NF-κB2/p100 induces Bcl-2 expression

P Viatoir, M Bentires-Alj, A Chariot, V Deregowski, L de Leval, M-P Merville and V Bours

1 Center for Cellular and Molecular Therapy, University of Liège, Liège, Belgium; and 2 Experimental Cancerology Research Center, University of Liège, Liège, Belgium

The NF-κB2/p100 and bcl-3 genes are involved in chromosomal translocations described in chronic lymphocytic leukemias (CLL) and non-Hodgkin’s lymphomas, and nuclear factor kappaB (NF-κB) protects cancer cells against apoptosis. Therefore, we investigated whether this transcription factor could modulate the expression of the Bcl-2 antiapoptotic protein. Bcl-2 promoter analysis showed multiple putative NF-κB binding sites. Transfection assays of bcl-2 promoter constructs in HCT116 cells showed that NF-κB can indeed transactivate bcl-2. We identified a κB site located at position –180 that can only be bound and transactivated by p50 or p52 homodimers. As p50 and p52 homodimers are devoid of any transactivating domains, we showed that they can transactivate the bcl-2 promoter through association with Bcl-3. We also observed that stable overexpression of p100 and its processed product p52 can induce endogenous Bcl-2 expression in MCF7AZ breast cancer cells. Finally, we demonstrated that, in breast cancer and leukemic cells (CLL), high NF-κB2/p100 expression was associated with high Bcl-2 expression. Our data suggest that Bcl-2 could be an in vivo target gene for NF-κB2/p100.

Keywords: NF-κB; Bcl-3; Bcl-2; oncogene; apoptosis; cancer

Introduction

Apoptosis or programmed cell death is a vital process that controls cell number in multicellular organisms. Mechanisms regulating apoptosis are complex and their dysfunction represents a central step in tumorigenesis and conditions cancer cell response to radiotherapy and chemotherapy. Members of the Bcl-2 protein family are key regulators of apoptosis and their implication in cancer development has been largely documented. At least 15 proteins of this family have been identified in mammalian cells. They are divided into proapoptotic (Bax, Bak, Bad, Bimd and others) and antiapoptotic proteins (Bcl-2, Bcl-XL, A1/BI-1 and others).

Nuclear factor kappaB (NF-κB) is a ubiquitous transcription factor involved in a large variety of events such as inflammation, immune response and cell survival. NF-κB consists of dimers of subunits (RelA, RelB, c-Rel, p50, p52) that all contain a Rel homology domain. Among the Rel-related proteins, p65 (RelA), RelB and c-Rel contain one or two transactivating domains, while p50 and p52 are derived from large cytoplasmic precursors, p105 and p100, respectively, and do not possess any transactivating domain. NF-κB is sequestered in the cytoplasm of resting cells through binding with inhibitors belonging to the IκB family (IκBα, IκBβ, IκBε, p100 and p105). Following several stimuli, the ubiquitous IκB-α inhibitor is degraded and NF-κB translocates to the nucleus.

Several NF-κB proteins are found to be overexpressed in several cancers, following chromosomal translocation or gene amplification. The rearrangement affecting the NF-κB2 locus at 10q24, found in many lymphomas, results in the deletion of the C-terminal part of p100, thus enhancing p52 expression, notably in the nucleus. Mice lacking NF-κB2/p100 develop normally, but exhibit impaired immune response and disruption in splenic and lymph node architecture.

Bcl-3 is a member of the IκB protein family. Unlike the other IκB proteins, which are mostly localized in the cytoplasm, Bcl-3 is predominantly present in the nucleus where it forms transactivating complexes with p50 or p52 homodimers. bcl-3 gene was originally identified at the breakpoint in the t(14;19) translocation in chronic lymphocytic leukemias (CLL), resulting in a constitutive enhanced Bcl-3 protein expression and a bad prognosis. bcl-3 knockout (KO) mice develop normally, but exhibit severe defects in humoral immune response and impaired germinal center reaction. Interestingly, KO mice for p52 or Bcl-3 show similar phenotypes, suggesting common in vivo target genes. On the contrary, bcl-3 transgenic animals develop splenomegaly and an accumulation of mature B cells in lymph nodes, bone marrow and peritoneal cavity. Despite these in vivo models, Bcl-3 biological functions and target genes remain largely unknown. An in vitro effect on the human P-selectin promoter has been reported and, recently, Westerheide et al showed that Bcl-3, in association with p52, could enhance cyclin D1 expression in breast cancer cells. Finally, Mitchell et al demonstrated that immunological adjuvant-induced Bcl-3 could promote T-cell survival.

Similar to bcl-3, the bcl-2 gene was identified in a t(14;18) translocation in B-cell lymphomas. It codes for the Bcl-2 antiapoptotic protein, a key factor in the regulation of apoptosis. The bcl-2 gene comprises three exons with a very long second intron. The promoter is composed of two distinct regions (P1 and P2). P1 is located 1400 bp upstream of the translation initiation site and is a TATA less, GC-rich promoter that displays multiple initiation sites. Several regulatory regions have already been identified in this promoter and are bound by the transcription factors CREB, II1, WT1, E2, etc. The second promoter (P2) is located 1.3 kb downstream to P1. It contains a CCAAT box, a TATA element and a negative responsive element.

In this paper, we show that (i) NF-κB transactivates the bcl-2 promoter; (ii) p50 and p52 homodimers, in collaboration with Bcl-3, bind to and transactivate the bcl-2 promoter, and (iii) p100 stable overexpression in a breast cancer cell line enhances endogenous Bcl-2 expression. In addition, breast cancer tissue and B cells from leukemic patients (CLL) showed an association between NF-κB2/p100 expression and enhanced Bcl-2 protein level.

Materials and methods

Cell culture and biological samples

The HCT116 human colon carcinoma cells (ATCC CCL 247) were grown in McCoy’s 5A modified medium supplemented

Correspondence: Dr V Bours, Department of Genetics, CHU B35, University of Liège, Liège 4000 Belgium; Fax: +32 4 3668 146
Received 20 January 2003; accepted 14 March 2003

Journal: LEU
Article: npg_leu_2402982
Pages: 1–8
Op: thilakam
Ed: Ch
with 1% l-glutamine 200 mM, 10% (v/v) fetal bovine serum (FCS, Life Technologies, Grand Island, NY, USA), penicillin (100 IU/ml) and streptomycin (100 μg/ml). The MCF7AZ breast cancer cell line (from Professor M Mareel, University of Gent, Belgium) was maintained in RPMI 1640 supplemented with 10% FCS, 1% l-glutamine 200 mM, penicillin (100 IU/ml) and streptomycin (100 μg/ml).

The stably transfected cell lines were cultivated in the same medium as parental cells plus genetin (500 μg/ml of G418 active concentration, Roche, Belgium).

Blood (samples 1–6) or bone marrow (samples 7,8) primary samples identified as CLL according to the REAL classification and with (samples 5–8) or without (samples 1–4) a t(14;19) chromosomal translocation were obtained from Professor A Hagemeijer (University of Leuven, Belgium) or from Dr F Lambert (Laboratory of Medical Chemistry, CHU Liège, Belgium). Breast cancer samples were obtained from the Department of Surgery, CHU Liège. The samples were immediately frozen in liquid nitrogen and reduced to powder. Total cellular extracts were made by using SDS 1%.

**Sequence analysis**

Analysis of the bcl-2 promoter sequence was performed with the Ben (Belgian Emnet Node) software using the mapping program and the TF sites database.

**Immunoblots**

Whole-cell protein extracts (10 μg) obtained by SDS lysis were separated on a 14% SDS-PAGE gel. After transfer to a nylon membrane (Immobilon-P, Millipore, Bedford, MA, USA) and overnight blocking at 4°C, the membranes were incubated for 1 h with antibodies directed against human actin, Bcl-2, Bcl-3 (Santa Cruz, CA, USA), respectively; the c-Rel polyclonal antibody was a gift from Dr Nancy Rice (NCI, Frederick, MD, USA). Mismatched nucleotides are underlined.

**Plasmids**

The bcl-2 Luc-reporter plasmids containing the fragments from −3934 to −8, −751 to −8 and −178 to −8 from the bcl-2 promoter cloned upstream of the luciferase-reporter gene were generous gifts from Dr L Boxer (Stanford University Medical Center, Stanford, CA, USA). The bcl-2 β-Gal-reporter plasmids containing the fragments from −760 to −8, −178 to −8 and −182 to −8 were constructed by insertion of PCR products corresponding to these fragments in a pBlue-TOPO TA Cloning vector (Invitrogen, Groningen, The Netherlands). The Pmt2T-Bcl-3, Pmt2T-p100, Pmt2T-p50, Pmt2T-p52 and Pmt2T-RelA expression vectors have been previously described.14,29

**Transient transfections**

Expression vectors and Luc- or β-Gal-reporter plasmids were transfected into HCT116 cells using the FuGENE transfection reagent (Roche, Mannheim, Germany). Cellular extracts were prepared 24 h later and the luciferase or β-galactosidase activities were measured with the Luciferase-reporter gene assay (Roche) or the β-Gal-reporter gene assay (Roche). Enzyme activities were normalized to the protein concentration of the extracts.

**Nuclear protein extraction and electrophoretic mobility shift assay**

Nuclear protein extraction for electrophoretic mobility shift assay (EMSA) has been previously described. Briefly, the pelleted nuclei were resuspended in nuclear buffer (20 mM HEPES pH 7.9, 1.5 mM MgCl2, 0.2 mM EDTA, 0.63 mM NaCl, 25% glycerol, protease inhibitors (Protease inhibitor kit, Roche), incubated for 20 min at 4°C and centrifuged for 30 min at 14,000 g. Protein amounts were quantified with the Micro BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, USA). EMSAs were performed as described with 5 μg of nuclear protein extracts. The prototypical palindromic xB probe was (upper strand) 5’- TTGGCAACGGAGGGAATTCCTCTCTCTTA-3’ and (lower strand) TTGGTAGAGAGGAGGAGAATTCCTCTCTTA-3’.

**Results**

**Analysis of the bcl-2 promoter and identification of κB sites**

Analysis of the P1 bcl-2 promoter sequence revealed two putative NF-κB binding sites that we named xB (GGGGCGTCCC) and xBII (GGGAGCCCCC), located at positions −2306 and −1896, respectively, as well as five other putative xB sites, numbered 1–5, that all harbor one mismatched

<table>
<thead>
<tr>
<th>Table 1 Alignment of κB binding sites from the bcl-2 gene promoter and comparison with a consensus (CS) binding site</th>
</tr>
</thead>
<tbody>
<tr>
<td>κB1</td>
</tr>
<tr>
<td>κBII</td>
</tr>
<tr>
<td>κB1</td>
</tr>
<tr>
<td>κB2</td>
</tr>
<tr>
<td>κB3</td>
</tr>
<tr>
<td>κB4</td>
</tr>
<tr>
<td>κB5</td>
</tr>
<tr>
<td>κB6</td>
</tr>
<tr>
<td>κB7</td>
</tr>
<tr>
<td>CS</td>
</tr>
</tbody>
</table>

Mismatched nucleotides are underlined.
nucleotide when compared to the consensus sequence (Figure 1). Several double-stranded oligonucleotides containing these sequences were synthesized and used to detect NF-κB binding in nuclear extracts from unstimulated and TNF-α-treated HCT116 cells. Stimulation of these cells rapidly induced a strong DNA binding to a control palindromic κB probe, and supershifting experiments showed that the main shifted band contained p50 and p65 proteins (Figure 2a, lanes 7–11). However, under the same experimental conditions, we could not detect any NF-κB DNA binding to the seven κB probes from the bcl-2 P1 promoter (Figure 2a, lanes 1–6, and data not shown). This was surprising, as another study characterized a p50/p50 binding in the P1. 30,31 These results indicate that, in our cell system, NF-κB dimers do not bind to the P1 promoter through these identified κB sites.

While the P2 promoter does not harbor any perfect NF-κB binding site, it contains two sites presenting one mismatch with the NF-κB consensus sequence. To test whether NF-κB could bind one of these sites, EMSAs were performed with probes κB6 and κB7 harboring these sites using HCT116-derived nuclear extracts. As shown in Figure 2b and c, an NF-κB binding activity to probe 7 was observed and the shifted complex comigrated with the lower complex observed using the palindromic consensus κB probe (Figure 2c, left and middle panel), and thus contained probably p50 homodimers as this palindromic probe is very efficiently bound by p50 or p52 homodimers. 29 Supershifting experiments confirmed that only p50 binds to probe 7, while we could not detect any supershift by the p65/RelA antibody (Figure 2c, middle panel and data not shown). Under the same conditions, we showed that NF-κB cannot bind probe 7 where the κB site is mutated (TTTAAACACC instead of GGGAAACACC) (Figure 2c, right panel). Moreover, supershifting experiments performed with nuclear extracts from MCF7AZ cells (which express p52) showed that p52 homodimers are also able to bind to probe 7 (Figure 2d). Indeed, we have reproducibly observed that the p52 antibody could reduce the intensity of a p52-containing retarded complex, without inducing a ‘supershift’ (data not shown).

Regulation of the bcl-2 gene promoter by NF-κB

To further address the control of the bcl-2 gene transcription by NF-κB, HCT116 cells were transiently transfected with several reporter plasmid constructs corresponding to the P2 (from −750 to −8) or the P1+P2 (from −3934 to −8) promoters (see Figure 1) as well as one construct containing only the κB7 site. The P1 promoter was weakly transactivated in response to p50/p65 overexpression and the deletion of the two consensus κB sites did not modify this activity (data not shown), confirming that NF-κB could not bind to these sites and suggesting an indirect effect. The P2-reporter and the κB7-reporter constructs were transcriptionally activated by p50/p65 complexes but also following transfection of p50 alone (Figure 3a and b). Mutation of the κB7 site suppressed transcription in the presence of transfected p50 (Figure 3b), thus demonstrating that the p50 effect on this fragment relies on the integrity of this degenerated κB response element. Finally, we showed that p52 or p100 was also able to transactivate the κB7 promoter (Figure 3c).

Figure 1 bcl-2 promoter constructs. The human bcl-2 upstream region contains two promoters: P1 and P2. P1, 1400 bp upstream of the open reading frame (ORF), displays multiple initiation sites (black arrows); P2 is located just upstream of the ORF (from −750 to −8) and displays one initiation site. Two κB consensus sites are located at −2306 (κBI) and −1896 (κBII) (black circles). The seven sites presenting one mismatch (1–7) are located at positions −3980, −3423, −2581, −1666, −1341, −629 and −170 and are represented as gray boxes. Plasmid constructs are illustrated as follows: P1+P2Luc, P2Luc and κB7Luc (white boxes) were cloned upstream of a Luc gene (a generous gift from L Boxer). P2Gal, κB7Gal and κB7mGal were cloned upstream of a β-gal-reporter gene (gray boxes). κB7m contains a PCR-generated mutated κB7 site (TTTAAACACC instead of GGGAAACACC).
Bcl-3 forms a transactivating complex with p50 or p52 homodimers on the bcl-2 promoter

As shown above, (i) p50 or p52 can bind to a site in the bcl-2 promoter, and (ii) p50 or p52 can transactivate the bcl-2 promoter in transient transfection assays. However, these two proteins are devoid of any transactivating domain and thus require an endogenous partner to exert these activities.

Bcl-3 can interact with p50 or p52 to form a transcriptionally active complex (see Introduction) and Bcl-3 is expressed at low but detectable levels in HCT116 cells (Figure 4b, third panel from the top, left lane).

We therefore determined whether such complexes could influence the activity of the bcl-2 promoter. We first cotransfected expression vectors for p50 and Bcl-3 together with the (−178/−8) reporter plasmid. As shown in Figure 4a, while p50 and Bcl-3 alone weakly transactivated the reporter plasmid, cotransfection of Bcl-3 expression vector resulted in a dose-dependent increase of luciferase activity. Moreover, immunoblot analyses performed in parallel with nuclear extracts from HCT116 cells transfected with the p50 expression vector alone or together with increasing amounts of the Bcl-3 expression vector showed that Bcl-3 increasing presence in the nucleus (lower panel) resulted in a dose-dependent increase of p50 protein expression in the nucleus (middle panel) (Figure 4b). The enhanced p50 nuclear expression was also observed by EMSA performed with these same nuclear extracts and a kB7 probe, indicating that increased p50 nuclear expression in the presence of Bcl-3 could lead to increased DNA-binding activity (upper panel) (Figure 4b).

Bcl-3 binds to the bcl-2 promoter. (a) NF-κB does not bind to kB1 and kBII sites from the bcl-2 promoter. Nuclear extracts were prepared from HCT116 cells either unstimulated or treated with TNF-α for 30 min. These extracts were analyzed for DNA-binding activity to a palindromic kB probe (kB) or to two kB probes derived from the bcl-2 promoter (kB1 and kBII). Supershift experiments were performed with antibodies directed against p50 (lane 9), p65 (lane 10) or c-Rel (lane 11), as indicated. Lanes 1–3: binding to the kB1 probe from the Bcl-2 promoter; lanes 4–6: binding to the kBII probe from the Bcl-2 promoter; lanes 7–11: binding to a palindromic kB probe. (b) NF-κB binds the kB7 probe. Nuclear extracts binding was tested with a palindromic kB probe, a kB7 probe (GGGAAACACC) or a mutated kB7 probe (TTTAAACC). Nuclear extracts from untreated cells (−) and cells treated with TNF-α for 30 or 60 min were tested, as indicated. Supershift experiments were performed with an antibody directed against p50. Upper and lower black arrows indicate p65/RelA and p50 complexes, respectively. The asterisk indicates the p50 supershift. (c) NF-κB does not bind the mutated kB7 probe. Nuclear extracts binding was tested with a palindromic kB probe, a kB7 probe (GGGAAACACC) or a mutated kB7 probe (TTTAAACC). Nuclear extracts from untreated cells (−) and cells treated with TNF-α for 30 or 60 min were tested, as indicated. Supershift experiments were performed with antibodies directed against p50 and p50, as indicated.

Figure 2

NF-κB induction of Bcl-2 expression

P Viatour et al

Leukemia
As we and others previously showed that NF-κB induces Bcl-2 expression in human cancers, we investigated whether this expression could be correlated with Bcl-2 expression levels. We thus generated an MCF7AZ stable transfectant overexpressing NF-κB/p100 and the p52 processed protein. This overexpression led to a clear enhanced Bcl-2 expression in p100-transfected cells compared to mock-transfected cells (Figure 5a).

We then studied the expression of p100/p52 and Bcl-2 in seven primary human breast cancers. Six out of seven samples showed a higher p100/p52 expression in the cancer tissue than in normal adjacent tissue (Figure 5b, upper panel), while p50 expression is similar (data not shown). This p100/p52 expression was associated, in five samples (sample numbers 1–6), with a significant Bcl-2 expression (Figure 5b, lower panel). Meanwhile, Bcl-3 expression was shown to be unmodified between adjacent or tumoral tissues (data not shown).

As previously mentioned, the bcl-3 gene was cloned at the breakpoint of the t(14;19) translocation in CLL, resulting in a high Bcl-3 expression. Western blotting analyses of eight CLL samples showed that samples bearing a t(14;19) translocation expressed high amounts of Bcl-3 (Figure 5c, samples 5–8). Another sample (lane 2) also showed a high Bcl-3 expression. Three out of eight samples (numbers 2, 5 and 6) also showed a significant p100/p52 expression. Interestingly, Bcl-2 expression was easily detectable in all samples where p100/p52 was observed. Since the amount of cells collected was very low, we were only able to obtain nuclear extracts from the four t(14;19)

Correlation between p52, Bcl-3 and Bcl-2 expression in human cancers

As we and others previously showed that NF-κB/p100 is overexpressed in breast cancers as compared to normal adjacent tissue, we investigated whether this expression could be correlated with Bcl-2 expression levels. We thus generated an MCF7AZ stable transfectant overexpressing NF-κB/p100 and the p52 processed protein. This overexpression led to a clear enhanced Bcl-2 expression in p100-transfected cells compared to mock-transfected cells (Figure 5a).

We then studied the expression of p100/p52 and Bcl-2 in seven primary human breast cancers. Six out of seven samples showed a higher p100/p52 expression in the cancer tissue than in normal adjacent tissue (Figure 5b, upper panel), while p50 expression is similar (data not shown). This p100/p52 expression was associated, in five samples (sample numbers 1–6), with a significant Bcl-2 expression (Figure 5b, lower panel). Meanwhile, Bcl-3 expression was shown to be unmodified between adjacent or tumoral tissues (data not shown).

As previously mentioned, the bcl-3 gene was cloned at the breakpoint of the t(14;19) translocation in CLL, resulting in a high Bcl-3 expression. Western blotting analyses of eight CLL samples showed that samples bearing a t(14;19) translocation expressed high amounts of Bcl-3 (Figure 5c, samples 5–8). Another sample (lane 2) also showed a high Bcl-3 expression. Three out of eight samples (numbers 2, 5 and 6) also showed a significant p100/p52 expression. Interestingly, Bcl-2 expression was easily detectable in all samples where p100/p52 was observed. Since the amount of cells collected was very low, we were only able to obtain nuclear extracts from the four t(14;19)

Correlation between p52, Bcl-3 and Bcl-2 expression in human cancers

As we and others previously showed that NF-κB/p100 is overexpressed in breast cancers as compared to normal adjacent tissue, we investigated whether this expression could be correlated with Bcl-2 expression levels. We thus generated an MCF7AZ stable transfectant overexpressing NF-κB/p100 and the p52 processed protein. This overexpression led to a clear enhanced Bcl-2 expression in p100-transfected cells compared to mock-transfected cells (Figure 5a).

We then studied the expression of p100/p52 and Bcl-2 in seven primary human breast cancers. Six out of seven samples showed a higher p100/p52 expression in the cancer tissue than in normal adjacent tissue (Figure 5b, upper panel), while p50 expression is similar (data not shown). This p100/p52 expression was associated, in five samples (sample numbers 1–6), with a significant Bcl-2 expression (Figure 5b, lower panel). Meanwhile, Bcl-3 expression was shown to be unmodified between adjacent or tumoral tissues (data not shown).

As previously mentioned, the bcl-3 gene was cloned at the breakpoint of the t(14;19) translocation in CLL, resulting in a high Bcl-3 expression. Western blotting analyses of eight CLL samples showed that samples bearing a t(14;19) translocation expressed high amounts of Bcl-3 (Figure 5c, samples 5–8). Another sample (lane 2) also showed a high Bcl-3 expression. Three out of eight samples (numbers 2, 5 and 6) also showed a significant p100/p52 expression. Interestingly, Bcl-2 expression was easily detectable in all samples where p100/p52 was observed. Since the amount of cells collected was very low, we were only able to obtain nuclear extracts from the four t(14;19)
a

AZ AZ p100

p100

p52

Bcl-2

ns

b

p100

p52

Bcl-2

ns

c

Untranslated

1(14;19)

Bcl-3

p100

p52

Bcl-2

ns

d

1(14;19)

Probe xB

5 6 7 8

Figure 5  p100/p52 and Bcl-3 expression correlates with Bcl-2 expression in human cancer cells and primary tumors. (a) p100 stable overexpression enhances endogenous Bcl-2 expression. Upper panel: p100/p52 expression in transfected (AZ p100) vs control MCF7A/Z cells. Lower panel: basal Bc-2 expression was examined by Western blotting in the same control and stably transfected cells. As a control for equivalent loadings, we showed a nonspecific band observed on the same gel (NS). (b) Bcl-2 expression correlates with p52 expression in breast cancer samples. Total cell lysates from tumor (T) or adjacent (N) breast tissue were assayed by Western blotting with specific antibodies against NF-κB2/p100 (upper panel) or Bcl-2 (lower panel). (c) Bcl-2 expression correlates with p52 expression in CLL. Total cell lysates from blood or bone marrow samples from eight CLL patients were assayed by Western blotting with specific antibodies against Bcl-2 (lower panel), Bcl-3 (upper panel) and p100/p52 (middle panel). Lanes 1–4: CLL patients; lanes 5–8 CLL patients with a t(14;19) translocation. (d) Binding to a xB probe: Nuclear extracts from t(14;19) CLLs (samples 5–8) were analyzed for DNA binding to a palindromic xB probe.

CLL samples (5–8). We performed EMSA with these nuclear extracts and showed that binding to a palindromic xB probe was stronger with samples 5 and 6, which precisely showed the highest Bcl-2 expression, whereas samples 7 and 8 that do not express high levels of Bcl-2 did not show detectable NF-κB binding activity in similar experiments (Figure 5d). Taken together, our results suggest a potential link between expression levels of p52 and Bcl-2, therefore providing evidence for bcl-2 being a target gene of this NF-κB protein.

Discussion

The role of NF-κB in the process of apoptosis has been vastly debated these last years. NF-κB has already been reported to regulate the expression of several genes controlling apoptosis. Bcl-2-like proteins are important regulators of programmed cell death and the expression of Bcl-XL and A1/Bfl-1, two antiapoptotic members of this family, is regulated by NF-κB, while NF-κB downregulates the expression of the proapoptotic Bax protein. Although several authors failed to see any regulation of Bcl-2 expression by NF-κB, a few studies recently showed that in some circumstances, Bcl-2 could indeed be induced by NF-κB. These studies are quite important in the context of CLL. Indeed, Bcl-2 expression in cells from CLL patients has been frequently observed and it has been reported that Bcl-2 expression correlates with prognosis, stage of the disease and resistance to chlorambucil. Moreover, constitutive and CD40-induced NF-κB activity has also been described in CLls and could inhibit apoptosis and participate in resistance to chemotherapy. This last point is very relevant as CD40 induces p100 processing into p52. Therefore, if a relation between these two events could be established, it would provide a new insight into molecular mechanisms leading to CLL progression and resistance to treatment and a new rationale for NF-κB targeting therapeutic agents.

The present report identifies a xB site in the P2 bcl-2 promoter that had already been identified in another study. However, in our hands, this xB site was found to be specifically bound by p50 and p52 homodimers and could be transactivated following exogenous expression of these complexes. These DNA-binding and transactivating activities are further increased by simultaneous Bcl-3 expression, an oncprotein known to interact with p50 and p52 in the nucleus and to contain transactivating domains. These data therefore provide a first insight into a putative biological role for p52 complexes, probably in association with Bcl-3. This is supported by several observations: (i) NF-κB2 stable overexpression led to an enhanced Bcl-2 level (Figure 5a), (ii) NF-κB2 and Bcl-2 expression levels correlate in two types of cancer (Figure 5b and c), and (iii) BAFF, probably by enhancing p100 processing, enhances Bcl-2 expression in transitional B cells.

There is a clear redundancy between p50 and p52 biological roles but also some new overlap between p52 and Bcl-3 functions, as indicated by the phenotypes of the KO mice. Simultaneous inactivation of the NF-κB1/p105/p50 and NF-κB2/p100/p52 genes generates mice with a decreased Bcl-2 expression in early B cells. Meanwhile, Bcl-2 expression is unmodified in NF-κB1/p105/p50 and NF-κB2/p100/p52 (U Siebenlist, personal communication) KO mice. These data suggest that p52 and p50 proteins regulate the bcl-2 gene in vivo in a redundant but essential way. Our data demonstrating an increased Bcl-2 expression in p100/p52 overexpressing MCF7 A/Z cells also indicate that p100/p52 regulates Bcl-2 expression.

The p30 and p52 interacting proteins that confer transactivation abilities to the homodimers for upregulation of Bcl-2 are currently unknown. According to our data, it could be p65 or Bcl-3. Indeed, Bcl-3 expression increases p50 and p52 nuclear translocation and their transactivating properties on the Bcl-2 promoter. As Bcl-3 and p52 are both required for a proper development of the splenic microarchitecture and germinal centers, it is likely that they form complexes that control the expression of survival and developmental genes.

The present data are also important for understanding the NF-κB biochemical function in cancer cells. Indeed, NF-κB is constitutively active in various cancers, such as multiple myelomas, CLL, or breast, ovarian, colon and skin cancers. Such activity was also found in Hodgkin's
lymphomas, probably as a consequence of a loss of IκBz activity or constitutive IKK activity. In some of these cancers, NF-κB inhibition by an IκBz-super-repressor is sufficient to induce apoptosis, thus demonstrating an antiapoptotic role for NF-κB in these cell systems. Moreover, in two different types of cancers, we observed a putative correlation between levels of p52 and Bcl-2 expression. If confirmed on larger series, this observation will demonstrate that p52, possibly in association with Bcl-3, plays a major role in the progression of very different cancers. Therefore, besides the ubiquitous NF-κB complexes, there exist other proteins from the same family that will have to be considered in our understanding of the cancer antiapoptotic pathways and in our design of specific treatments.

Indeed, as NF-κB is considered as a target for anticancer therapy and novel therapeutic agents are currently being developed in this prospect, a precise understanding of the regulation of NF-κB antiapoptotic target genes in cancer cells will be very helpful to delineate the optimal targets for these treatments and to understand their successes and failures. It is indeed possible that proteasome or IKK inhibitors will not be as efficient on cells expressing high levels of p52 and Bcl-3 than on cells constitutively expressing nuclear p50/p65 complexes. The exact activity of these novel inhibitors and their effect on distinct NF-κB complexes and on target gene expression, including Bcl-2, will thus have to be studied in various cell types expressing different NF-κB-related complexes.

Acknowledgements

We thank Dr U Siebenlist (NIH, Bethesda, MD, USA) for his helpful comments on the manuscript. Dr L Boxer (Stanford, CA, USA) for the Bcl-2 Luc plasmids. Dr Hagemeier (Leuven, Belgium) and Dr Lambert (CHU Liège, Belgium) for LLC samples. We are most thankful to Professor J Gielien (University of Liège, Belgium) for his continuous support during this work. M-P Viatour, M Bentires-Alj and V Deregowski are supported by the National Foundation for Scientific Research (FNRS, Belgium). P Viatour, M Bentires-Alj and V Deregowski are supported by grants from the ‘Centre Anti-Cancereux’ (University of Liège, Belgium) and the FRSM (FNRS, Belgium).

References

NF-κB induces Bcl-2 expression

P Vlahou et al

31 Cogswell PC, Guttridge DC, Funkhouser WK, Baldwin AS. Selective activation of NF-κB subunits in human breast cancer: potential roles for NF-κBp2p2 in mouse.

32 Dejardin E, Bonizzi G, Behlache C, Canuto G, Merville MP, Bouras V. Highly expressed p100/p105 (NFKB2) sequesters other NF-κB-related proteins in the cytoplasm of human breast cancer cells.


36 Jones PL, Ping D, Boss JM. Tumor necrosis factor alpha and B cell lines.


