated apoptosis similarly to sensitization by rituximab. The sensitization was specific, because control IgG had no effect (Fig. 1B).

Treatment of Ramos cells with rituximab up-regulated surface Fas expression as determined by flow cytometry. Time-kinetcis analysis of treatment showed that significant up-regulation of Fas occurred as early as 6 h after rituximab treatment and returned to baseline levels at 24 h (Fig. 1C). The up-regulation of surface Fas expression was confirmed at the total protein level by Western blot (Fig. 1D).

These findings demonstrate that rituximab treatment of B-NHL cells sensitizes the cells to Fas-induced apoptosis, and synergy is achieved. Rituximab treatment also up-regulated Fas expression in these cells.

Rituximab-mediated inhibition of NF-κB activity correlates with down-regulation of Bcl-xL expression and up-regulation of Fas expression

Treatment of Ramos cells with rituximab resulted in inhibition of NF-κB activity, as assessed by EMSA. The inhibition was significant at 3 h after treatment and was maintained for up to 24 h. The specificity was confirmed by use of the NF-κB-specific inhibitors Bay 11-7085 and DHMEQ (Fig. 2A). The specificity of these inhibitors has been previously established (10, 14, 20). In addition, rituximab inhibited IKK and IκB phosphorylation in a time-dependent manner beginning 3 h after treatment, and the inhibition was maintained for up to 24 h. Levels of the nonphosphorylated IKK and IκB were not affected by rituximab (Fig. 2B). These findings demonstrate that rituximab inhibits the NF-κB signaling pathway via inhibition of phosphorylated IKK (p-IKK) and p-IκB. Previous studies have reported that NF-κB regulates Bcl-xL transcription (15, 22–24). Hence, treatment of Ramos cells with rituximab inhibited Bcl-xL expression beginning at 3 h after treatment, and the inhibition was maintained for up to 24 h. The NF-κB-specific inhibitor DHMEQ (20) also inhibited Bcl-xL expression (Fig. 2C).

The above findings demonstrated that rituximab inhibits the NF-κB pathway (Fig. 2A) and down-regulates Bcl-xL expression (Fig. 2C). Thus, we examined the direct relationship between inhibition of the NF-κB pathway and Bcl-xL expression by rituximab. To this end, Ramos cells with a functional block in the NF-κB signaling pathway were established. The strategy involved the overexpression of a chimeric fusion protein consisting of a dominant-active IκB-α mutant (S32/36A) fused to a mutated ER (15). The IκB mutant is incapable of being phosphorylated at the critical serine residues and thus is not targeted for proteasomal degradation upon activation of IKK. The fused ER confers inducible activation of the gene of interest upon exposure to the synthetic estrogen 4-hydroxytamoxifen (4-OHT). The FLAG-IκB-mutant-ER construct was cloned into the pCDNA3 expression vector and was stably transfected into the cells. Single clones expressing the construct were isolated and used for additional analysis (15). The Ramos-IκB-ER cells were either untreated or pre-treated with 4-OHT (200 nM; 8 h). Thereafter, the cell lysates were subjected to immunoblot analysis. As depicted, treatment of Ramos-IκB-ER cells with 4-OHT inhibited NF-κB activity, similar to findings observed with rituximab treatment (Fig. 3A), and also reduced the basal level of Bcl-xL protein expression in these cells (Fig. 3B). These findings demonstrate that inhibition of NF-κB correlated with inhibition of Bcl-xL expression. In addition, treatment of the Bcl-xL-overexpressing Ramos cells, HA-Bcl-xL with rituximab resulted in down-regulation of endogenous Bcl-xL, but that not driven by the overexpressing plasmid (Fig. 3C). Together, these findings establish a strong correlation between rituximab-induced inhibition of NF-κB activity and inhibition of Bcl-xL expression in Ramos B-NHL cells.

We have shown above that rituximab sensitizes B-NHL cells to CH-11-induced apoptosis and also up-regulates Fas expression (Fig. 1, C and D). Because rituximab inhibits NF-κB activity (Fig. 2), the role of NF-κB in the regulation of Fas expression was examined. Treatment of Ramos IκB-ER cells with rituximab resulted in up-regulation of Fas expression, comparable to treatment of Ramos cells, as determined by flow cytometry (Fig. 4A) and Western blot (Fig. 4B). However, when Ramos IκB-ER cells were treated with 4-OHT to inhibit NF-κB activity and in the absence of rituximab, there was a much higher up-regulation of Fas expression compared with that in untreated cells as determined by both flow cytometry (Fig. 4A) and Western blot (Fig. 4B). Treatment of Ramos IκB-ER cells with the combination of rituximab and 4-OHT did not further augment Fas expression compared with the effect of treatment with either rituximab or 4-OHT alone. These findings demonstrate the regulation of Fas expression correlated with the sensitization of tumor cells to CH-11-induced apoptosis. Thus, although treatment of Ramos IκB-ER cells with rituximab sensitized the cells to CH-11-induced apoptosis, 4-OHT treatment was more efficient in sensitizing the cells to CH-11-induced apoptosis than treatment with rituximab (Fig. 4C). These findings demonstrate that NF-κB participates in the negative regulation of Fas expression and resistance to CH-11-induced apoptosis in Ramos cells.

Bcl-xL expression regulates drug resistance, but fails to regulate Fas resistance

We examined the role of Bcl-xL in regulation of the sensitivity of Ramos cells to CH-11-induced apoptosis. Treatment of Bcl-xL-overexpressing Ramos cells (HA-Bcl-xL) with rituximab resulted in significant sensitization to CH-11-induced apoptosis, comparable to that of wild-type Ramos cells. The extent of apoptosis was a function of the CH-11 mAb concentration used (Fig. 5A). These findings demonstrate that Bcl-xL expression does not influence the sensitivity of Ramos cells to CH-11-induced apoptosis.
We then examined the role of Bcl-xL in the sensitization to cis-platinum (CDDP)-mediated apoptosis by comparing rituximab-mediated chemosensitization in Ramos and Ramos HA-Bcl-x cells. Although treatment of Ramos cells with rituximab sensitized the cells to CDDP-mediated apoptosis, rituximab treatment of Ramos HA-Bcl-x cells, in contrast, failed to significantly sensitize to CDDP-induced apoptosis (Fig. 5B). Treatment of Ramos IxB-ER cells with CDDP resulted in moderate cytotoxicity; however, inhibition of NF-kB by 4-OHT and consequently by down-regulation of Bcl-xL sensitized the cells to CDDP-mediated apoptosis (Fig. 5C). These findings clearly demonstrate that Bcl-xL expression regulates tumor cell sensitivity to drug-induced apoptosis, but not to Fas-induced apoptosis.

YY1 expression/activity regulates Fas expression and resistance to CH-11-induced apoptosis

We have previously reported that YY1 negatively regulates Fas expression through its interaction with the silencer region of the
Fas promoter. Inhibition of YY1 by NO results in up-regulation of Fas expression and sensitization of ovarian carcinoma cells to Fas-induced apoptosis (12). Thus, we examined whether rituximab-induced up-regulation of Fas expression in B-NHL cells was also regulated by YY1. Treatment of Ramos cells with rituximab or specific NF-κB inhibitors (e.g., Bay 11-7085 and DHMEQ) and/or inhibition of YY1 (by the NO donor DETANONate) resulted in significant up-regulation of surface Fas expression (Fig. 6A) and sensitization of Ramos cells to CH-11-induced apoptosis. The extent of apoptosis was a function of the CH-11 concentration used (Fig. 6B). These findings suggested that YY1 inhibition by rituximab in B-NHL cells regulates Fas expression and sensitizes the cells to Fas-induced apoptosis.

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The direct role of YY1 in rituximab-mediated sensitization to CH-11-induced apoptosis was examined. Treatment of Ramos cells with rituximab resulted in inhibition of YY1 protein expression (Fig. 6C) and YY1 DNA-binding activity (Fig. 6D). Likewise, treatment of the cells with the NF-κB inhibitors (Bay11-7085 and DHMEQ) or the NO donor, DETANONOate, resulted in inhibition of both YY1 expression (Fig. 6C) and YY1 DNA-binding activity (Fig. 6D). These findings demonstrate that rituximab inhibits both YY1 expression and DNA-binding activity and correlated with rituximab-mediated inhibition of NF-κB activity (Fig. 4A). The direct role of YY1 in the negative regulation of Fas expression and sensitivity to CH-11-induced apoptosis was corroborated by the use of cells transfected with YY1 siRNA, whereby the transfectants showed significantly lower levels of YY1 expression and were significantly sensitized to CH-11-induced apoptosis, and the extent of apoptosis was a function of the CH-11 concentration used. The specificity of the siRNA was shown by transfection of the cells with a negative siRNA control, which did not show any sensitization to CH-11 (Fig. 6E).

The findings presented above provide clear evidence that rituximab-mediated up-regulation of Fas expression and sensitization to Fas agonist-induced apoptosis are the results of rituximab-mediated inhibition of YY1 expression and DNA-binding activity.

**Discussion**

The present study provides evidence demonstrating that chemoresistance and Fas resistance in B-NHL cell lines are commonly regulated by constitutive NF-κB activation. However, downstream of NF-κB, chemoresistance and Fas resistance are differentially regulated by Bcl-xL and YY1, respectively. Rituximab-mediated inhibition of NF-κB activity resulted in both the inhibition of Bcl-xL expression and chemosensitization and the inhibition of the transcription repressor YY1 and sensitization to CH-11-induced apoptosis. These differentially regulated mechanisms for chemotherapeutic and CH-11-induced apoptosis emanated from findings making use of both biologically engineered cell lines and specific chemical inhibitors. Treatment with rituximab or specific inhibitors of NF-κB sensitized NHL cells to both drug- and CH-11-induced apoptosis. The role of Bcl-xL expression in the regulation of drug resistance, but not Fas resistance, was demonstrated by the failure of rituximab to sensitize Bcl-xL-overexpressing Ramos cells (Ramos HA-Bcl-x) to drug-induced apoptosis, although these same cells were sensitized by rituximab, like the wild type, to CH-11-induced apoptosis. However, inhibition of Bcl-xL expression in Ramos-IκB-ER or by various NF-κB inhibitors sensitized the cells to CDDP-induced apoptosis. Like rituximab, inhibition of NF-κB either by specific chemical inhibitors or by 4-OHT treatment of Ramos-IκB-ER cells sensitized the cells to CH-11-induced apoptosis. The resistance to Fas-induced apoptosis was found to be regulated by the transcription repressor YY1, whereby inhibition of YY1 expression or activity by rituximab, chemicals, or YY1 siRNA resulted in up-regulation of Fas expression and sensitization to CH-11-induced apoptosis. These findings clearly establish distinct regulatory mechanisms modulated by rituximab in B-NHL cells, downstream of NF-κB for the sensitization to
caspase 3 activation as described in Fig. 4. B. Role of Bcl-xL in resistance to drug-induced apoptosis. Ramos and Ramos-HA-Bcl-x cells were treated with rituximab (20 μg/ml) for 6 h, treated with different concentrations of CH-11 for 18 h, and tested for apoptosis by caspase 3 activation as described in Fig. 4C. Results are presented as the mean ± SD of duplicate samples. B. Role of Bcl-xL in resistance to drug-induced apoptosis. Ramos and Ramos-HA-Bcl-x cells were treated with rituximab (20 μg/ml), CDDP (2 and 5 μg/ml), and their combination for 24 h. The cells were analyzed for apoptosis by caspase 3 activation. Results are presented as the mean ± SD of duplicate samples. C. Sensitization of Ramos cells to CDDP-induced apoptosis via inhibition of NF-κB. Ramos-IκB-ER cells were left untreated or treated with CDDP (2 and 5 μg/ml), 4-OHT (200 nM), or their combination for 18 h and tested for apoptosis. Results are presented as the mean ± SD of duplicate samples. * p < 0.05. C. Sensitization of Ramos cells to CDDP-induced apoptosis via inhibition of NF-κB. Ramos-IκB-ER cells were left untreated or treated with CDDP (2 and 5 μg/ml), 4-OHT (200 nM), or their combination for 18 h and tested for apoptosis. Results are presented as the mean ± SD of duplicate samples. * p < 0.05.
FIGURE 6. Sensitization to CH-11-induced apoptosis by inhibition of NF-κB and YY1. A, Surface Fas expression. Ramos cells were treated with rituximab (20 μg/ml), Bay 11-7085 (5 μM), DHMEQ (10 μg/ml), or NO donor DETANONOate (1000 μM) for 6 h, and surface Fas expression was examined by flow cytometry. *, p < 0.05. B, Sensitization of Ramos to CH-11-induced apoptosis by inhibition of NF-κB. Ramos cells were treated with rituximab (20 μg/ml), Bay-11-7085 (6 μM), DHMEQ (10 μg/ml), or the NO donor DETANONOate (1000 μM) for 6 h, followed by treatment with different concentrations of CH-11 (5, 10, and 20 ng/ml) for 18 h. *, p < 0.01. C, Inhibition of YY1 expression by rituximab. Ramos cells were left untreated or treated with rituximab (20 μg/ml), Bay-11-7085 (6 μM), DHMEQ (10 μg/ml), or the NO donor DETANONOate (1000 μM) for 18 h. The cell lysates were tested for YY1 expression by Western blot (top panel) and by densitometric analysis (bottom panel). D, Inhibition of YY1 DNA-binding activity by rituximab. Ramos cells were treated as described above, and nuclear lysates were analyzed for YY1-DNA-binding activity by EMSA. The data are representative of two independent experiments. E, Role of YY1 in the resistance to CH-11-induced apoptosis. PC3 cells (2.5 × 10⁶) were transfected with 10 μl of Superfect and 10 μl of YY1 siRNA or control siRNA. After 48 h, the cells were treated with CH-11 (5 and 10 ng/ml), and apoptosis was measured. The inhibition of YY1 siRNA was tested by RT-PCR (top panel). *, p < 0.03 compared with control nontransfected cells.
resulted in inhibition of YY1 expression and YY1 DNA-binding activity concomitant with the inhibition of NF-κB activity. The inhibition of NF-κB and YY1 activities by chemical inhibitors mimicked the inhibition produced by rituximab. The involvement of YY1 in the regulation of Fas expression and sensitivity of Ramos cells to CH-11-induced apoptosis was demonstrated by inhibition of YY1 by chemicals (such as NO or NF-κB inhibitors) or YY1 siRNA, all of which resulted in up-regulation of Fas expression and sensitization to CH-11-induced apoptosis in the absence of rituximab. These findings establish YY1 as a negative regulator of Bcl-xL expression and as a resistance factor in B-NHL cells, where its inhibition by rituximab sensitizes the cells to Fas-induced apoptosis.

Activation of NF-κB is emerging as one of the major mechanisms of tumor cell resistance to drugs (5, 22–24, 30, 31). Thus, interruption of this pathway is a target for therapeutic intervention for the treatment of tumors that has proven successful in enhancing the apoptotic effects of anticancer agents (e.g., TNF-α and carnitine palmitoyl transferase-11), resulting in tumor regression in vivo (32). Targeted suppression of Bcl-xL expression facilitated drug-induced B cell leukemia tumor regression in the SCID/NOD-Hu in vivo model (33). Thus, NF-κB and Bcl-xL inhibition augments drug-, Fas-, and TNF-α-induced apoptosis in various tumor model systems. Currently, rituximab in combination with chemotherapy improves the clinical response in patients significantly compared with treatment with either agent alone (34). However, several reports demonstrate that certain patients are not responsive to treatment by rituximab or a combination of drugs, and the underlying mechanisms of resistance are not clear.

It is possible that resistance of the tumor cells may have resulted from the overexpression of antiapoptotic gene products, such as Bcl-xL, which was shown in this study in Ramos cells and other drug-induced B-NHL cells. It is possible to consider therapies aimed at reducing or inhibiting the expression of antiapoptotic gene products to reverse acquired or induced resistance. Based on our studies, this may be achieved by inhibition of NF-κB activity directly or inhibition of the antiapoptotic members of the Bcl-2 family. The underlying mechanisms of resistance of B-NHL by rituximab are not clear. There have been several reports demonstrating that resistance may be due to loss of the CD20 receptor, overexpression of complement inhibitors, inhibition of signaling for apoptosis, and inhibition of Ab-dependent cellular cytotoxicity due to the polymorphism of the FcRs (5, 35, 36). It is also possible that tumor cells overexpress antiapoptosis regulatory proteins, such as Bcl-2 or Bcl-xL (7–11, 14, 37). The long, alternatively spliced variant of the Bcl-x gene, Bcl-xL, shares a high degree of structural and functional similarity with the antiapoptotic protein Bcl-2 and exerts its protective effects mainly in the membrane of mitochondria by preventing loss of membrane potential, cytochrome c efflux upon apoptotic stimuli, and the initiation of apoptosis (38). Bcl-xL is predominantly expressed in lymphomas (39, 40) and antagonizes DNA-damaging agents, metabolic, microtubule, and topoisomerase inhibitors. Through modulation of apoptosis, Bcl-xL plays a major role in determination of the cellular response to a wide variety of apoptosis-inducing stimuli (41, 42). It is not clear whether overexpression of such molecules can also affect immune-mediated apoptosis of rituximab-treated cells. We show in this study that overexpression of Bcl-xL does not inhibit Fas-induced apoptosis in Ramos NHL cells.

The present findings demonstrate that rituximab sensitizes B-NHL cell lines to apoptotic stimuli by two different and complementary signaling pathways downstream of NF-κB. The down-regulation of Bcl-xL sensitizes to drug-induced apoptosis, and inhibition of YY1 sensitizes to Fas-induced apoptosis (see scheme in Fig. 7). Clearly, these findings establish that inhibition of NF-κB will sensitize B-NHL cells to both drugs and immunotherapy. Furthermore, although rituximab fails to chemosensitize Bcl-xL-overexpressing cells, it can sensitize these cells to Fas-induced apoptosis. The present findings provide new evidence that chemoresistance in B-NHL cells after treatment with rituximab and drugs in combination may still be amenable for immunotherapeutic intervention in combination with rituximab. Our findings strongly suggest that rituximab sensitizes drug-resistant tumor cells to Fas-induced apoptosis. Therefore, it may be conceivable that treatment with rituximab in combination with activation of host immune cells to express death-inducing TNF family ligands may result in host-induced killing of the resistant tumor cells. These findings establish several targets (e.g., NF-κB, YY1, or Bcl-xL) for intervention in rituximab/drug refractory cells.

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**Disclosures**

The authors have no financial conflict of interest.