Inactivating mutations and overexpression of *BCL10*, a caspase recruitment domain-containing gene, in MALT lymphoma with t(1;14)(p22;q32)

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Mucosa-associated lymphoid tissue (MALT) lymphomas most frequently involve the gastrointestinal tract and are the most common subset of extranodal non-Hodgkin lymphoma[1] (NHL). Here we describe overexpression of *BCL10*, a novel apoptotic signalling gene that encodes an amino-terminal caspase recruitment domain (CARD; ref. 2), in MALT lymphomas due to the recurrent t(1;14)(p22;q32) (ref. 3). *BCL10* cDNAs from t(1;14)-positive MALT tumours contained a variety of mutations, most resulting in truncations either in or carboxy terminal to the CARD. Wild-type BCL10 activated NF-κB but induced apoptosis of MCF7 and 293 cells. CARD-truncation mutants were unable to induce cell death or activate NF-κB, whereas mutants with C-terminal truncations retained NF-κB activation but did not induce apoptosis. Mutant BCL10 overexpression might have a twofold lymphomagenic effect: loss of BCL10 pro-apoptosis may confer a survival advantage to MALT B-cells, and constitutive NF-κB activation may provide both anti-apoptotic and proliferative signals mediated via its transcriptional targets.

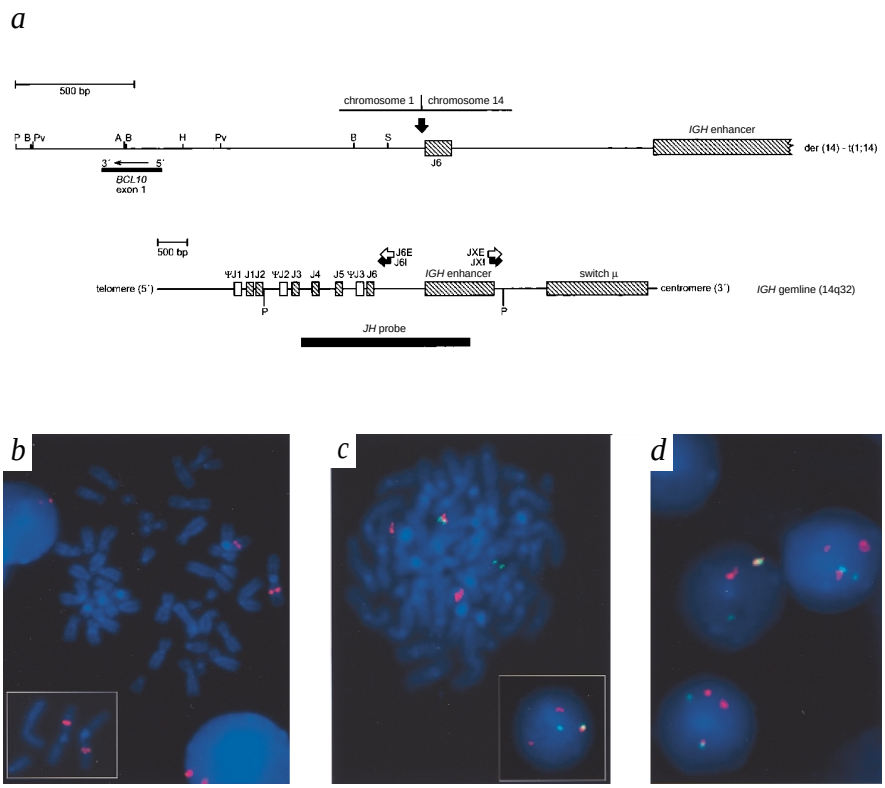

Fig. 1 Genomic cloning of t(1;14)(p22;q32). ***a***, Schematic of t(1;14)(p22;q32) genomic breakpoint. The restriction map shows the chimaeric der(14) chromosome fragment generated by the t(1;14); the breakpoint is indicated by an arrow. '*BCL10* exon 1' indicates the 255-bp segment that showed identity to EST sequences and is the most 5' exon of *BCL10*. Bottom, corresponding segment from normal *IGH*. J6E/JXE and J6I/JXI are primer pairs used for LDI-PCR of rearranged *Pst*I fragments identified in MALT lymphoma DNAs with the *JH* probe. *Pst*I sites only are shown for the *IGH* locus. A, *Apa*I; B, *Bst*XI; H, *Hin*cII; P, *Pst*I; Pv, *Pvu*II; S, *Sma*I. *b–d*, FISH analysis. ***b***, Chromosomal assignment of *BCL10*-containing genomic clones. FISH of normal metaphase and interphase cells with pooled non-chimaeric YACs (876g12, 769e11 and 937c11) or PACH 211K24 (inset), showing bright signals at chromosome 1p22. Detection of t(1;14) in MALT lymphomas from patients 1 (***c***) and 2 (***d***) by FISH on metaphase and interphase cells is shown. The pooled *BCL10*-specific YAC probes 876g12, 769e11 and 937c11 hybridize to 1p22 (red) and the *IGH* constant region cosmid probe COS-cα1 to 14q32 (green). The translocation splits the signal derived from the 1p22 YAC pool, with one signal remaining on der(1) and the other co-localizing with the COS-cα1 signal on der(14). Thus, metaphase and interphase cells containing the t(1;14) display 3 red signals for 1p22 on intact chromosome 1, der(1) and der(14), respectively, and 2 green signals for the *IGH*-constant region on intact and derivative chromosomes 14. Co-localization of one signal each for 1p22 and 14q32 (red-green or yellowish hybrid signal) indicates the der(14) *BCL10-IGH* junction.

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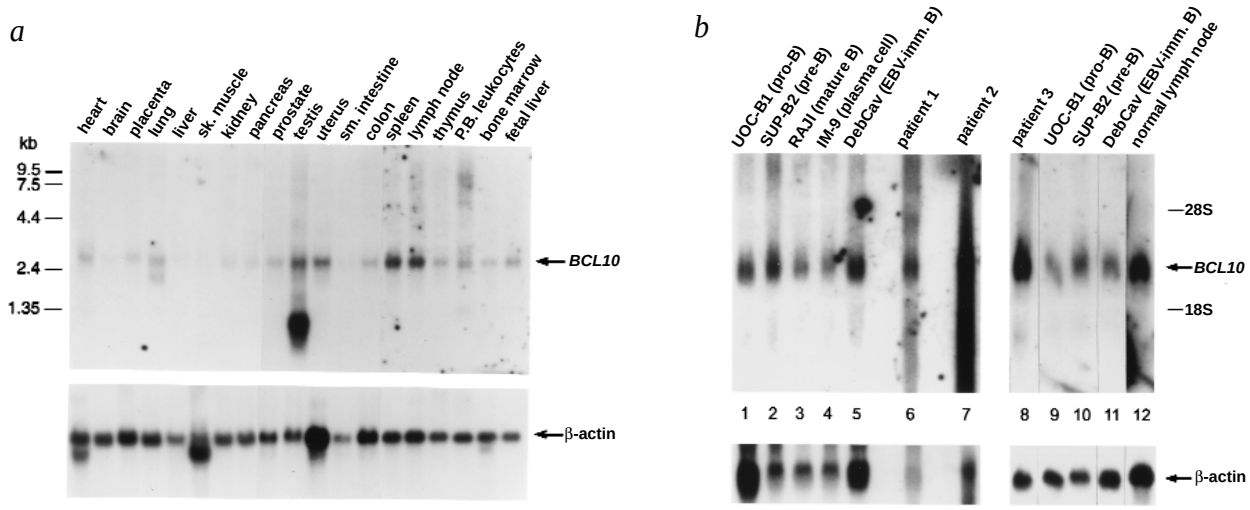


Fig. 2 *BCL10* expression. ***a***, Northern-blot analysis of *BCL10* expression in normal human tissues. Poly(A)$^+$ RNAs from various adult tissues (except fetal liver) were hybridized with *BCL10* cDNA. ***b***, Overexpression of *BCL10* in t(1;14)-positive MALT lymphomas. Normal human lymph-node poly(A)$^+$ RNA (1 μg), total RNAs (10 μg for patient 1; 25 μg for all others) from B-cell lines lacking the t(1;14) (UOC-B1, SUP-B2, RAJI, IM-9, DebCav) and three t(1;14)-positive MALT lymphomas (patients 1, 2 and 3) were analysed by northern-blot hybridization using a *BCL10* cDNA probe. Results for patient 3 are shown separately because the hybridizations were performed at a later time. B-cell lines represent cells transformed at various stages of differentiation (UOC-B1, pro-B acute lymphocytic leukaemia; SUP-B2, pre-B acute lymphocytic leukaemia; RAJI, Burkitt lymphoma; IM-9, multiple myeloma) and an EBV-immortalized human lymphoblastoid line (DebCav). Normalization of *BCL10* transcript levels against β-actin revealed *BCL10* overexpression in the tumour of patient 2 and a lesser but significant increase in *BCL10* transcription in the MALT lymphoma from patient 1, compared with t(1;14)-negative cell lines (for example, compare signal intensity of *BCL10* to β-actin for lane 6 with that for lane 1). Northern analysis of RNA from patient 3 also demonstrated significant *BCL10* overexpression (lane 8), especially given that less than 30% of cells in the tumour sample from this patient contained t(1;14), as determined by FISH.

Recurrent chromosome 1p22 abnormalities occur in MALT and other lymphoma subtypes, including 8% of large-cell diffuse NHL cases, 10% of follicular NHL cases and up to 30% of Hodgkin disease cases[3–6]. To identify the 1p22 gene involved in lymphomagenesis, we cloned the t(1;14)(p22;q32) genomic breakpoint from two MALT tumours (patients 1 and 2) using long-distance inverse PCR (LDI-PCR; Fig. 1*a*). In both cases, the chromosome 14 breaks occurred 5′ of *IGHJ* exon 6, juxtaposing a chromosome 1 DNA segment containing a 255-bp region that has identity to several expressed sequence tags from a germinal center B-cell cDNA library.

To further characterize the chromosome 1 breakpoint region, including the segment translocated to der(1), we isolated large genomic clones spanning this area by PCR-based screening. One clone, YAC876g12 (CEPHyG12876), had been assigned (Whitehead Institute physical mapping project) to chromosome 1, position 119 cM (genetic map) and approximately 240 cR (radiation hybrid map), confirming that we had isolated the region encompassing chromosome 1p22. As the overlapping YAC clones 769e11, 956e11, 937c11 and 852g5 also contained the STS defined by our PCR primers, the most likely position of the breakpoint region is between STS WI-10259 (*D1S3417*) and WI-2403 (*D1S2449*), according to the Whitehead contig WC1.14. Fluorescence *in situ* hybridization (FISH) using these clones and the one BAC and three PAC clones isolated established 1p22 assignment (Fig. 1*b*, and data not shown). FISH analysis of tumour metaphases from patients 1 and 2 revealed co-localization of 1p22-specific and COS-cα1 *IGH* constant region probes on der(14), indicative of t(1;14) (Fig. 1*c*, and data not shown). Our analysis of tumour interphases showed that signals for 1p22 and *IGH* co-localized for 59.9% (197/329) and 72.8% (190/261) of nuclei for patients 1 and 2, respectively (Fig. 1*c*,*d*). In patient 3, t(1;14) was observed in 27.9% (39/140) of bone marrow and 17.8% (71/399) of peripheral blood cells. A fourth MALT lymphoma patient was found to contain the translocation in 17.9% (68/380) of bone marrow cells (data not shown).

We identified a candidate gene, which we called *BCL10* to denote its involvement in B-cell lymphoid malignancy, within the region of chromosome 1 translocated in t(1;14)-positive tumours. Northern-blot analysis with a partial-length cDNA sequence of *BCL10* identified a 2.8-kb transcript that is expressed at relatively low levels in all normal tissues (Fig. 2*a*). We established that *BCL10* is overexpressed in three t(1;14)-positive MALT lymphomas (Fig. 2*b*).

The 2.8-kb full-length sequence of *BCL10* was isolated by 3′-RACE and contains a 699-bp ORF, with 5′ and 3′ UTRs of 706 bp and 1,373 bp. To determine *BCL10* genomic structure, we sequenced the approximately 140-kb insert of the 1p22-specific PAC clone (PACH-211K24), which we have demonstrated by FISH to span the breakpoint (data not shown). *BCL10* is composed of 4 exons (of 255 bp, 501 bp, 289 bp and 1,733 bp, 5′ to 3′) within an approximately 11.7-kb genomic segment. The entire *BCL10* locus is present in the chromosome 1 region translocated to der(14). No potential target genes remaining on the der(1) that might be transcriptionally upregulated by juxtaposition downstream of 5′ *IGH* sequences were located on PACH-211K24 in the approximately 110-kb chromosome 1 region centromic to the breakpoint. *BCL10* is oriented 5′ (centromeric)–3′ (telomeric); thus it is juxtaposed intact adjacent to *IGH* on der(14), with the genes in opposite orientations.

Database searches revealed that BCL10 (233 aa, predicted mass of 26 kD) has 29% identity (35.5% similarity) with ORF E10 of γ herpesvirus equine herpesvirus 2 (EHV-2; Fig. 3*a*; ref. 7). Although not known to be tumorigenic, EHV-2 is highly related to herpesvirus saimiri, which causes lymphoma in New World primates, and to Epstein-Barr virus and human herpesvirus 8, which are implicated in Burkitt lymphoma and lymphomas in immunocompromised patients[8]. Comparisons among apoptotic signalling proteins (including pro-apoptotic RAIDD/CRADD, Ced4/Apaf-1, caspase-1, caspase-2, caspase-9, CED-3 and anti-apoptotic c-IAP1, c-IAP2 and ARC) recently revealed that EHV-2 E10 contains a CARD, a motif that medi-

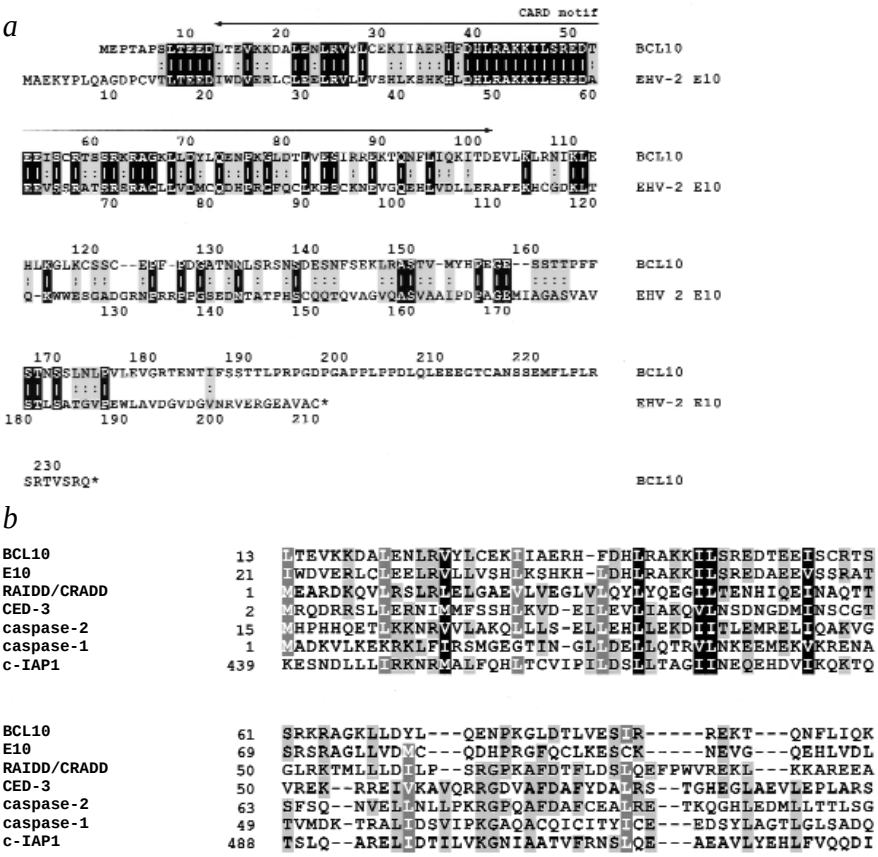
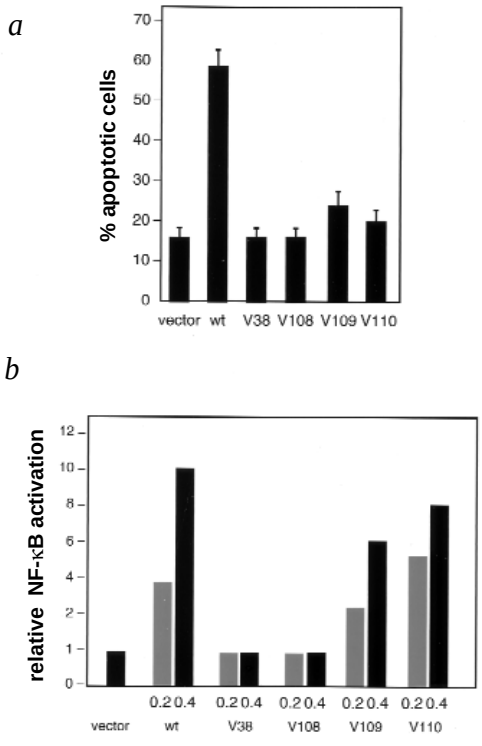


Fig. 3 Alignment of BCL10 with EHV-2 E10 (*a*) and comparison of the BCL10 CARD motif with other apoptotic signalling proteins (*b*). ***a***, Sequence identity, reverse lettering; amino acid similarity, shading and double dots (:). The CARD motifs of the proteins are indicated. The 210-aa EHV-2 E10 sequence has been reported[7]. ***b***, A CARD motif alignment of BCL10, E10 and several known apoptotic signalling proteins is shown. Numbers indicate the position of the domain within the protein sequence. All proteins are human except CED-3 (*Caenorhabditis elegans*) and E10 (equine herpesvirus-2).

ates interactions between CARD-containing proteins and which bears sequence similarities to the death domain and death-effector domain of cell-death proteins[2,9]. BCL10 also contains a CARD, with highest homology between BCL10 and E10 in this region (Fig. 3*a*,*b*).



On the basis of the predicted function of E10 (ref. 2) and analogous to BCL2 involvement in NHL, we expected BCL10 to be anti-apoptotic. Expression of wild-type *BCL10* in MCF7 cells (Fig. 4*a*) or 293 (data not shown), however, resulted in apoptotic death. Furthermore, we found that *BCL10*, unlike *BCL2*, failed to enhance BaF3 pro-B cell survival upon interleukin 3 (IL-3) withdrawal, nor did it protect BJAB lymphoma cells from Fas ligand-induced apoptosis (data not shown). Similar to many cell-death signalling proteins, wild-type BCL10 was capable of NF-κB activation (Fig. 4*b*).

Given the pro-apoptotic function of BCL10, we scrutinized *BCL10* transcripts expressed by t(1;14)-positive MALT lymphomas for mutations within their ORFs and identified many abnormalities (Table 1). The regions of *BCL10* found to be recurrently mutated include the junction of exons 3 and 4 (at which loss of a splice acceptor at the exon 4 5′ boundary produced splicing aberrations with 16- or 33-bp deletions involving codons 116–121 or 116–126), as well as 2 mononucleotide stretches (8 adenines beginning at nt 129, codon 43, and 7 thymidines beginning at nt 493, codon 165) at which deletions or insertions of 1 or 2 adenines or thymidines resulted in frameshifts. These mutations tended to produce two subsets of truncated BCL10: proteins (of 33–101 aa) truncated within the CARD and proteins (of 141–222 aa) truncated C terminal to the CARD (Table 1). We also identified several *BCL10* clones encod-

Fig. 4 Cell death (*a*) and NF-κB activation (*b*) assays of wild-type *BCL10* and selected mutants. ***a***, Note that *BCL10* mutants with truncations affecting the CARD (V38, V108), as well as those with truncations C terminal to the CARD (V109, V110), failed to induce apoptotic death. ***b***, NF-κB activation data are shown for a representative experiment. Mutants with CARD truncations (V38, V108) failed to activate NF-κB, whereas those containing truncations C terminal to the CARD (V109, V110) retained the ability to activate NF-κB almost as well as wild-type *BCL10*.

Table 1 *BCL10* mutations identified in t(1;14)-positive MALT lymphomas

Patient	RT-PCR clone	Nucleotide alteration[1]	Mutation	Exon	Codon	Type of mutation	Size of BCL10 protein (full-length=233 aa)
1	RN1	13G→T	GCA →TCA	2	5	Ala → Ser	
		del 347–379	33-bp deletion	4	116–126	splicing abnormality	222 aa
		400T→C	TCC → CCC	4	134	Ser →Pro	
	RN2	13G→T	GCA →TCA	2	5	Ala →Ser	
		47T→A	GTG→GAG	2	16	Val→Glu	
		del 347–379	33-bp deletion	4	116–126	splicing abnormality	
		400T→C	TCC→CCC	4	134	Ser → Pro	
		del 455T	1-bp deletion	4	152	truncation	160 aa
	RN13,RN21	del 129,130AA	2-bp deletion	3	43/44	truncation	48 aa
	RN109, RN112	13G→T	GCA→TCA	2	5	Ala→Ser	
		del 129A	1-bp deletion	3	43	truncation	69 aa
	RN114	91A→G	AAA→GAA	3	31	Lys→Glu	
		169T→C	TGT→CGT	3	57	Cys →Arg	
		del 347–379	33-bp deletion	4	116–126	splicing abnormality	
		ins 493T	1-bp insertion	4	165	truncation	157 aa
	RN120	13G→T	GCA→TCA	2	5	Ala→Ser	
		del beginning with 557T	279-bp deletion	4	186–233	truncation	196 aa
2	V31,V32,V40	del 347–362	16-bp deletion	4	116–121	splicing abnormality	141 aa
	V38	del 129A	1-bp deletion	3	43	truncation	69 aa
	V105	ins AG between 90G and 91A	2-bp insertion	3	30	truncation	33 aa
	V106	ins 493AA	2-bp insertion	4	165	truncation	172 aa
	V108	del 129,130AA	2-bp deletion	3	43/44	truncation	48 aa
		502 A→G	ACT→GCT	4	168	Thr→Ala	
		638 G→A	GGA→GAA	4	213	Gly→Glu	
	V109	del 351A	1-bp deletion	4	117	truncation	146 aa
	V110	ins 493A	1-bp insertion	4	165	truncation	168 aa
		638 G→A	GGA→GAA	4	213	Gly→Glu	
	V112	del 129A	1-bp deletion	3	43	truncation	69 aa
		del 347–362	16-bp deletion	4	116–121	splicing abnormality	
		638G→A	GGA→GAA	4	213	Gly→Glu	
	V115	ins 493A	1-bp insertion	4	165	truncation	168 aa
	V116	303T→A	GAT→GAA	3	101	Asp→Glu	
		ins 493AA	2-bp insertion	4	165	truncation	172 aa
		638G→A	GGA→GAA	4	213	Gly→Glu	
		688G→A	GTT→ATT	4	230	Val→Ile	
	V119	ins 271A	1-bp insertion	3	91	truncation	101 aa
		del 347–362	16-bp deletion	4	116–121	splicing abnormality	
		638 G→A	GGA→GAA	4	213	Gly→Glu	
3	LY1	521T→C	TTG→TCG	4	174	Leu→Ser	
		700 T→C	TGA→CGA	4	stop	stop→Arg	239 aa
	LY3, LY19	ins 136TT	2-bp insertion	3	46	truncation	49 aa
	LY4, LY11, LY18	del 129A	1-bp deletion	3	43	truncation	69 aa
	LY16	del 129A	1-bp deletion	3	43	truncation	69 aa
		del 347–379	33-bp deletion	4	116–126	splicing abnormality	
	LY24	del 129A	1-bp deletion	3	43	truncation	69 aa
		del 347–362	16-bp deletion	4	116–121	splicing abnormality	
	LY28, LY29, LY45	del 347–379	33-bp deletion	4	116–126	splicing abnormality	222 aa
	LY49	191G→A	AGG→AAG	3	64	Arg→Lys	
		ins 271A	1-bp insertion	3	91	truncation	101 aa

Nucleotide numbering begins at the *BCL10* initiation codon. *BCL10* coding sequences are present in exons 2, 3 and 4, with most of exon 4 being 3′ UTR. RN2 and RN112 also contain a 279-bp deletion (beginning with nt 557T) that removes codons 186–233 and a portion of the 3′ UTR. This mutation was also found in clone RN120. V106 also contains a 521T→A mutation in codon 174 resulting in a Leu→stop (TTG→TAG) change. A partial listing of all of the RT-PCR clones sequenced is shown, with clones containing a representative spectrum of the mutations identified in each tumour sample. A complete listing of all mutations identified, including silent mutations and mutations within the 3′ UTR, is available on request. The predicted sizes of the BCL10 proteins encoded by a number of the clones listed (RN1, RN2, RN21, V38, V40, V105, V108, V109 and V110) were confirmed by *in vitro* transcription/translation (data not shown).

ing full-length proteins (233 aa) with up to 3 amino acids altered; the significance of these alterations remains to be determined because they did not consistently involve a specific codon, and some may represent polymorphisms. Several clones from an individual tumour shared identical mutations, in addition to containing unique abnormalities (for example, clones V31, V112 and V119), suggesting that additional mutations accumulate in *BCL10* during tumorigenesis.

To assess the functional effects of these mutations, we analysed cell death and NF-κB activation using clones V38 and V108, which contain CARD truncations, and V109 and V110, with C-terminal truncations. We found that neither apoptosis nor NF-κB activity were induced by clones V38 and V108 (Fig. 4). Likewise, V109 and V110 failed to show induction of apoptosis; however, both retained NF-κB activation capabilities similar to wild-type BCL10 (Fig. 4*b*). Thus, full-length BCL10 is required for pro-apoptotic function but not for NF-κB activation, and an intact CARD is essential for both functions.

BCL10 is the first CARD-containing gene implicated in oncogenesis. In contrast to other lymphomagenic genes, wild-type *BCL10* does not appear to confer a growth or survival advantage. Additional analysis of BCL10 (referred to as mE10) has indicated that its C terminus binds pro-caspase-9, promoting autoproteolytic activation of the zymogen due to BCL10-mediated

oligomerization[10]. No BCL10 interactions with other CARD-containing proteins (caspase-1, caspase-2, Apaf-1, RAIDD) besides itself were identified[10]. BCL10 pro-apoptosis may normally suppress tumour formation, similar to p53 and BAX (ref. 11). Although t(1;14) may directly expose *BCL10* to *IG* somatic hypermutation (which can produce mutations in lymphomagenic genes like *BCL6* even in the absence of aberrant *IG* rearrangement[12,13]), it is likely that mutation precedes or occurs concomitant with translocation, because wild-type *BCL10* overexpression would probably be deleterious to cell survival. Additional studies are required to determine whether both *BCL10* alleles (translocated and non-translocated) in a given tumour cell are mutated, completely abolishing pro-apoptosis, or if selected mono-allelic mutations confer dominant-negative inhibition of death mediated by a remaining wild-type allele. Furthermore, the role in oncogenesis of constitutive NF-κB activation induced by C-terminal truncated BCL10 remains to be elucidated, although NF-κB may theoretically inhibit physiologic inducers of B-cell apoptosis[14,15] and the pro-apoptotic effects of genes such as *MYC* or *RAS* that might cooperate with *BCL10* in tumour development[16–19]. In addition, the NF-κB target genes *MYC* and *IL6* may be upregulated to enhance MALT B-cell growth[20–23]. Although these issues are largely unanswered, some insight has been provided by Willis and colleagues, who have also recently demonstrated *BCL10* to be the t(1;14) target gene and identified mutations in other lymphoid malignancies, such as follicular NHL and selected non-haematopoietic tumours, which all lacked chromosomal rearrangements involving the locus[24]. Although these investigators did not examine the functional consequences of BCL10 CARD truncations, they performed studies which demonstrated that wild-type BCL10 can suppress primary rat-embryo fibroblast transformation by an activated *RAS* and cooperating adenovirus *E1a*, papillomavirus *E7*, or mutant *Trp53* genes, and that C-terminal truncation mutants enhance this transformation. Whether the enhanced transformation exhibited by these mutants is due solely to NF-κB activation or involves other mechanisms remains to be determined.

Methods

Clinical cases. Tumour samples with histopathological and immunological features typical for MALT-type lymphoma[1] were studied from three patients. Cytogenetic characterization showed the following tumour karyotypes: patient 1, 50,XX,t(1;14)(p22;q32),+3,+7,+del(18)(q21),+mar; patient 2, 48,XX,t(1;14)(p22;q32),+3,+18; and patient 3, 47–49,X,–X,t(1;14)(p22;q32),+3,+12,–13,–17,+18,–21,+mar.

LDI-PCR. Rearranged *IGHJ* fragments were identified by Southern-blot hybridization using joining-region probe C76R51A. LDI-PCR was performed to clone rearranged *IGHJ* fragments using minor modifications to described methods[25]. High molecular-weight genomic DNA from the MALT lymphomas of patients 1 and 2 was digested with *Pst*I, then ligated at low concentration (0.8 ng/μl) to circularize monomeric fragments. Ligated DNA (10 ng) was used as template for a nested PCR using primers homologous to *JH* and *IGH* enhancer sequences (primer pair J6E and JXE, followed by J6I and JXI). PCR bands of expected sizes were purified, then ligated after restriction endonuclease digestion into pBluescript SK+ (Stratagene).

BAC, PAC and YAC clone isolation. Large insert genomic clones from the *BCL10* locus were identified by PCR-based screening using a primer pair (RN-C, 5′–TACAGCCTCATCCTACTTCCCTGT–3′, and RN-D, 5′–ACACACCACCACGCCCAGCTAATT–3′) derived from single-copy intronic chromosome 1 sequence located approximately 1 kb from the genomic breakpoint on der(14) of the tumours from patients 1 and 2. These primers, which amplify a 234-bp product, were used to screen human BAC and PAC libraries (Genome Systems); a single BAC clone (BACH-179L7) and three PAC clones (PACH-33c22, PACH-224i4 and PACH-211K24) were identified. YAC clones were isolated by PCR screening of the CEPH-YAC library (obtained through the German Resource Center) using RN-C/RN-D primers.

FISH analysis. Metaphase spreads and interphase nuclei of tumour cells were prepared from short-term cultures of lymphoma tissue and digested with pepsin. Control experiments were performed on metaphases from PHA-stimulated peripheral blood cultures of healthy individuals. FISH with *BCL10*-specific BAC, PAC and YAC probes and cosmid probe COS-cα1, which spans the *IGHA1* gene on chromosome 14q32, was performed as described[26].

Northern-blot analysis. RNA was isolated using acid guanidinium thiocyanate-phenol-chloroform extraction (RNA STAT-60, Tel-Test "B"). Total RNAs from t(1;14)-negative cell lines (UOC-B1, SUP-B2, RAJI, IM-9, DebCav), t(1;14)-positive MALT lymphomas and poly(A)$^+$ RNA from normal human lymph node (Clontech) were electrophoresed in a 1% agarose gel containing formaldehyde (2.2 M), then transferred to a nylon membrane by capillary transfer. Multiple-tissue northern blots (Clontech) containing poly(A)$^+$ RNA (2 μg) prepared from normal human tissues were also used. We incubated blots at 68 °C for 2 h in ExpressHyb buffer (Clontech) with a partial-length 1.46-kb *BCL10* cDNA that contains the complete coding sequence but lacks portions of the 5′ and 3′ UTRs, extending from nt 676 to 2,136. Filters were autoradiographed at –80 °C with two intensifying screens for 7 d for *BCL10* and 2 (normal tissue) or 16 (MALT lymphomas) h for β-actin.

RACE. The full-length 2.8-kb *BCL10* cDNA was cloned by 3′-RACE using Marathon Ready human uterus and spleen cDNAs (Clontech) as templates and the Marathon cDNA Amplification kit (Clontech). Sequence of the most 5′ portion of *BCL10* was determined from several overlapping sequences in the human EST database and by our sequencing of the entire 1.1-kb inserts of two clones from which these ESTs were derived (IMAGE Consortium clones 1184134 and 1252017). To obtain the full 3′ extent of *BCL10*, RACE was performed using a nested primer set (5′–CCGACCCGCTCCGCCTCCTCTCCTTCTT–3′, nt 586–613 of *BCL10*, for the initial amplification, and 5′–CCCGGAAGAAGCGCCATCTCCCGCCTCC–3′, *BCL10* nt 676–703, for the nested reaction). Separate amplifications using uterus or spleen cDNA each yielded approximately 2.1-kb products that were cloned into TA cloning vector pCR2.1 (Invitrogen) for sequencing.

Identification of *BCL10* mutations. Total RNA prepared from the MALT lymphomas of patients 1, 2 and 3 was reverse transcribed using an oligo (dT) primer, and the cDNAs used as templates for PCR with a primer pair from the 5′ UTR or 3′ UTR of *BCL10* (5′ UTR primer, 5′–GACCCGGAAGAAGCGCCATCTC–3′, nt 674–695, and 3′ UTR primer, 5′–CGT TCTTCCTAGTAAGGGTCTATTC–3′, reverse complement of nt 1,711–1,735) to amplify the entire ORF. The PCR products were subcloned into pCR2.1 (Invitrogen) for sequencing. At least two individual reverse transcriptions and two PCR amplifications using the cDNAs from these RT steps were performed with each RNA sample to reduce the possibility that spurious nucleotide alterations might be introduced. Independent clones from the tumour samples of patients 1 and 2 (30 each), and patient 3 (25), were analysed for mutations. Normal clones containing wild-type *BCL10* sequence (derived at least in part from the non-malignant cells present in each tumour specimen) and mutant clones were identified from each of the three cases. All clones were sequenced at least twice and the sequences analysed independently by two groups of investigators (Q.Z., L.X., S.W.M.; and B.H., G.B., A.R.) to ensure correct identification of all mutations.

BCL10 functional assays. Cell death and NF-κB activation assays were performed with wild-type and mutant *BCL10* cDNAs in mammalian expression plasmid pcDNA3 (Invitrogen). For apoptosis assays, MCF7 or 293 cells were transiently transfected with reporter gene β-galactosidase plasmid (0.15 μg) together with the indicated expression constructs (0.4 μg). The total amount of transfected DNA was kept constant (at 1 μg) by supplementation with empty vector. Following transfection at

30–36 h, cells were fixed with 0.5% glutaraldehyde and stained with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside. Apoptotic cells were distinguished by morphological changes including cell rounding, condensation and detachment from the cell-culture dish, as described[27]. NF-κB activation assays were performed with 293 cells seeded into 12-well plates. The cells were transfected with ELAM-luciferase reporter gene plasmid (0.25 μg), pRL-TK (25 ng) and the indicated amounts of each expression construct (either 0.2 or 0.4 μg); total transfected DNA was kept constant (at 1 μg) by empty vector supplementation. Cells were collected 40 h after transfection and reporter gene activity was determined using the Dual-Luciferase Reporter Assay system (Promega).

GenBank accession numbers. *BCL10* genomic locus from PAC clone PACH-211K24, AF097732; *BCL10* cDNA, AF082283; ESTs, AA654174, AA731881, AA761849 and AA767451.

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