available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Analysis of 11q22–q23 deletion target genes in B-cell chronic lymphocytic leukaemia: Evidence for a pathogenic role of *NPAT*, *CUL5*, and *PPP2R1B*

Claudia Kalla[a,*], *Markus O. Scheuermann*[a], *Ina Kube*[a], *Magdalena Schlotter*[a], *Daniel Mertens*[a], *Hartmut Döhner*[b], *Stephan Stilgenbauer*[b], *Peter Lichter*[a]

[a]*Division of Molecular Genetics, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany*
[b]*Department of Internal Medicine III, Medizinische Klinik der Universität Ulm, Robert-Koch-Strasse 8, D-89081 Ulm, Germany*

ARTICLE INFO

Article history:
Received 9 August 2006
Received in revised form
31 January 2007
Accepted 21 February 2007
Available online 20 April 2007

Keywords:
B-cell chronic lymphocytic leukaemia
11q22–q23
NPAT
CUL5
PPP2R1B
PP2A
Cell cycle
Apoptosis

ABSTRACT

Deletion of 11q22–q23 is associated with an aggressive course of B-cell chronic lymphocytic leukaemia (B-CLL). Since only in a subset of these cases biallelic inactivation of *ATM* was observed, we sought to identify other disease-associated genes within 11q22–q23 by analysing *NPAT* (cell-cycle regulation), *CUL5* (ubiquitin-dependent apoptosis regulation) and *PPP2R1B* (component of the cell-cycle and apoptosis regulating PP2A) for point mutations and their expression in B-CLL by single-strand conformation polymorphism/sequence analysis of the transcripts and real-time polymerase chain reaction. Though none of the genes were affected by deleterious mutations, we observed a significant down-regulation of *NPAT* in B-CLL versus CD19+ B cells and of *CUL5* in 11q deletion versus non-deletion B-CLL samples and measured reduced *PPP2R1B* transcript levels in a subset of B-CLL cases. Alternative splicing of *PPP2R1B* transcripts (skipping of exons 2/3, 3, 9) was associated with a reduced activity of protein phosphatase 2A. Together, these results implicate deregulation of the cell-cycle and apoptosis regulators *NPAT*, *CUL5* and *PPP2R1B* and a role for these genes in the pathogenesis of B-CLL.

1. Introduction

B-cell chronic lymphocytic leukaemia (B-CLL) is the most common leukaemia in adults presenting with a highly variable clinical course. Since in B-CLL genomic aberrations are important independent predictors of disease progression and survival, the identification of the involved genes has implications for the design of risk-adapted treatment strategies.[1] Deletion of chromosome region 11q22–q23 represents the second most common chromosome aberration in B-CLL and defines a subgroup of patients characterised by poor survival.[2] B-CLL tumour cells show increased survival rates, possibly due to inhibited apoptosis and genetic alteration of genes involved in cell-cycle control and cell survival.[3] The consensus 11q deletion region comprises the tumour suppressor gene *ATM*, but biallelic inactivation of *ATM* was detected only in about one-third of B-CLL cases with 11q22–q23 deletion, which is in marked contrast to the situation in mantle cell lymphoma, where *ATM* inactivation was identified as a frequent genetic alteration.[4–8] To identify a second disease-associated gene, we investigated three candidate genes within the 11q22–q23 consensus deletion region

* *Corresponding author:* Tel.: +49 6221 424619; fax: +49 6221 424639.
E-mail address: c.schaffner@dkfz.de (C. Kalla).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2007.02.005

Fig. 1 – Schematic illustration of the critical region within chromosomal bands 11q22–q23. Genetic map with genes and YAC contig: YAC clones (black) 801E11, 975H6, and 755B11 represent the consensus deletion region in B-CLL.[16]

(Fig. 1): (i) *PPP2R1B* was found mutated in sporadic cases of lung, colon[9,10] and breast cancer.[11] *PPP2R1B* (PP2A-Aβ) encodes the beta isoform of the constant regulatory subunit A of the protein phosphatase 2A (PP2A), which is implicated in the regulation of many cellular processes including cell-cycle progression and apoptosis by controlling the activity of at least 50 kinases.[12,13] (ii) *NPAT* plays a critical role in promoting cell-cycle progression and contributes to cell-cycle regulated histone gene expression.[14] (iii) *CUL5* is a component of E3 ubiquitin ligase complexes and is thereby involved in the ubiquitin-dependent degradation and control of the p53 tumour suppressor protein.[15] In order to assess the pathogenic role of *PPP2R1B*, *NPAT* and *CUL5* in B-CLL, we analysed the genes with respect to their expression and the presence of point mutations in a series of B-CLL cases.

2. Materials and methods

2.1. Patient and control material

The study comprised 66 patients with B-CLL that were diagnosed according to established morphological and immunophenotypical criteria of the German CLL study group. Dual-colour interphase FISH with YACs 801E11, 975H6 and 755B11 detected 11q22–q23 deletions in 42 B-CLLs and 11q23 translocations (not affecting *NPAT*, *CUL5* and *PPP2R1B*) in two B-CLLs.[16] Sorted peripheral blood B-cells from six unrelated healthy donors (CD19 antibody-coupled beads; Miltenyi, Bergisch Gladbach, Germany) and peripheral blood cells from 51 unrelated healthy probands served as control material. Samples were collected with the patients' consent.

2.2. RNA and DNA preparation

RNA and genomic DNA were extracted from lymphocytes of B-CLL patients, from sorted peripheral blood B-cells, and from mononuclear cells of healthy controls with Trizol reagent according to the manufacturer's instructions (Gibco BRL, Eggenstein, Germany).

2.3. Mutation analyses

By use of gene specific primers, the coding regions of the *NPAT* and *CUL5* transcripts were analysed by SSCP and sequence analysis of RT-PCR products as described previously,[4] whilst the coding region of *PPP2R1B* was searched for mutations by direct sequencing of RT-PCR products. All detected nucleotide alterations were verified by analysing the corresponding genomic DNA by PCR amplification and sequence analysis of the affected exons as described previously.[4] Primer sequences are shown in Table 1.

2.4. Real-time polymerase chain reaction

After DNaseI digestion, real-time RT-PCR was carried out as described previously.[17] As internal standards, the housekeeping genes lamin B1 (*LMNB1*; GDB512284), phosphoglycerokinase (*PGK*; CAA23835), and cyclophilin (*PPI*; EC5.2.1.8) were used, which had been proven to represent suitable internal controls for normalising B-CLL samples.[18] Each cDNA sample was analysed in triplicate. Primer sequences are shown in Table 1.

2.5. PP2A activity assay

Cell extracts used for the phosphatase assay were prepared as described by Yan and Mymby.[19] Protein phosphatase activity was determined with the Serine/Threonine Phosphatase Assay System (Promega, Mannheim, Germany) according to the manufacturer's instructions. PP2A activity was assessed by 5 nM okadaic acid inhibition; as a control, protein phosphatase activity was also measured in the presence of 5 μM okadaic acid, which completely inhibits both PP1 and PP2A. Each sample was analysed in triplicate.

3. Results and discussion

Based on FISH analysis, 17 B-CLL cases with monoallelic deletions of chromosome region 11q22–q23 were selected for mutation screening in the remaining copy of the *PPP2R1B* gene. By direct sequencing of RT-PCR fragments, a silent nucleotide change (216T > C; Y72Y) was detected in one B-CLL and in 1/13 controls, but no deleterious mutation was found. This confirms the results by Zhu and colleagues,[20] who did not detect deleterious mutations in B-CLL. Quantitative expression analysis of the gene, however, revealed lower transcript amounts as compared with sorted B cells from probands in about half of 31 B-CLLs analysed (on average, a 3-fold down-regulation), whilst the other half showed expression levels comparable to control cells. The down-regulation was independent of the copy number of the gene (Fig. 2a).

In addition to correctly spliced *PPP2R1B* transcripts, 7/16 B-CLLs showed alternative splice variants: Skipping of exon 9 from 20% to 50% of the transcripts in five B-CLLs, skipping of exon 3 (60%) and of exons 2/3 (50%) each in one B-CLL. Transcripts lacking exon 3 or exon 9 were previously found in 1/10 B-CLL and two breast tumour samples, respectively.[11,20] However, the expression of those splicing variants

Table 1 – Oligonucleotide primers for PCR amplifications, sequence and expression analyses

Primer	Gene	Oligonucleotide sequence (5′ → 3′)
Mutation analysis (RT-PCR, PCR)		
NPAT_REF1A	NPAT (Exon 1)	TTTGCTTTAACCTGAGTCTTG
NPAT_REF1B	*NPAT (Exon 11)*	ATGCTGGGTCTGATTCTGTC
NPAT1	*NPAT (Exon 11)*	CTGAAGAAGCTATACAGGAC
NPAT2	*NPAT (Exon 13)*	AGTAGAAGACAGCGAATCTC
NPAT_REF3A	*NPAT (Exon 13)*	GTGAAATACTACCTGTGTCTG
NPAT_REF3B	*NPAT (Exon 16)*	AACTGAATGACCTGACGAATC
NPAT_REF4A	*NPAT (Exon 15)*	AGCACCTGTATGCAATAGAAG
NPAT_REF4B-4	*NPAT (Exon 17)*	AGGACGAGAGTTTCGCTCAC
PPP_2A	*PPP2R1B (Exon 1)*	CGCATCAGAGCTCGGGACCG
PPP_2B	*PPP2R1B (Exon 15)*	TCTCCACCCAGTTAAGAAC
PPP_4A	*PPP2R1B (Exon 1)*	GAGCAGCGGGTGGAGATG
PPP_4B	*PPP2R1B (Exon 15)*	GTGACAAGAATCTAGTAAAGG
PPP_NPCR_2	*PPP2R1B (Exon 17)*	TAAATCTGGGGTATTAGTCTG
PPP_NPCR_4	*PPP2R1B (Exon 14)*	CAAAAGATTGGACCAATTCTAG
CUL_REF1_1	*CUL5 (Exon 1)*	GGAGCGCCACGAATTCTCG
CUL_REF1_4	*CUL5 (Exon 12)*	GCAACATGTCACAGTAATTGG
CUL_REF2_1	*CUL5 (Exon 10)*	CGTTGAGCAGTTACTTAGAC
CUL_REF2_4	*CUL5 (Exon 19)*	ATGCTAACTAATGTGATTGGC
CUL_Ex3_4	*CUL5 (Intron 3)*	TGACAGAAGTATCACTGTGG
CUL_Ex3_5	*CUL5 (Intron 2/Exon 3)*	ATTCACAGGGATGTGCATGC
Mutation analysis (sequence analysis)		
NPAT_S1	*NPAT*	CCTTCTAGCAACAACTCAG
NPAT_S2	*NPAT*	CTGAGTTGTTGCTAGAAGG
NPAT_S3	*NPAT*	GTGACTGATCCAACAGCG
NPAT_S4	*NPAT*	CGCTGTTGGATCAGTCAC
NPAT_S5	*NPAT*	ACCATACACTTTCTCAGAT
NPAT_S6	*NPAT*	ATCTGAGAAAGTGTATGGTC
NPAT_S7	*NPAT*	GTCATTCCTGGTGCTCAG
NPAT_S8	*NPAT*	CTGAGCACCAGGAATGAC
NPAT_S9	*NPAT*	AGCAACAACCATGATGTGC
NPAT_S10	*NPAT*	GCACATCATGGTTGTTGCT
NPAT_S11	*NPAT*	CTGATCAGCCTGATATACC
NPAT_S12	*NPAT*	GGTATATCAGGCTGATCAG
NPAT_S13	*NPAT*	CCAATGTGTCCTCCACCT
NPAT_S14	*NPAT*	AGGTGGAGGACACATTGG
NPAT_S15	*NPAT*	TATACAGAGGCACAGCTC
NPAT_S16	*NPAT*	GAGCTGTGCCTCTGTATA
PPP_S1	*PPP2R1B*	TTCACCTCTCGCACATCTG
PPP_S2	*PPP2R1B*	GACTGTGAAGCTGAAGTCC
PPP_S3	*PPP2R1B*	TATGGCTTGGCTCGTGGAC
PPP_S4	*PPP2R1B*	GGACTTCAGCTTCACAGTC
PPP_S7	*PPP2R1B*	TATGGCTTGGCTCGTGGAC
PPP_S8	*PPP2R1B*	GTCCACGAGCCAAGCCATA
CUL_S1	*CUL5*	AGTACTGAGCCATCAAGATG
CUL_S2	*CUL5*	TTTCGAACAATACTGTCTTCC
CUL_S3	*CUL5*	GCAATGAAGCTGGTACATGC
CUL_S4	*CUL5*	GCATGTACCAGCTTCATTGC
Real-time polymerase chain reaction		
209NPATp2959fwd	*NPAT*	CCTCGGCAGGTTCTTCATATG
210NPATp3113rev	*NPAT*	TGACCTGACGAATCCACCAA
211NPATp990fwd	*NPAT*	TGAAGAAGCTATACAGGACATATTGGAA
212NPATp1165rev	*NPAT*	CTAGATTAGTTTCATCTGCTAAGACTATACTGG
ATM_TM_1	*ATM*	TAAGTTTACAGGATCTTCTC
ATM_TM_2	*ATM*	GTTCGTAGTCTAGTAATGG
ATM_TM_3	*ATM*	GGAGCATTTTGGCATCTAAC
ATM_TM_4	*ATM*	ATAAGGATCAGCCTCAAGC
191KIAA	*ARHGAP20*	GAGCGCTCCATCTCTTATCCTG
192KIAA	*ARHGAP20*	TCAACACTAGCAGAAGGACTGTCC
KIAA_TM_2	*ARHGAP20*	GGACTGTCCCTGGTAGTAGG
KIAA_TM_5	*ARHGAP20*	AGGCAGCTGCACCAAGAAG

Table 1 – *continued*

Primer	Gene	Oligonucleotide sequence (5′ → 3′)
Real-time polymerase chain reaction		
PPP_TM_1	*PPP2R1B*	CAGACAGCAATTCCGTTC
PPP_TM_3	*PPP2R1B*	AAGGAGGCGCACTGAATC
PPP_TM_2	*PPP2R1B*	TTCCACTGTTCACTAGTC
PPP_TM_5	*PPP2R1B*	CCAAAGTCTCAAGGTCATC
CUL_TM_13	*CUL5*	GCACCCTCGTATTTACAAC
CUL_TM_14	*CUL5*	TCCATGAGTGCTTCAACG
CUL_TM_15	*CUL5*	CTGCTTGCCAATTACTGTG
CUL_TM_18	*CUL5*	AATGAGCTTTATGATACCTC
84CYCLOPHILINfwd	*Cyclophilin*	GCTCGTGCCGTTTTGCA
85CYCLOPHILINrev	*Cyclophilin*	GCAAACAGCTCAAAGGAGACG
55PGKfwd	*PGK*	AAGTGAAGCTCGGAAAGCTTCTAT
56PGKrev	*PGK*	TGGGAAAAGATGCTTCTGGG
13LAMIN_B1e1fwd	*LaminB1*	GCTGCTCCTCAACTATGCTAAGAA
14LAMIN_B1e2rev	*LaminB1*	TCTTTCGAATTCAGTGCTGCTTC

seems not to be restricted to tumour cells: skipping of exon 3 and exons 2/3 was reported by Baysal and colleagues[21] for samples of control cases as well, and traces of transcripts with skipped exon 9 were found in 2/13 of our control cases. Possibly, the relative amount of alternative transcripts is critical for a pathogenic effect. All three splicing variants are expected to interfere with the binding properties of PP2A-Aβ, which coordinates the assembly of the regulatory subunit B and the catalytic C subunit, thereby preventing the assembly of functional trimeric PP2A holoenzymes: (i) in case of skipping of exon 2/3 or exon 9, subunit B cannot be bound to the PP2A holo-enzyme complex, because different parts of the subunit B binding domain of PP2A-Aβ are lost, though heterodimers of subunits A and C still may be formed; (ii) loss of exon 3 results in premature translation termination and thereby to the loss of the domains for interaction with subunits B and C. To answer the question of whether the expression of altered PP2A-Aβ protein disturbs the function of the holoenzyme, the activity of PP2A was measured in five B-CLLs with *PPP2R1B* exon skipping (three cases with exon 9 skipping; one case with exon 2/3 and exon 3 skipping each) and compared to three B-CLLs without exon skipping and also to sorted control B cells. The analysis of these B-CLL cases revealed (i) a lower PP2A activity in most of the analysed tumour samples as compared to non-malignant B lymphocytes and (ii) reduced PP2A activities in all cases with alternative transcripts as compared to the activity in B-CLLs expressing intact transcripts (Fig. 3a). The reduced PP2A activities in the cases with exon skipping are attributable either to the altered function of the variant proteins (most obvious for B30) or at least partly caused by reduced transcript amounts that we observed in 4/5 cases (B3, B19, B14, B15; Fig. 3b). In addition, regulatory mechanisms acting posttranscriptionally/posttranslationally cannot be excluded. The latter mechanisms could also be responsible for a non-correlation of the PP2A activity with the amount of *PPP2R1B* transcript as observed for the non-skipping cases.

Recent investigations have identified PP2A as a regulator of multiple signalling pathways (including cell division and apoptosis) and as a tumour suppressor: (i) inactivation of PP2A plays a crucial role in the experimental transformation of human cells (induced by SV40 or okadaic acid); (ii) several studies found alterations of subunits PP2A-Aα (*PPP2R1A*), PP2A-Aβ (*PPP2R1B*) and PP2A-B′ (*PPP2R5C*) in human cancers (for review see [12]). Our data indicate that not mutational inactivation but down-regulated gene expression and alternative splicing of *PPP2R1B* and PP2A inactivation are associated with the tumourigenesis of B-CLL in a subset of cases. Recently, *PPP2R5C* was identified as a progression marker in B-CLL down-regulated in progressive as compared to stable B-CLL.[22] To elucidate a potential involvement of *PPP2R1B* deregulation and PP2A inactivation in the progression of B-CLL and to confirm the results of this small-sample sized study, an extended study including clinical data is called for.

In addition to *PPP2R1B*, the candidate genes *NPAT* and *CUL5* were investigated for point mutations and their expression in B-CLL. By mutation analysis of *NPAT* in 32 and *CUL5* in 20 B-CLL cases with monoallelic 11q22–q23 deletions, several nucleotide substitutions were detected; mutations of obvious deleterious character, however, were not found: in *NPAT*, three polymorphic changes with comparable allele frequencies in B-CLL ($n = 32$) and healthy controls ($n = 51$) were observed, which are, thus, not regarded to be disease associated: 1620 A/T (L540F; allele A: 0.81 in B-CLL, 0.80 in controls), 1723 A/G (I575V; allele A: 0.56 in B-CLL, 0.59 in controls), 2997 C/A (N999K; allele C: 0.94 in B-CLL, 0.92 in controls). In *CUL5*, we detected the known polymorphism 225G/A (Q75Q) with a frequency of allele A of 0.35 in B-CLL ($n = 29$) and of 0.38 in healthy controls ($n = 47$), indicating that this genetic variant is not disease associated and not B-CLL predisposing as well. In addition to correctly spliced *CUL5* transcripts, all B-CLLs expressed transcripts that lacked exon 4. Although this alternative transcript variant comprised a high proportion of the *CUL5* transcripts (50–80% in 18/20 B-CLLs, 10–20% in 2/20 B-CLLs), it is regarded to be rather B cell than tumour specific, since it was found expressed at comparable levels (60–80%) in 4/4 samples of sorted normal B lymphocytes. In addition, the removal of amino acids 79–137 from the protein caused by skipping of exon 4 is not supposed to inactivate CUL5, since several N-terminal internal deletion mutants (e.g. deletion of amino acids 65–93) did not affect the normal (HIV-1 Vif) function of Cul5.[23]

Whilst disease associated structural alterations of the *NPAT* and *CUL5* transcripts were not detected in this study,

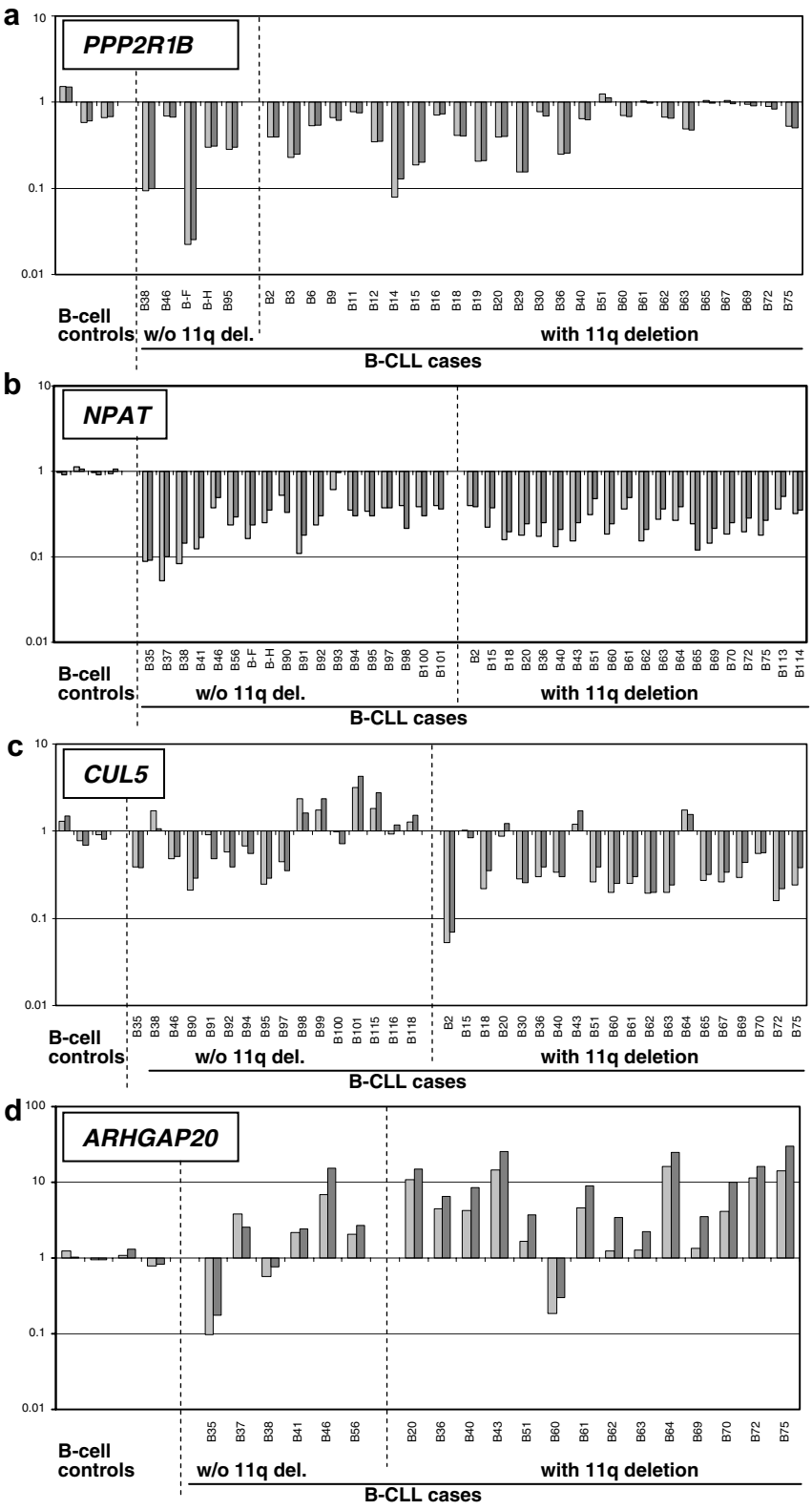

Fig. 2 – Quantitative expression analysis of *PPP2R1B*, *NPAT*, *CUL5*, *ARHGAP20*, and *ATM* in B-CLL. Expression measured by real-time RT-PCR, normalised to three housekeeping genes, where the height of the bars represents the relative gene expression for individual patient and control samples (CD19+ B cells) on a logarithmic scale. From each sample, two regions of *PPP2R1B* (a), *NPAT* (b), *CUL5* (c), *ARHGAP20* (d) and *ATM* (e) were amplified.

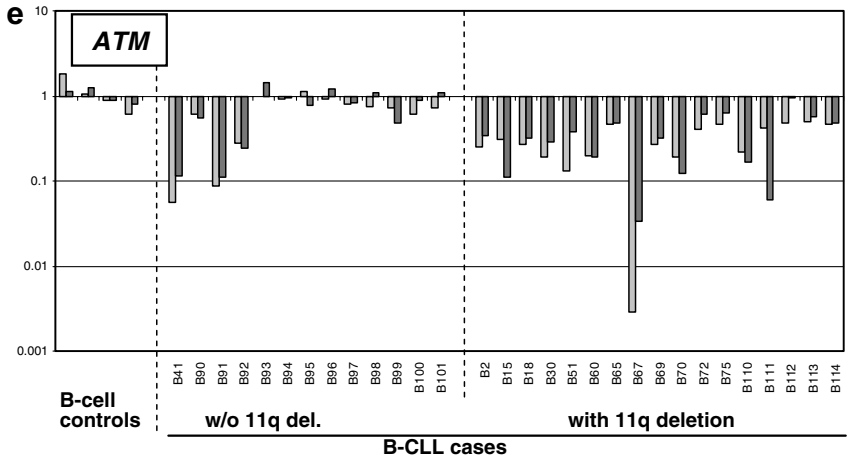

Fig. 2 – *continued*

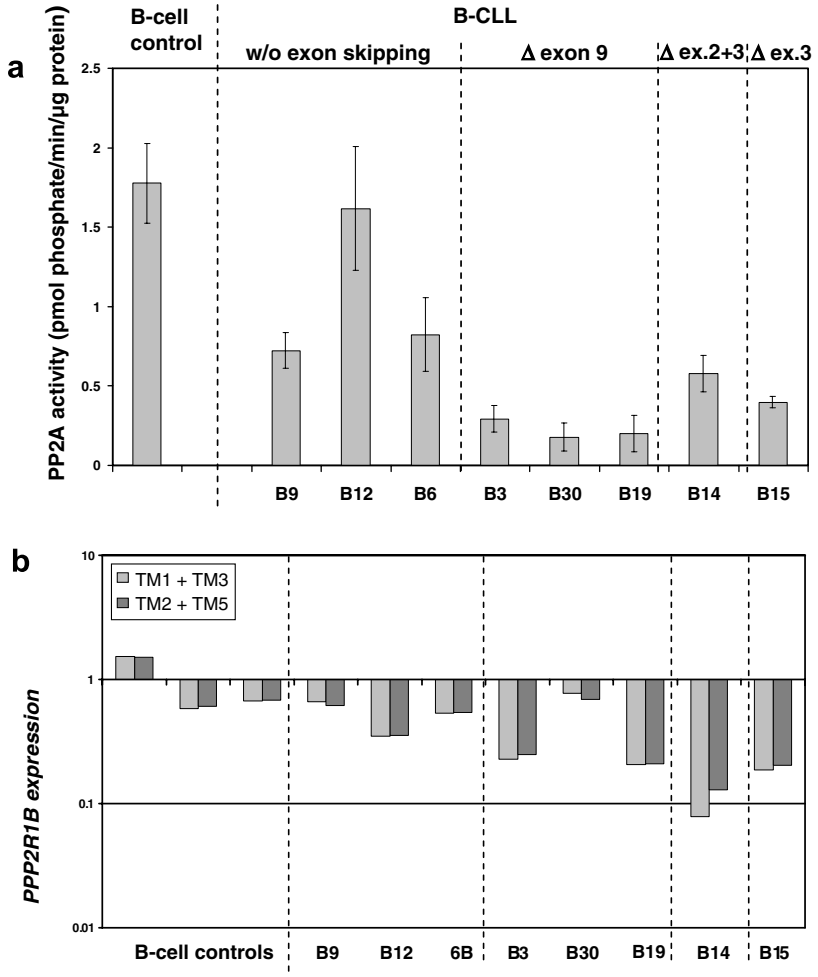

Fig. 3 – Effects of *PPP2R1B* exon skipping on the activity of the holoenzyme PP2A. (a) PP2A activity was determined in cell extracts of five B-CLL samples that expressed *PPP2R1B* transcripts lacking exon 9 (B3, B30, B19), exons 2/3 (B14), or exon 3 (B15) in comparison with three B-CLLs expressing intact *PPP2R1B* transcripts (B9, B12, B6) and with sorted B cell controls. The data shown represent means ± SD of triplicate assays. (b) *PPP2R1B* gene expression measured by real-time RT-PCR, where the height of the bars represents the relative gene expression for individual patient and control samples (CD19+ B cells) on a logarithmic scale. Two regions of *PPP2R1B* were amplified (primer pairs PPP_TM_1/PPP_TM_3; PPP_TM_2/PPP_TM_5). The relative amount of alternatively to correctly spliced transcripts was about 20% in B3 and B19, 20–30% in B30, 40% in B11, 50% in B16 (all Δ exon 9), 50% in B14 (Δ exon 2 + 3), 60% in B15 (Δ exon 3).

the expression of both genes was found to be altered in B-CLL cells: (i) *NPAT* was highly significantly down-regulated in B-CLL cells as compared with sorted normal B cells ($n = 38$; Mann–Whitney U statistic, $p = 0.0006$); the down-regulation was independent of the copy number of the gene ($p = 0.77$; Fig. 2b). (ii) In most patients with monoallelic loss of *CUL5*, this gene was expressed at a lower level than in control B cells ($p = 0.034$) and in B-CLLs biallelic for *CUL5* ($p = 0.0017$; Fig. 2c). A comparative expression analysis of *ARHGAP20* and *ATM* as two further genes within the critical 11q22–q23 deletion region revealed a general, gene-dosage independent up-regulation of *ARHGAP20* ($p = 0{,}015$; Fig. 2d) and a down-regulation of *ATM* ($p = 0{,}0081$; Fig. 2e) in B-CLL versus control B cells. Though sharing a common promoter region with *NPAT*, the expression of *ATM* was gene-dosage dependent ($p = 0.0024$) and not independent from the copy number as observed for *NPAT*, which points to an independent regulation of both genes. Together, the expression data of this study indicate, that the observed down-regulation of *PPP2R1B*, *NPAT* and *CUL5* is, obviously, not caused by a general lowering of expression from all genes in the deletion region, but is the result of gene specific regulation.

Whilst the down-regulation of the cell-cycle regulator *NPAT* seems to be a common mechanism of B-CLL leukaemogenesis, decreased expression of *CUL5* might contribute to the characteristic tumour progression in patients with 11q22–q23 deletions: as component of E3 ubiquitin ligase complexes, *CUL5* is involved in the regulation of the stability of p53 and in the ubiquitin-dependent control of the apoptotic cell death of normal human lymphocytes.[15,24] The change in the expression of *CUL5* may conceivably contribute to the impaired response of B-CLL lymphocytes to apoptotic death activation and in particular to the tumour progression in high-risk B-CLL. Thus, together with *PPP2R1B*, *NPAT* and *CUL5* constitute novel candidate genes potentially involved in the tumourigenic development of B-CLL cells via deregulation of cell cycle and apoptosis.

Conflict of interest statement

None declared.

Acknowledgements

This work was funded in part by grants from the Deutsche Krebshilfe (70-3183-Li) and the Deutsche Forschungsgemeinschaft (STI 296/1-1).

REFERENCES

1. Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *New Engl J Med* 2000;**343**:1910–6.
2. Döhner H, Stilgenbauer S, James MR, et al. 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. *Blood* 1997;**89**:2516–22.
3. Korz C, Pscherer A, Benner A, et al. Evidence for distinct pathomechanisms in B-cell chronic lymphocytic leukemia and mantle cell lymphoma by quantitative expression analysis of cell cycle and apoptosis-associated genes. *Blood* 2002;**99**:4554–61.
4. Schaffner C, Stilgenbauer S, Rappold GA, Döhner H, Lichter P. Somatic ATM mutations indicate a pathogenic role of ATM in B-cell chronic lymphocytic leukemia. *Blood* 1999;**94**:748–53.
5. Schaffner C, Idler I, Stilgenbauer S, Döhner H, Lichter P. Mantle cell lymphoma is characterized by inactivation of the ATM gene. *Proc Natl Acad Sci USA* 2000;**97**:2773–8.
6. Austen B, Powell JE, Alvi A, et al. Mutations in the ATM gene lead to impaired overall and treatment-free survival that is independent of IGVH mutation status in patients with B-CLL. *Blood* 2005;**106**:3175–82.
7. Bullrich F, Rasio D, Kitada S, et al. ATM mutations in B-cell chronic lymphocytic leukemia. *Cancer Res* 1999;**59**:24–7.
8. Stankovic T, Weber P, Stewart G, et al. Inactivation of ataxia telangiectasia mutated gene in B-cell chronic lymphocytic leukaemia. *Lancet* 1999;**353**:26–9.
9. Wang SS, Esplin ED, Li JL, et al. Alterations of the PPP2R1B gene in human lung and colon cancer. *Science* 1998;**282**:284–7.
10. Takagi Y, Futamura M, Yamaguchi K, Aoki S, Takahashi T, Saji S. Alterations of the PPP2R1B gene located at 11q23 in human colorectal cancers. *Gut* 2000;**47**:268–71.
11. Calin GA, di Iasio MG, Caprini E, et al. Low frequency of alterations of the alpha (PPP2R1A) and beta (PPP2R1B) isoforms of the subunit A of the serine–threonine phosphatase 2A in human neoplasms. *Oncogene* 2000;**19**:1191–5.
12. Janssens V, Goris J, Van Hoof C. PP2A: the expected tumor suppressor. *Curr Opin Genet Dev* 2005;**15**:34–41.
13. Van Hoof C, Goris J. Phosphatases in apoptosis: to be or not to be, PP2A is in the heart of the question. *Biochim Biophys Acta* 2003;**1640**:97–104.
14. Miele A, Braastad CD, Holmes WF, et al. HiNF-P directly links the cyclin E/CDK2/p220NPAT pathway to histone H4 gene regulation at the G1/S phase cell cycle transition. *Mol Cell Biol* 2005;**25**:6140–53.
15. Querido E, Blanchette P, Yan Q, et al. Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. *Genes Dev* 2001;**15**:3104–17.
16. Stilgenbauer S, Liebisch P, James MR, et al. Molecular cytogenetic delineation of a novel critical genomic region in chromosome bands 11q223–923.1 in lymphoproliferative disorders. *Proc Natl Acad Sci USA* 1996;**93**:11837–41.
17. Kalla C, Nentwich H, Schlotter M, et al. Translocation t(X; 11)(q13; q23) in B-cell chronic lymphocytic leukemia disrupts two novel genes. *Genes Chromosome Cancer* 2005;**42**:128–43.
18. Mertens D, Wolf S, Schroeter P, et al. Down-regulation of candidate tumor suppressor genes within chromosome band 13q14.3 is independent of the DNA methylation pattern in B-cell chronic lymphocytic leukemia. *Blood* 2002;**99**:4116–21.
19. Yan Y, Mumby MC. Distinct roles for PP1 and PP2A in phosphorylation of the retinoblastoma protein.PP2a regulates the activities of G(1) cyclin-dependent kinases. *J Biol Chem* 1999;**274**:31917–24.
20. Zhu Y, Loukola A, Monni O, et al. PPP2R1B gene in chronic lymphocytic leukemias and mantle cell lymphomas. *Leukemia Lymphoma* 2001;**41**:177–83.
21. Baysal BE, Farr JE, Goss JR, Devlin B, Richard 3rd CW. Genomic organization and precise physical location of protein

phosphatase 2A regulatory subunit A beta isoform gene on chromosome band 11q23. *Gene* 1998;**217**:107–16.
22. Fält S, Merup M, Gahrton G, Lambert B, Wennborg A. Identification of progression markers in B-CLL by gene expression profiling. *Exp Hematol* 2005;**33**: 883–93.
23. Yu X, Yu Y, Liu B, et al. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. *Science* 2003;**302**:1056–60.
24. Blaise R, Masdehors P, Lauge A, et al. Chromosomal DNA and p53 stability, ubiquitin system and apoptosis in B-CLL lymphocytes. *Leukemia Lymphoma* 2001;**42**:1173–80.