Analysis of a regulatory element in the 5′-untranslated region of the *bcl-2* gene

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Abstract The *bcl-2* gene is an important antagonist of apoptosis, the programmed cell death. *Bcl-2* is highly expressed in a variety of lymphomas. Lymphocytes of patients with chronic lymphocytic leukemia (CLL) express high amounts of *bcl-2* even in the absence of the t(14;18) translocation, resulting in a strong resistance towards corticosteroid induced apoptosis. Within the 5′-untranslated region of the *bcl-2* gene a p53 dependent negative response element has been described. Genetic alterations within this element could lead to uncontrolled overexpression of *bcl-2* and subsequent resistance towards apoptosis. We therefore analyzed the mRNA from the 5′-untranslated region −279 to −85 bp of the *bcl-2* gene by direct PCR sequencing from peripheral blood derived lymphocytes from patients with CLL and normal healthy donors. Compared to published sequences (Tsujimoto and Croce (1986) Proc. Natl. Acad. Sci. USA 83, 5214), we consistently found an exchange at position 1271 from A to G and at position 1284 from G to A in all CLL as well as normal donor derived samples analyzed. Thus, CLL specific alterations compared to normal cells could not be found and deregulated expression of *bcl-2* in CLL cells does not appear to be due to alterations in the p53 dependent negative response element of the *bcl-2* gene. However, our data add information to published sequence data of this region.

Key words: *bcl-2*; p53; Chronic lymphocytic leukemia

1. Introduction

bcl-2 is an important regulator of cell death [1]. Overexpression has been found originally in the context of t(14;18) translocations in follicular lymphoma [2,3]. However, high levels of *bcl-2* are found even in the absence of t(14;18) in a variety of lymphoma tissues [4–6] as well as normal tissue progenitors [7,8]. Lymphocytes from patients with chronic lymphocytic leukemia (CLL) express high amounts of *bcl-2*, although a t(14;18) is not present in this disease entity [9]. Furthermore, CLL cells are resistant towards induction of apoptosis, e.g. with corticosteroids [10].

Recently, a negative regulatory element (NRE) in the 5′-untranslated region of *bcl-2* has been described [11]. This element inhibits expression from an upstream promoter and is responsive to the effects of p53 [12]. Experimentally induced mutations within this untranslated region corresponding to position −279 to −85 bp of the *bcl-2* gene abolish the effect of the NRE leading to uncontrolled overexpression of *bcl-2*.

The aim of our study was the analysis of CLL cells in order to look for mutations within this part of the *bcl-2* gene in correlation with a specific state of disease. By using direct PCR amplification we found sequence variations compared to published data. However, base pair exchanges were consistently found in CLL as well as normal cells.

2. Material and methods

Peripheral blood mononuclear cells (PBMNCs) were obtained from healthy normal donors and patients with CLL, as leftovers from diagnostic samples. CLL patients presented with various stages of disease (RAI I–IV). PBMNCs were prepared after Ficoll centrifugation and were frozen at −80°C.

2.1. RNA extraction, cDNA preparation and PCR

Extraction of RNA was performed using a guanidinium-isothiocyanate and phenol based method with a commercially available kit (Ultraspec RNA Isolation System, Campro Sci.) followed by DNase digestion (RQ1 RNase-free DNase, Promega). 20 μl DNase was used in a total volume of 200 μl for 30 min at 37°C followed by phenol/chloroform extraction and ethanol precipitation. 3 μg RNA was transcribed into cDNA using SuperScript RNase H-Reverse Transcriptase (Gibco BRL) for 10 min at 23°C, 60 min at 45°C, and 5 min at 95°C. PCR amplification of cDNA was performed using the GeneAmp PCR Reagent Kit with AmpliTaq DNA Polymerase (Perkin Elmer) and FPLC purified primers. In 50 μl final volume 0.4 μl of AmpliTaq Polymerase (5 U/μl) 30 pmol primer each and 1% DMSO were used for the amplification of a 194 bp large fragment corresponding to position −279 to −85 5′ of the *bcl-2* gene. Primer *bcl-2*-279S (5′A-CAGAGGAAG TAGACTGATA TTAAC) and *bcl-2*-85A (5′A-GAGGAGTTA TAATCCAGCT ATTT) [12] were used with an annealing temperature of 63°C for 35 cycles. For the quality control of cDNA the housekeeping gene PBGD was used [5].

2.2. PCR sequencing

PCR fragments were separated in a 2% agarose gel and after staining with ethidium bromide bands were cut out and the DNA was extracted using a kit (QIAquick, QIAgen). Direct sequencing using 33p end labeling was performed with a SequiTherm Cycle Sequencing Kit (Biozym). Each probe was analyzed twice and in sense and antisense directions. Sequences were read into a computer program (DNASIS) and compared to published sequences [13,14].

3. Results and discussion

Samples from 15 patients with CLL and two healthy normal donors were analyzed. By repeating the sequencing analysis at least once using the sense and antisense primers, respectively, the sequence from position −279 to −85 of the 5′-untranslated region of *bcl-2* for nine individual patients could be read reliably and only fully interpretable unequivocal data were used for the final analysis. In six patients the RNA was of poor quality not allowing reliable sequence analysis. No major deletions were found within this region. However, in all the other nine CLL and in both normal donor samples adenine was substituted for guanine at position 1271 of the *bcl-2* mRNA and guanine was substituted for adenine at po-

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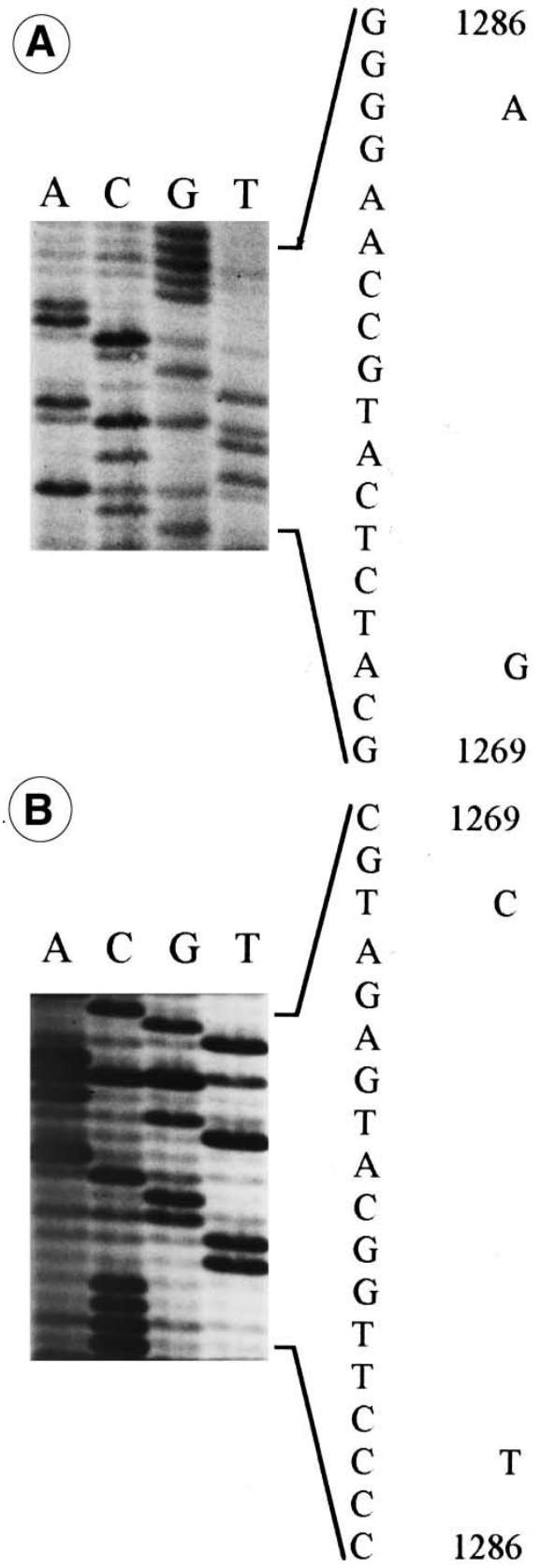

Fig. 1. Direct PCR sequencing using sense (A) or antisense (B) primer from CLL patient No. 15 (A) and normal donor No. 2 (B).

sition 1284 (Fig. 1). No other consistent sequence deviation compared to Tsujimoto et al. [13] was detected.

The finding of a negative regulatory element in the 5′-untranslated region of the *bcl-2* gene which is responsive to p53 is of major interest. The relevance for a specific state of disease, however, has not been documented so far. In our attempt to look for genetic abnormalities in lymphoma cells of patients with CLL we found two sequence deviations compared to Tsujimoto et al. [13]. However, analysis of PBMNC from healthy donors showed the same base substitutes. Therefore these changes can not be regarded as disease specific. The complete mRNA sequence including the 5′-untranslated region has been published by two groups [13,14]. Within the region of the NRE Seto et al. [14] showed several base substitutes compared to the sequence published by Tsujimoto and Croce. We can confirm the exchanges at positions 1271 and 1285, but did not find any other sequence deviations from the data of Tsujimoto and Croce [13].

In conclusion, within the p53 dependent negative response element 5′ to the *bcl-2* gene two base pair exchanges were found in comparison to the reference sequence [13]. Thus, no disease specific changes in this area could be seen in samples from patients in CLL and other causes may contribute to the high levels of *bcl-2* expression in chronic lymphocytic leukemia.

References

[1] S.J. Korsmeyer, Blood 80 (1992) 879–886.
[2] M.L. Cleary, J. Sklar, Proc. Natl. Acad. Sci. USA 82 (1985) 7439–7443.
[3] Y. Tsujimoto, J. Cossman, E. Jaffe, C.M. Croce, Science 228 (1985) 1440–1443.
[4] F. Pezzella, A.G.D. Tse, J.L. Cordell, K.A.F. Pulford, K.C. Gatter, D.Y. Mason, Am. J. Pathol. 137 (1990) 225–232.
[5] J. Finke, R. Fritzen, P. Ternes, P. Trivedi, K.J. Bross, W. Lange, R. Mertelsmann, G. Dölken, Blood 80 (1992) 459–469.
[6] D.M. Hockenbery, M. Zutter, W. Hickey, M. Nahm, S.J. Korsmeyer, Proc. Natl. Acad. Sci. USA 88 (1991) 6961–6965.
[7] D. Delia, A. Aiello, D. Soligo, E. Fontanella, C. Melani, F. Pezzella, M.A. Pierotti, G. Della Porta, Blood 79 (1992) 1291–1298.
[8] D. De Jong, F.A. Prins, D.Y. Mason, J.C. Reed, G.B. Van Ommen, P.M. Kluin, Cancer Res. 54 (1994) 256–260.
[9] M. Schena, L.-G. Larsson, D. Gottardi, G. Gaidano, M. Carlsson, K. Nilsson, F. Caligaris-Cappio, Blood 79 (1992) 2981–2989.
[10] M.Y. Mapara, R. Bargou, C. Zugck, H. Döhner, F. Ustaoglu, R.R. Jonker, P.H. Krammer, B. Dörken, Eur. J. Immunol. 23 (1993) 702–708.
[11] R.L. Young, S.J. Korsmeyer, Mol. Cell. Biol. 13 (1993) 3686–3697.
[12] T. Miyashita, M. Harigai, M. Hanada, J.C. Reed, Cancer Res. 54 (1994) 3131–3135.
[13] Y. Tsujimoto, C.M. Croce, Proc. Natl. Acad. Sci. USA 83 (1986) 5214–5218.
[14] M. Seto, U. Jaeger, R.D. Hockett, W. Graninger, S. Bennett, P. Goldman, S.J. Korsmeyer, EMBO J. 7 (1988) 123–131.