British Journal of Haematology, 2003, **120**, 434–441

Abnormal expression of apoptosis-related genes in haematological malignancies: overexpression of MYC is poor prognostic sign in mantle cell lymphoma

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Received 18 March 2002; accepted for publication 4 September 2002

Summary. The expression of apoptosis-related genes *BCL2*, *BAX*, *BCL2L1*, *BCL2A1*, *MCL1*, *DAPK1* and *MYC* was studied by quantitative real-time polymerase chain reaction on total RNA samples from patients with acute lymphoblastic leukaemia (ALL, $n = 16$), acute myeloid leukaemia (AML, $n = 27$), chronic myeloid leukaemia (CML, $n = 12$), mantle cell lymphoma (MCL, $n = 19$) and chronic lymphoid leukaemia (CLL, $n = 32$). *BCL2*, *BAX*, *BCL2A1*, *MCL1*, *DAPK1* and *MYC* were overexpressed in all patient groups. *BCL2L1* was underexpressed in CLL and CML, but not in AML, ALL and MCL. *MCL1* levels were significantly higher in CD13 and CD33-positive ALL, and in CD56-positive AML samples. *BCL2*, *BCL2L1*, *BCL2A1* and *MCL1* were overexpressed and *DAPK1* was underexpressed in CLL samples with a 11q23 deletion. *MYC* overexpression was significantly associated with shorter overall survival in MCL ($P < 0{\cdot}01$). AML patients with a normal karyotype showed a higher frequency of *BCL2A1* overexpression ($P < 0{\cdot}001$) than those with an abnormal karyotype.

Keywords: apoptotic genes, gene expression, haematological malignancies, quantitative real-time PCR, prognosis.

Apoptosis plays a critical role in the normal development of tissue homeostasis as well as in defence against infections. Deregulation of apoptosis has been shown to contribute to the development of cancer, autoimmune diseases and degenerative disorders (Reed, 1998, 1999, 2000). A number of genes are involved in the regulation of apoptosis, among them the family of *BCL2* genes. They regulate cellular responsiveness to a wide variety of death-inducing stimuli, including growth factor deprivation, the presence of glucocorticoids, receptor antibodies, and radio- or chemotherapeutical agents (Reed, 1998, 1999, 2000). Some of the *BCL2* family members are antiapoptotic, such as *BCL2*, *MCL1*, *BCL2A1* and *BCL2L1*, and others are pro-apoptotic such as *BAX*, *BAK*, *BAD* and *BCLXS* (Oltvai *et al*, 1993; Chao & Korsmeyer, 1998; Srivastava *et al*, 1999). The pro- and antiapoptotic proteins can form heterodimers and the ratio of these determines the execution of apoptosis (Oltvai *et al*, 1993; Adams & Cory, 1998; Schimmer *et al*, 2001). Another important regulator in apoptosis is the *MYC* oncogene. It has maintained a highly conserved sequence through evolution and encodes a transcription factor that plays an important role in a number of biological functions, including cell proliferation, differentiation and apoptosis (Cole, 1986).

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Aberration of apoptosis has been observed in haematological neoplasms. Chromosomal translocations affecting apoptosis regulator genes, such as t(14;18) of the *BCL2* gene, are well defined in haematological malignancies (Tsujimoto *et al*, 1985). The *bcl2* protein inhibits apoptosis by forming dimers with *bax* and *bak* (Schimmer *et al,* 2001). It regulates cell death by controlling mitochondrial membrane permeability via inhibition of caspase activity and prevents the release of *cytochrome c* (Jacobson *et al*, 1993; Shimizu *et al*, 1999). *BAX*, which is a pro-apoptotic gene, induces the release of *cytochrome c*. Furthermore, the ratio of *bcl2* to *bax* determines cell survival or death after an apoptotic stimulus (Oltvai *et al*, 1993). *BCL2L1* is a highly conserved gene encoding an alternative splicing of two isoforms with antagonistic functions, the shorter with a pro-apoptotic and the longer with an antiapoptotic function (Boise *et al*, 1993). *Bcl2L1* forms dimers with *bax* and *bak* and has been shown to be an independent regulator of programmed cell death by preventing apoptosis (Boise *et al*, 1993; Korsmeyer, 1999). *BCL2A1* prevents apoptosis induced by the p53 tumour suppressor protein (D'Sa-Eipper *et al*, 1996). *MCL1* participates in myeloid cell differentiation and has sequence homology with *BCL2* (Kozopas *et al*, 1993; Bae *et al,* 2000). Death-associated protein kinase (*DAPK1*) is a potential mediator of γ-interferon-induced cell death: when deregulated, it behaves as an oncogene or growth suppressor (Deiss *et al*, 1995). Overexpression of *MYC* has been found in numerous human tumours, including several haematological disorders with T- and B-cell lineage involvement (Marcu *et al*, 1992; Sakamuro & Prendergast, 1999).

Our recent studies of acute myeloid leukaemia (AML) and chronic lymphocytic leukaemia (CLL) using the microarray technology revealed altered expression of several apoptosis-related genes (Aalto *et al,* 2001; Larramendy *et al,* 2002).

In this study we determined the expression levels of seven genes (*BCL2, BAX, BCL2L1, BCL2A1, MCL1, DAPK1, MYC*) using quantitative real-time reverse transcription polymerase chain reaction (QRT-PCR). We set out to identify diagnostic and prognostic markers for acute lymphoid leukaemia (ALL), acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML), mantle cell lymphoma (MCL) and chronic lymphocytic leukaemia (CLL).

PATIENTS AND METHODS

ALL, AML and CML samples

Bone marrow samples were obtained from 16 ALL patients, 27 AML patients and 12 CML patients at the Helsinki University Central Hospital, Helsinki, Finland. Table I shows the key data of these patients. Bone marrow aspirates were diluted 1:10 in RNA/DNA stabilization reagent for blood/bone marrow (Boehringer Mannheim GmbH, Mannheim, Germany) containing guanidinium thiocyanate and in Triton® X-100 (Boehringer) for simultaneous cell lysis and stabilization of nucleic acids according to the supplier's instructions. Lysates were stored at −70°C until RNA extraction.

Mantle cell lymphoma (MCL)

Samples were obtained from 19 patients diagnosed at the Helsinki University Central Hospital (Table I). All patients were $CD5^+$, $CD19^+$, $CD20^+$, $CD23^-$ and cyclin $D1^+$. The International Prognostic Index (IPI) was used for risk grouping of the patients (Blay *et al*, 1998).

CLL

Peripheral blood specimens were obtained from 32 patients referred to the CLL out-patient clinic at Tampere University Central Hospital (Tampere, Finland) (Table I). All had a blood lymphocyte count of 30×10^9/l or higher. These patients were diagnosed and staged according to standard clinical, morphological and immunophenotypic criteria and the Binet system. All patients had a $CD19^+/CD5^+/CD23^+$ immunophenotype. The proportions of monocytes and polyclonal T and B lymphocytes were 1–13%, indicating that 87–99% of the isolated cells represented the leukaemic population.

Control samples

Bone marrows from four healthy individuals were used as controls for the ALL, AML and CML samples. References for MCL and CLL samples were $CD19^+$ B cells, which were purified from the adenoid palatine tonsils of six healthy children and pooled. B cells were purified using microbeads conjugated to a monoclonal CD19 antibody (Miltenyi Biotec, Bergisch Gladbach, Germany). The proportion of T lymphocytes was less than 5%, indicating that 95% of the isolated cells represented B-lymphocyte population.

RNA isolation

AML, ALL, CML and control samples. Total nucleic acid was isolated from samples using the mRNA isolation kit for blood/bone marrow (Boehringer) based on magnetic glass particle technology following the manufacturer's instructions.

CLL. Lymphocytes were isolated from patient blood using one-step density gradient centrifugation in Ficoll–Paque (Pharmacia Fine Chemicals, Uppsala, Sweden), and total RNA was extracted using the Trizol Reagent (Gibco BRL, Grand Island, NY, USA).

MCL. Total RNA was isolated from deep-frozen tissue specimens using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

DNase I treatment

All RNA samples were treated with DNase I (Boehringer) to remove genomic DNA contamination from the preparations according to the manufacturer's instructions. RNA quality and integrity were checked by electrophoresis using 1% agarose gel with ethidium bromide staining and UV transillumination. RNA concentrations were measured using a spectrophotometer at a wavelength of 260 nm.

QRT-PCR

QRT-PCR was performed on all samples to determine the expression of seven apoptosis-related genes (*BCL2, BAX, BCL2L1, BCL2A1, MCL1, DAPK1, MYC*) and one

Table I. Patients divided according to clinicopathological features.

Disease	Patients (*n*) M	F	Mean age (years)	Karyotype/altered gene expression			Risk group/FAB/ immunophenotype/altered gene expression		
Acute lymphoblastic leukaemia	8	8	5	Normal	1		HR	6	MCL1 ↑
				Hyperdiploid	5		IR	6	
				t(12;21)	3		SR	4	
				Other	7		$CD13^+/CD33^+$	8	BCL2L ↑ MCL1 ↑
Acute myeloid leukaemia	13	14	52	Normal	13	BCL2A1 ↑	M0	1	
				t(15;17)	1		M1	3	
				t(8;21)	5		M2	12	
				inv(16)	1		M3	2	
				Complex	2		M4	6	
				Other	5		M5	3	
							$CD56^+$	12	MCL1 ↑
Chronic myeloid leukaemia	9	3	38	t(9;22)	10				
				t(9;22) variant	2				
Mantle cell lymphoma	12	7	71	Not studied			IPI 1	2	
							IPI 2	6	
							IPI 3	5	
							IPI 4	3	
							Unclassified	2	
							Typical morph.	12	
							Blastoid morph.	6	BCL2L1 ↑
Chronic lymphoid leukaemia	23	9	64	Normal	12		Binet A	13	
				+12	4		Binet B	10	
				del 11q	5	BCL2 ↑ BCL2L1 ↑ BCL2A1 ↑ MCL1 ↑ DAPK1 ↓	Binet C	9	
				del 13q	4				
				Complex	9				

HR, high risk; IR, intermediate risk; SR, standard risk; INPI, International Prognosis Index.

Gene expressions that showed statistically significant association with any features are marked for overexpression (↑) and underexpression (↓). Other clinicopathological parameters did not show statistically significant association with gene expression. Statistical tests were performed using the SPSS program package for Windows.

housekeeping gene for β-actin (*ACTB*) (Table II). Complementary DNA (cDNA) was synthesized using 0·5 μg of DNase-I-treated total RNA and a First Strand cDNA Synthesis kit for RT-PCR (Roche Diagnostics, Indianapolis, IN, USA). Gene-specific primers were designed and synthesized by TIB MOLBIOL (Berlin, Germany). PCR was performed in a LightCycler thermal cycler (Roche). Each PCR reaction consisted of 1 μl of DNA Master SYBR Green I mix (LightCycler-FastStart DNA Master SYBR Green I kit, Roche; containing *Taq* polymerase, dNTP, $MgCl_2$, and SYBR Green I dye), 1 μl of cDNA and 2·5–5·0 pmol of primers. The amplification programme included an initial denaturation at 95°C for 8 min, 45–55 cycles with denaturation at 95°C for 10 s, annealing at 58–62°C for 5 s and extension at 72°C for 20 s. Amplifications were followed by melting curve analysis using one cycle at 95°C for 0 s, 65°C for 10 s, and 95°C for 0 s at the acquisition step mode. A negative control without a cDNA template was run simultaneously with every assay. For each cDNA sample the PCR was run in duplicate. Standard curves were obtained using serial dilutions of the beta-globulin gene (DNA Control kit; Roche) according to the supplier's instructions. The concentration of each gene product was determined on the basis of a kinetic approach using the LIGHTCYCLER software (Roche). The levels of *ACTB* were used for normalization of RNA quantity and quality differences in all samples.

Statistical analysis of data

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) for Windows (Version 9·0), with the Student's *t*-test and Levene's test for the gene expression comparisons. The overall survival related to gene expression was analysed by the Kaplan–Meier method.

RESULTS

The expression levels of *BCL2*, *BAX*, *BCL2L1*, *BCL2A1*, *MCL1*, *DAPK1* and *MYC* were determined by QRT-PCR (Table III). Figure 1A and B shows the ratios of expression levels in each sample. ALL, AML and CML samples were compared with RNA isolated from the bone marrow of healthy donors, whereas MCL and CLL samples were compared with RNA isolated from tonsillar B cells.

BCL2 and *BAX* were overexpressed in all patients (Table III). The *BCL2*/*BAX* ratio was highest in CLL (1·844), followed by ALL (0·895), MCL (0·44), AML (0·067) and CML (0·036). The *BCL2L1* gene was underexpressed in CLL and CML, but the levels were higher than those of controls in AML, ALL and MCL. *BCL2A1*, *MCL1*, *DAPK1* and *MYC* were overexpressed in all patient groups (Table III).

Risk groups and the gene expression

We observed statistically significant differences in the expression of *MCL1* between high risk (HR) (53·35 ± 38·82 ng/μl, $P < 0·05$), intermediate risk (IR) (9·93 ± 4·32 ng/μl) and standard risk (SR) 10·43 ± 2·58 ng/μl groups of ALL. Gene expression levels in the MCL subgroups (IPI), and in the CLL subgroups (Binet) did not

Table II. Studied genes and primer sequences used.

Name of the gene	Chromosomal location	GenBank code	Primer sequences used	
			Forward	Reverse
BCL2	18q21.3	M14745	AggAAgTgAACATTTCggTgAC	gCTCAgTTCCAggACCAgg
BCL2-associated X protein (BAX)	19q13.3-q13.4	L22474	TgCTTCAgggTTTCATCCAg	ggCggCAATCATCCTCTg
BCL2-like 1 (BCL2L1)	20pter-p12.1	Z23115	ATggCAgCAgTAAAgCAAgC	CggAAgAgTTCATTCACTACCTgT
BCL2-related protein A1 (BCL2A1)	15q24.3	U29680	CgCATCAggAAggCTAgAgTT	TCggTCTCCTAAAAgCAggC
Myeloid cell leukaemia sequence 1 (BCL2-related) (MCL1)	1q21	L08246	gATgATCCATgTTTTCAgCgAC	CTCCACAAACCCATCCCAg
Death-associated protein kinase 1 (DAPK1)	9q34.1	P53355	CAgTgTTgTTgCTCTAggAAg	gggACTgCCACAAATgATgAgC
MYC oncogene	8q24.12-q24.13	V00568	ggCAAAAggTCAgAgTCTgg	gTgCATTTTCggTTgTTgC
Beta-actin (ACTB)	7p15-p12	X00351	AgCCTCgCCTTTgCCgA	CTggTgCCTggggCg

Table III. Concentration of different gene products determined by quantitative real-time polymerase chain reaction (QRT-PCR) in groups of patients with haematological malignancy and in healthy controls (ng/μl).

Gene	ALL ($n = 16$)	P	AML ($n = 27$)	P	CML ($n = 12$)	P	Controls (bone marrow) ($n = 4$)	MCL ($n = 19$)	P	CLL ($n = 32$)	P	Controls (pooled tonsils)
BCL2	5·856 ± 4·8408	< 0·01	1·1164 ± 2·1637	NS	0·7118 ± 0·6602	NS	0·12 ± 0·10	7·4896 ± 4·4326	< 0·05	6·3703 ± 10·8490	< 0·05	0·0702
BAX	6·5378 ± 7·9300	NS	16·0526 ± 13·6911	NS	19·3065 ± 25·5333	NS	1·6631 ± 0·9979	16·8789 ± 10·7624	< 0·001	3·2199 ± 2·7497	NS	1·5445
BCL2L1	0·1647 ± 0·4596	NS	0·1230 ± 0·2342	NS	0·0613 ± 0·0476	NS	0·1017 ± 0·4871	0·0164 ± 0·0244	< 0·05	0·0004 ± 0·0008	NS	0·045
BCL2A1	3·9577 ± 4·1441	NS	10·5792 ± 16·8301	NS	2·8781 ± 3·8174	NS	0·3517 ± 0·5484	13·3684 ± 9·7509	< 0·01	5·7302 ± 7·2322	NS	0·02943
MCL1	26·341 ± 31·60	NS	49·3977 ± 56·3773	NS	138·593 ± 102·8256	< 0·001	6·7522 ± 4·675	52·5789 ± 50·0541	NS	82·4199 ± 172·4837	NS	7·7365
DAPK1	5·7795 ± 6·7748	NS	19·4635 ± 36·0556	NS	0·5877 ± 0·4596	NS	0·1393 ± 0·1854	0·7436 ± 0·7710	< 0·01	0·0316 ± 0·0340	NS	0·0032
MYC	76·6152 ± 60·900	NS	117·9831 ± 195·664	NS	149·3558 ± 191·6201	NS	4·8538 ± 1·915	265·720 ± 279·8153	< 0·01	25·0391 ± 45·405	NS	5·9059

NS, not significant.
The Student t-test was used to calculate significance levels.

differ. With the exception of French-American-British (FAB) types AML M2 and M4, other subtype groups were too small to enable statistical analyses. Subtypes M2 and M4 did not show statistically significant alterations in gene expression.

Immunophenotypes and gene expression

There was a statistically significant difference in the expression of *BCL2L1* and *MCL1* in the $CD13^+/CD33^+$ and negative ALL samples (*BCL2L1*: 0·2812 ± 0·6482 ng/μl and 0·048 ± 0·0328 ng/μl, $P = 0·049$; *MCL1*: 35·9220 ± 38·9558 ng/μl and 16·7599 ± 19·1491 ng/μl; $P = 0·026$). The CD56-positive and -negative AML patients showed a statistically significant difference in the level of *MCL1* (54·22 ± 74·94 ng/μl and 35·43 ± 22·96 ng/μl; $P = 0·027$).

Cytogenetic alterations and gene expression

We observed that CLL patients with the 11q23 deletion showed significantly higher expression of *BCL2* than patients without the deletion ($11q23^+$: 3·38 ± 4·62 ng/μl versus $11q23^-$: 20·74 ± 21·63 ng/μl; $P = 0·0001$), *BCL2L1* ($11q23^+$: 0·0002 ± 0·0004 ng/μl versus $11q23^-$: 0·0009 ± 0·0018 ng/μl; $P = 0·001$), *BCL2A1* ($11q23^+$: 3·75 ± 3·63 ng/μl versus $11q23^-$: 15·40 ± 13·15 ng/μl; $P = 0·0001$) and *MCL1* ($11q23^+$: 43·01 ± 88·53 ng/μl versus $11q23^-$: 291·15 ± 344·74 ng/μl; $P = 0·001$), and lower expression of *DAPK1* ($11q23^+$: 0·0342 ± 0·0037 ng/μl versus $11q23^-$: 0·0289 ± 0·0170 ng/μl; $P < 0·05$). In AML, the expression of *BCL2A1* was different between samples with normal ($n = 13$) and abnormal karyotypes ($n = 14$) (16·0407 ± 21·0988 ng/μl versus 4·6976 ± 7·6931 ng/μl, $P = 0·001$).

Survival and gene expression

We compared the expression of genes with the overall survival in MCL. There was significant negative association between the level of *MYC* and the overall survival ($P = 0·01$) (Fig 2), but not with the IPI ($P = 0·36$), age ($P = 0·27$) or gender ($P = 0·39$). The patients alive at the end of the study had significantly lower levels of *MYC* than those who had died (109·05 ± 101·28 ng/μl, 286·48 ± 252·78 ng/μl; $P < 0·05$).

The overall survival was lower in patients with the blastoid form of MCL (blastoid variant 17·37 ± 17·04 months and normal variant 32·19 ± 24·9 months) and the expression of *BCL2L1* was significantly higher in blastoid subtypes (0·0341 ± 0·0361 ng/μl and 0·0085 ± 0·0361 ng/μl, $P = 0·03$).

DISCUSSION

In the present study, we analysed the expression of *BCL2*, *BAX*, *BCL2L1*, *BCL2A1*, *MCL1*, *DAPK1* and *MYC* in different types of haematological malignancies by QRT-PCR using the SYBR Green I method for the quantification of PCR products.

As controls, we used bone marrow samples from healthy donors for the ALL, AML and CML bone marrow samples. $CD19^+$ B cells were pooled from six normal adenoids and

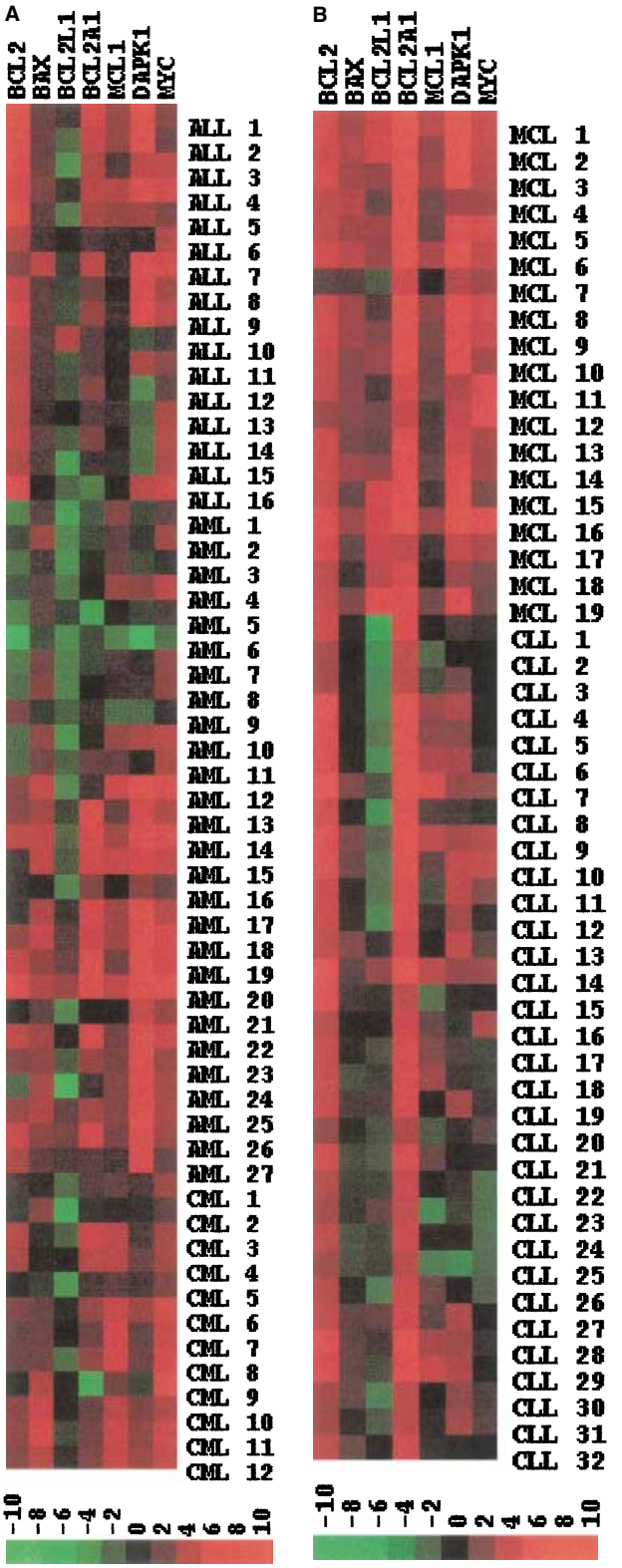

Fig 1. (A) The expression profile of seven apoptosis-related genes in acute lymphoblastic (ALL) ($n = 16$), acute myeloid (AML) ($n = 27$) and chronic myeloid leukaemia (CML) samples. Measured gene expressions compared with healthy bone marrow controls. Red represents upregulated genes and green represents downregulated genes. The samples are presented on the right side of the image and the studied genes on the top. Grade of colour correlates to the degree of expression as shown on the scale at the bottom of the image. The scale is shown from −10 to 10 in log. (B) The expression profile of seven apoptosis genes in mantle cell lymphoma ($n = 19$) and chronic lymphoid leukaemia ($n = 32$) samples. Red represents upregulated genes and green represents downregulated genes. Measured gene expressions were compared with $CD19^+$ cells from adenoids. The samples are presented on the right side of the image and the studied genes on the top. Grade of colour correlates to the degree of expression as shown on the scale at the bottom of the image. The scale is from −10 to 10 in log.

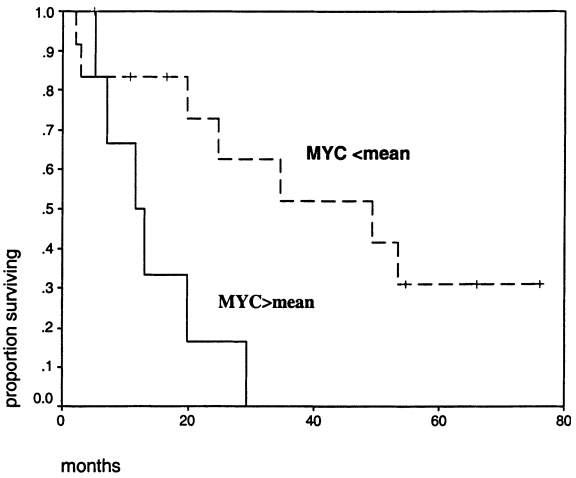

Fig 2. Overall survival of mantle cell lymphoma patients in relation to the detected *MYC* concentrations. Patients expressing *MYC* < mean *MYC* concentration (- - - -): overall survival 43·13 months; median 49·36 months. Patients expressing *MYC* > mean *MYC* concentration (——): overall survival 14·30 months; median 11·57 months. Overall survival versus *MYC* $P = 0{\cdot}01$.

used as reference for the CLL and MCL samples. Neither control is a perfect means to verify whether or not gene expression differences in the cancer samples are attributable merely to the disease *per se* and not to the maturation stage or lineage involvement of haematopoietic cells. Nevertheless, although the stem cell from which the malignancy originates is unidentified, the selection of controls is a disputable issue. Therefore, the results must be interpreted with caution. For example, $CD19^+$ B cells from children, which we used as controls for CLL and MCL, are likely to be active, and therefore less apoptotic, than $CD19^+$ lymph nodes from adults or peripheral blood B cells. It should, however, be noted that the control we used was the same for all patients of each disease group. Thus the selection of controls did not have an effect on gene expression differences within the same disease, and any associations between gene expression and clinical parameters can be interpreted reliably.

Patient groups and gene expression

BCL2 was overexpressed in all patients. This gene is involved in both normal lymphoid development and lymphomas with the t(14;18) translocation (Kozopas *et al*, 1993). High levels of the *bcl2* protein have been reported even in the absence of t(14;18), for example in CLL together with a high level of the *mcl-1* protein (Gottardi *et al*, 1996) and in diffuse large B-cell lymphoma (Monni *et al*, 1999).

BAX showed the highest overexpression in MCL. Mutations in *BAX* have been documented in up to 20% of haematopoietic malignancies (Meijerink *et al*, 1998). The *bcl2:bax* ratio was found to be inversely related with drug-induced apoptosis *in vitro* and with clinical response to chemotherapy (Pepper *et al*, 1996). In our study, the ratio was highest in CLL, followed by ALL, MCL, AML and CML. The functions of *BCL2L1* (underexpressed in CML and CLL) and *DAPK1* (overexpressed in MCL) in haematological malignancies are still largely unknown, although *BCL2L1* has been found to be overexpressed in about half of human cancers associated with resistance to radiotherapy and chemotherapy (Nicot *et al,* 2000).

Overexpression of *MCL1* (in our CLL and CML patients) has been indicated to inhibit cell death in human cell lines less effectively than *BCL2* (Krajewski *et al*, 1995) and to have an important role in the regulation of B cells (Lomo *et al*, 1997).

Our finding of overexpressed *MYC* and *BCL2L1* in MCL is in concordance with two previous microarray analyses (Hofmann *et al*, 2001; Zhu *et al*, 2002). Furthermore, the present results and those by Zhu and colleagues give evidence that the overexpression of these genes is mainly associated with a blastoid morphology of MCL cells.

BCL2L1 and *MCL1* were significantly overexpressed in $CD13^+/CD33^+$ ALL patients. *MCL1* levels in $CD56^+$ AML patients were significantly higher than in $CD56^-$ patients. Recently, it was reported that high expression of MCL1 correlates with resistance to chemotherapeutical agents (Kaufmann *et al*, 1998). CD56 positivity is otherwise associated with a poor prognosis in AML (Di Bona *et al*, 2002). Whether a cause-and-effect relationship exists between these two phenomena is still open to question. An important role for *MCL1* in the co-ordination of the *BCL2* gene family in cell phenotype differentiation has recently been identified (Craig 2002).

BCL2A1 levels in AML were significantly higher in samples with normal rather than abnormal karyotypes. It is generally acknowledged that approximately 40% of AML cases have a normal karyotype. To identify prognostic and follow-up markers for these patients, characterization of gene expression alterations is considered to be important. Our finding that overexpression of *BCL2A1* is a characteristic phenomenon for a subtype of AML patients with a normal karyotype needs be confirmed with a larger series of patients (Grimwade *et al*, 1998).

The antiapoptotic *BCL2*, *BCL2L1*, *BCL2A1 and MCL1* genes were significantly overexpressed and the pro-apoptotic DAPK1 was underexpressed in CLL patients with a 11q23 deletion. The loss of 11q23 is one of the most common structural chromosomal changes in CLL and is associated with the progression of the disease (Zhu *et al,* 2000). The alterations in expression levels of anti- and pro-apoptotic genes we observed support the results of previous cDNA array studies by Aalto and colleagues who showed that patients with a 11q23 deletion form a subtype of CLL (Aalto *et al*, 2001).

The follow up for all patient groups other than MCL was too short to apply the Kaplan–Meier survival curve assessment. In this study, the most important novel clinical finding was the significant association of *MYC* overexpression with shorter overall survival in MCL. Many molecular and clinicopathological variables have been investigated in this lymphoma type but few prognostical markers have been found. Other than tumour-related factors, blastoid morphology has also been shown to be associated with poor prognosis (Bernard *et al,* 2001). In the present study six patients with blastoid morphology showed shorter overall survival and a higher frequency of *MYC* overexpression than patients with typical MCL morphology. However, these differences were not statistically significant. Recently, *MYC* overexpression has been observed in association with blastoid morphology (Bea *et al*, 1999; Hernandez *et al*, 1999; Zhu *et al,* 2002). However, survival had not been studied previously. Whether *MYC* overexpression is an independent prognostic marker in MCL needs to be studied in a larger patient group.

To conclude, the aberrant expression of the apoptotic genes was observed to be associated with immunophenotypic and cytogenetic findings and with overall survival in leukaemias and lymphomas.

ACKNOWLEDGMENTS

This work was supported by grants from the Sigrid Jusélius Foundation, Finnish Cancer Society, Tampere University Hospital Research Fund (J.V), Paulo Foundation and Finnish Cultural Foundation, all in Finland. We are grateful for the excellent technical assistance provided by Leena Vilpo.

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