Microarray Analysis of B-Cell Lymphoma Cell Lines with the t(14;18)

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The t(14;18) is the most common genetic alteration in follicular lymphoma, and is detectable in a subset of diffuse large B-cell lymphomas (DLBCL), resulting in over-expression of the anti-apoptotic protein BCL-2. Although the t(14;18)-induced over-expression of BCL-2 is an important step in lymphomagenesis, this aberration alone is not sufficient to produce malignant lymphoma. Further analysis of these tumors is needed to identify additional genes that might be involved in the genesis of follicular lymphoma and progression to DLBCL. To address this issue, we analyzed the gene expression profiles of four t(14;18)-positive cell lines and two t(11;14)-positive mantle-cell lymphoma cell lines using cDNA microarrays containing 4364 genes, and compared them to the genetic profile of phenotypically purified B-cells obtained from hyperplastic tonsils. A total of 137 genes were differentially expressed by approximately twofold or more in the t(14;18) cell lines relative to tonsillar B-cells. 68 genes were up-regulated, 69 genes were down-regulated, and approximately 20% of the differentially regulated genes had no known function. The up-regulated genes included a number of genes involved in the promotion of cellular proliferation and survival, as well as cell metabolism. Down-regulated genes included mediators of cell adhesion and negative regulators of cell activation and growth. Hierarchical clustering analysis separated the t(14;18) and mantle-cell lines into distinct groups based on their gene expression profiles. We confirmed the differential expression of approximately 80% of selected up- and down-regulated genes identified by microarray analysis by quantitative real-time fluorescence reverse transcriptase polymerase chain reaction (RT-PCR) analysis and/or immunoblotting. This study demonstrates the utility of cDNA microarray analysis for the assessment of global transcriptional changes that characterize t(14;18)-positive cell lines, and also for the iden-

tification of novel genes that could potentially contribute to the genesis and progression of non-Hodgkin's lymphomas with this translocation. (*J Mol Diagn* 2002, 4:123–136)

The t(14;18) is a frequent chromosomal alteration in non-Hodgkin's lymphomas (NHL), occurring in up to 90% of follicular lymphomas¹ and 20 to 30% of diffuse large B-cell lymphomas (DLBCL).^{2,3} This translocation is acquired early in B-cell development and results in juxtaposition of the *bcl-2* gene on chromosome 18 with the immunoglobulin heavy-chain locus on chromosome 14, resulting deregulated expression of a structurally intact BCL-2 protein.^{4–8} BCL-2 is located in the inner mitochondrial membrane and functions as an anti-apoptotic protein that inhibits programmed cell death,⁹ resulting in accumulation of B-lymphocytes by virtue of increased cell survival.^{9–11}

Although the t(14;18)-induced over-expression of *bcl-2* is an important step in lymphomagenesis, this alteration alone is not sufficient to produce malignant lymphoma. This has been demonstrated in *in vitro* gene transfer experiments using cell lines and transgenic mice studies in which forced over-expression of *bcl-2* was insufficient to cause lymphoma.^{12,13} Indeed, low numbers of t(14;18)-carrying cells can be detected in benign lymphoid tissues such as follicular hyperplasias and the hyperplastic tonsils of young children.¹⁴ These studies indicate that cumulative genetic and cellular alterations superimposed on the t(14;18) are necessary for the development of lymphoma and underscore the need for further analysis of t(14;18)-containing DLBCLs to identify additional genes involved in lymphomagenesis and progression.

cDNA microarray technology allows large scale parallel analyses of gene expression and permits simultaneous comparison of the relative gene expression levels of several thousand genes in different cell types.^{15,16} This approach has now been used successfully by a number

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of groups to identify distinct gene expression patterns in various tumors, tumor cell lines, and disease states.^{17–24} In this study, we use cDNA microarrays to examine the global transcriptional profiles of four t(14;18)-positive lymphoma-derived cell lines and demonstrate that they can be readily distinguished from two t(11;14)-positive mantle-cell lymphoma cell lines on the basis of their respective expression profiles. We also demonstrate the utility of real-time quantitative fluorescence RT-PCR for validation of microarray expression data and show how this approach may be used to identify differentially expressed genes that could be involved in the development of t(14;18)-positive NHLs.

Materials and Methods

Cell Lines

Stanford University diffuse histiocytic lymphoma (SUDHL) cell lines SUDHL-4 and SUDHL-6²⁵ were generously provided by Dr. Lynn Sorbara, National Cancer Institute, National Institutes of Health, Bethesda, MD. Karpas 422,²⁶ Ontario Cancer Institute (OCI)-LY1,²⁷ and NCEB-1²⁸ were kindly provided by Dr. Neil Berinstein, University of Toronto, Ontario, Canada. Granta 519²⁹ was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Each of these cell lines has a complex karyotype that has previously been published.^{26,28–32} All cell lines were maintained in RPMI-1640 medium (Nova-Tech, Inc., Grand Island, NE) supplemented with 20% (v/v) heat-inactivated fetal calf serum and penicillin/streptomycin/amphotericin B solution (Antibiotic-Antimycotic, Life Technologies, Rockville, MD).

Tonsillar B-Cells

Isolation of phenotypically enriched tonsillar B-lymphocytes was performed as previously described.³³ Briefly, a routine tonsillectomy sample was obtained from a single patient (with informed consent) who underwent surgical extirpation for chronic tonsillitis. A single specimen was processed to minimize the heterogeneity of the purified B-cell population. Tonsillar tissue was finely minced and the resulting cell suspension was depleted of non-B-cells by plastic adherence and rosetting with sheep erythrocytes. This method routinely yields tonsillar B-lymphocytes at approximately 98% purity as determined by immunophenotypic analysis with CD3, CD19, CD20, and CD45 antibodies.

RNA Preparation

Total RNA was isolated from cell lines and purified tonsillar B-cells using TRIzol reagent (Life Technologies, Rockville, MD) according to the manufacturer's instructions. Residual DNA was removed by treatment with RNase-free DNase in the presence of the ribonuclease inhibitor RNasin. The RNA was phenol/chloroform-extracted, ethanol-precipitated, and then resuspended in

DEPC-treated RNase-free distilled water. mRNA was purified from total RNA using the Qiagen Oligotex mRNA purification kit as described by the manufacturer (Qiagen, Valencia, CA).

DNA Microarray Analysis

Microarray analysis was performed in the Huntsman Cancer Institute Microarray Core Facility at the University of Utah using Molecular Dynamics/Amersham Pharmacia Biotech (Piscataway, NJ) instrumentation to print and scan the microarray slides. Microarray slides contained 4364 sequence-verified clones obtained from Research Genetics (Huntsville, AL). Differential gene expression was investigated using a simultaneous two-color hybridization scheme essentially as described by Schena et al.¹⁵ Fluorescently labeled cDNA from each of the cell populations to be compared was prepared from mRNA samples by oligo dT-primed synthesis in a 20 μ l reaction using SuperScript II reverse transcriptase (Life Technologies, Rockville, MD) in the presence of 50 μ mol/L dGTP, dATP, dTTP, 5 μ mol/L dCTP, and 12.5 μ mol/L of either Cy3-dCTP (green fluorescence) or Cy5-dCTP (red fluorescence) (Amersham Pharmacia Biotech, Piscataway, NJ). After incubation at 42°C for 2.5 hours, the reaction was stopped by the addition of 1 μ l of 5 mol/L NaOH and incubation at 37°C for 10 minutes. The resulting alkaline solution was partially neutralized with 2.5 μ l of 2 mol/L Tris-HCL (pH 7.5) and 1 μ l of 5 mol/L HCL, and unincorporated nucleotides were removed using the Qiagen PCR clean-up kit (Qiagen, Valencia, CA). The two fluorescent probes were mixed, vacuum-concentrated, resuspended in hybridization buffer, and then hybridized to the microarray slides. Hybridization patterns were captured electronically using a two-color confocal laser microscope. Analysis of array images was performed by both visual inspection and electronic quantitation of the fluorescence intensities of individual microarray spots.

Four separate hybridizations were performed for all samples. Expression data from replicate cDNA microarray hybridizations showed a high degree of reproducibility with correlation coefficients ranging from approximately 0.85 to 0.90 (data not shown). Our cDNA microarrays tended to show a lower dynamic range among over-expressed genes than under-expressed genes, resulting in identification of fewer differentially over-expressed genes than under-expressed genes at a given threshold of expression. Therefore, to identify sufficient genes for further analysis, differential expression of a gene was considered to be present between cell line and tonsillar B-cell samples when the Cy5/Cy3 ratio was greater than or equal to a 1.5-fold difference from the mean signal ratio of the entire microarray slide for over-expressed genes, and a twofold or more difference for under-expressed genes. In accordance with the recommendations of Lee et al,³⁴ gene expression patterns were considered reproducible only if they were maintained in at least three replicate hybridizations (from a total of four hybridizations). Visual inspection of microarray images was accomplished with the assistance of ImageQuant NT

Table 1. Primers Used for Quantitative Fluorescence RT-PCR Analysis of Selected Genes

Gene	Primer	Sequence
Glyceraldehyde-3-phosphate dehydrogenase	Forward	5'-CGACCACTTTGTCAAGCTCA-3'
	Reverse	5'-AGGGGAGATTCAAGTGTGGTG-3'
Stearoyl-CoA desaturase (delta-9-desaturase)	Forward	5'-CCCAGCTGTCAAAGAGAAGG-3'
	Reverse	5'-CCACAGCATATCGCAAGAAA-3'
v-Myb	Forward	5'-GTCCGAAACGTTGGTCTGT-3'
	Reverse	5'-TTCGTCCAGGCAGTAGCTTT-3'
Neurotrophic tyrosine kinase, receptor-related 1	Forward	5'-ACCTCGACACCACAGACACA-3'
	Reverse	5'-GTGCGGTTGCCAATAAATCT-3'
Cyclin-dependent kinase 8	Forward	5'-GCAGATTTGGATCCAGTGGT-3'
	Reverse	5'-TCCAGCTGGTCATGGTGATA-3'
Human receptor-like tyrosine kinase (H-Ryk)	Forward	5'-TGCCCTCTCCAGAGACTTGT-3'
	Reverse	5'-CATCTCGAAGGGGTCAATGT-3'
EST 1	Forward	5'-CGAATCTCCTCTTGCTGT-3'
	Reverse	5'-GGCGAAATGCCAGCTTATAC-3'
EST 2	Forward	5'-CAGCCACACCAAGAAGTCAA-3'
	Reverse	5'-TCTGAGCGATCTGTGTTTGC-3'
EST 3	Forward	5'-GGCAGGAATACACAGCAACA-3'
	Reverse	5'-AGGCTGGCTGGAATAAAGGT-3'
JunB	Reverse	5'-TCTCTCAAGCTCGCCTCTTC-3'
	Forward	5'-ACGTGGTTCATCTTGTGCAG-3'
JunD	Forward	5'-AAGTCCTCAGCCACGTCAAC-3'
	Reverse	5'-TCTGAGTTCCTGGGCACACT-3'
FosB	Forward	5'-GACTCAAGGGGGTGACAGAA-3'
	Reverse	5'-AAAATGTCACAGCCCCTCAC-3'

software (Molecular Dynamics, Sunnyvale, CA). Electronic spot quantification was achieved using ArrayVision 4 software from Imaging Research (Ontario, Canada). This program permitted assignment of numerical values representative of the fluorescence intensities of the individual spots. Manipulation of raw fluorescence data were accomplished using GeneSpring software (Redwood City, CA).

Quantitative Fluorescence RT-PCR Analysis

Total RNA was isolated from cell lines and purified tonsillar B-cells using TRIzol reagent (Life Technologies, Rockville, MD) according to the manufacturer's instructions. First-strand cDNA synthesis was performed using 1.0 μ g of total RNA and SuperScript II RNase H-reverse transcriptase (Life Technologies, Rockville, MD) according to the manufacturer's instructions. Fluorescence PCR analysis was performed using the LightCycler (Roche Molecular Biochemicals, Indianapolis, IN) and SYBR Green I (Molecular Probes, Eugene, OR) essentially as described.³⁵ Briefly, 1.0 μ l of each first-strand cDNA reaction was amplified by primer pairs specific for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), stearoyl-CoA desaturase, v-myb, neurotrophic tyrosine kinase receptor-related 1, cyclin-dependent kinase 8, junB, junD, and fosB genes, and selected ESTs (summarized in Table 1) in a 10 μ l reaction containing 1X PCR buffer (50 mmol/L Tris [pH 8.3], 250 μ g/ml bovine serum albumin, 2% sucrose, 3.0 mmol/L $MgCl_2$), dNTPs at 200 μ mol each, 0.5 μ mol of each primer, 0.4 units of *Taq*DNA polymerase (Promega, Madison, WI), 8.8 ng/ μ l TaqStart antibody (Clontech, Palo Alto, CA), and the double-stranded DNA binding dye SYBR Green I (1:30,000 dilution). Amplification reactions consisted of 40 to 50 cycles of denaturation at 94°C (0 seconds), annealing at 55°C (0

seconds), and extension at 72°C (15 seconds). Fluorescence signals were obtained once in each cycle by sequential fluorescence monitoring of each sample tube at the end of extension. A fractional cycle number or crossing threshold (CT) was determined from the exponential phase of the fluorescence amplification profiles using the second derivative maximum function of the Roche Light-Cycler software. The CT values so derived serve as indirect indicators of gene expression so that samples with high expression of a given gene exhibit lower CTs than samples showing low-level gene expression. Expression of the housekeeping gene GAPDH was used as a control for input cDNA in each LightCycler amplification reaction. Once the GAPDH CT was determined for each cDNA sample, it was used to normalize all other genes tested from the same cDNA samples. Calculation of fold increase or decrease in expression of selected genes relative to expression levels in tonsillar B-cells was accomplished using the following formula modified from Elenitoba-Johnson et al.³⁶

Fold increase or decrease in expression =

$$\frac{2^{\Delta - (\text{Cell line test gene CT} - \text{Tonsillar B-cell test gene CT})}}{2^{\Delta - (\text{Cell line GAPDH CT} - \text{Tonsillar B-cell GAPDH CT})}}$$

This formula permits an accurate estimation of the abundance of specific transcripts relative to a reference sample (phenotypically purified tonsillar B-cells) using a relatively stable transcript (GAPDH) for normalization of input cDNA in all four of the t(14;18)-positive cell lines.

Protein Analysis

To validate our microarray analysis by another modality, we also assessed all four t(14;18)-positive cell lines (SUDHL-4, SUDHL-6, Karpas 422, and OCI-LY1) and

both mantle-cell lymphoma lines (Granta 519 and NCEB-1) for BCL-2 protein expression by immunoblotting (data not shown). Total cell extracts were prepared by lysing cells in ice-cold lysis buffer containing 0.2% Nonidet P-40, 20 mmol/L Tris-HCl (pH 7.50), 0.25 mol/L NaCl, 1 mmol/L EDTA, and 2 μ l/ml mammalian cell extract protease inhibitor cocktail (Sigma, St. Louis, MO). Cell lysates were then subjected to three cycles of freezing (liquid N₂) and thawing (37°C water bath). Equal amounts of total cell extract (30 μ g) were separated by SDS-PAGE, transferred to Immobilon membranes (Millipore, Bedford, MA), and probed with monoclonal antibodies to BCL-2 (1:500, sc-509) and actin (1:100, sc-8432) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Protein was detected by enhanced chemiluminescence (Luminol Reagent, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and Kodak BioMax ML film.

Results

Microarray Analysis

In this study, we sought to identify transcriptional changes that might implicate specific genes in the development of DLBCLs harboring the t(14;18). To accomplish this goal, we analyzed the gene expression profiles of four B-cell lymphoma cell lines containing the t(14;18) and two mantle-cell lymphoma cell lines containing the t(11;14) using cDNA microarrays consisting of nearly 4500 genes. For this analysis, purified tonsillar B-cells were used to represent a normal B-cell population in which successive genetic alterations might be superimposed on the t(14;18) during development of DLBCL. The use of phenotypically purified tonsillar B-cells as a reference standard also allowed us to compare the expression profiles of tumor cell lines derived from neoplasms putatively arising from germinal center cells and naive (mantle) cells, since both of these cell populations are represented in the purified tonsillar B-cell sample. We were also interested to determine whether expression profiling could discriminate the t(14;18) cell lines from another genetically distinct aggressive B-cell lymphoma, specifically, t(11;14)-positive mantle-cell lymphoma cell lines, based on their gene expression profiles. We also sought to determine whether we could identify patterns of differential gene expression that were shared by the t(14;18)-positive cell lines.

Unsupervised hierarchical clustering using all of the genes in our microarray system revealed that the four t(14;18)-positive cell lines could be readily separated from the two mantle-cell lymphoma cell lines based on their respective gene expression profiles, with the two mantle-cell lymphoma cell lines clustering together in a group that was distinctly separate from the four t(14;18) cell lines (Figure 1). Further analysis of the genetic profiles of the t(14;18) cell lines revealed a total of 137 differentially expressed genes at the RNA level by approximately twofold or more. Relative to tonsillar B-cells, 68 genes were up-regulated in at least three of the four cell lines (range, 1.5- to 3.7-fold; Tables 2 and 3), 69

Figure 1. Unsupervised hierarchical clustering of gene expression data from t(14;18) and mantle-cell lymphoma cell lines using the total complement of 4364 genes. The dendrogram lists the various cell lines and also provides a measure of the relatedness of gene expression in each sample so that samples showing closely related expression profiles are juxtaposed in adjacent branches of the hierarchical dendrogram. Karpas 422, SUDHL-4, SUDHL-6, and OCI-LY1 are t(14;18)-containing B-cell lymphoma cell lines, and NCEB-1 and Granta 519 are t(11;14)-containing mantle-cell lymphoma cell lines. The t(11;14)-containing mantle-cell lymphoma cell lines are closely related and arise from the same branch of the dendrogram. The t(14;18)-containing cell lines arise from a different node and cluster separately from the t(11;14)-cell lines.

genes were down-regulated in all four cell lines (range, 2.0- to 15.9-fold; Table 4), and 29 (approximately 20%) of these genes consisted of expressed sequence tags (ESTs) with no known function (Tables 2 and 4). Among the up-regulated genes with known function, approximately 40% were involved in the promotion of cellular proliferation and survival, as well as cellular metabolism (Table 2, Groups 1 and 2). Interestingly, a number of these genes have previously been found to be over-expressed in a variety of carcinomas and lymphomas (see discussion). A number of the up-regulated genes in the t(14;18)-positive cell lines were also over-expressed in both mantle-cell lymphoma lines, including seven ESTs (Table 3). However, the global expression profiles of these two cell lines clearly separated them from the four t(14;18)-positive cell lines (Figure 1). As expected, the mantle-cell lymphoma lines also over-expressed cyclin D1 and a number of additional genes in the cyclin D1 pathway (data not shown). Genes that showed at least twofold decreased expression in all four of the t(14;18)-positive DLBCL cell lines relative to tonsillar B-cells included putative negative regulators of cell activation and growth, genes potentially involved in apoptosis, mediators of cell adhesion, cytoskeletal genes, major histocompatibility complex genes, and a wide variety of other genes (Table 4, Groups 1–5). The vast majority of these genes had known or presumed functions, with only four ESTs identified in this group. Additional hierarchical clustering analysis using only the 137 differentially expressed genes (Tables 2 and 4) revealed only minor differences in gene expression among the four t(14;18) cell lines (Figure 2).

Confirmation of Differential Gene Expression by Real-Time PCR

To confirm the differential expression of genes identified by cDNA microarray analysis in the various lymphoma

Table 2. Genes Overexpressed in at Least Three t(14;18) Cell Lines

Gene name	Gene symbol	Clone ID no.	GenBank no.	Locus	Mean fold Increase
Group 1: Cellular proliferation and survival					
v-Myc	MYC	812965	AA464600	8q24.12-q24.13	1.9
v-Myb	MYB	243549	N49526	6q22-q23	2.8
Bcl-2	BCL2	232714	H74208	18q21.3	3.7
BCL2/adenovirus E1B 19kD-interacting protein 3	BNIP3	359982	AA063521		1.8
Cyclin-dependent kinase 8	CDK8	42880	R59697	13q12	1.9
Mitogen-activated protein kinase 10	MAPK10	23173	T75436		1.9
Erythropoietin receptor	EPOR	49509	H15634	19p13.3-p13.2	1.6
Neurotrophic tyrosine kinase, receptor-related 1	NTRKR1	781097	AA430035	1p32-p31	1.8
RYK receptor-like tyrosine kinase (H-Ryk)	RYK	108815	T77810	3q22	2.3
Inositol 1,4,5-triphosphate receptor, type 2	ITPR2	753914	AA479093	12p11	1.5
Suppression of tumorigenicity 16 (melanoma differentiation)	ST16	712049	AA281635	1	1.8
EGF-like-domain, multiple 2	EGFL2	175103	H39187		1.6
Zinc finger protein 22 (KOX 15)	ZNF22	137638	R37224	10q11	1.8
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 3	SMARCA3	810974	AA459632	3q25.1-q26.1	1.6
Nuclear factor (erythroid-derived 2)-like 1	NFE2L1	755821	AA496576	17q21.3	1.7
Primase, polypeptide 1 (49kD)	PRIM1	365641	AA025937	12q13	1.6
Group 2: Cellular metabolism					
Stearoyl-CoA desaturase (delta-9-desaturase)	SCD	123474	R00707	10	3.1
Cytochrome c-1	CYC1	813830	AA447774	8q24.3	2.0
Glycine cleavage system protein H (aminomethyl carrier)	GCSH	134748	R28294		1.8
Methionine adenosyltransferase II, alpha	MAT2A	79502	T59286	2p11.2	1.7
Ornithine aminotransferase (gyrate atrophy)	OAT	783696	AA446819	10q26	1.6
Methylene tetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase	MTHFD2	814615	AA480995		1.7
Aldehyde dehydrogenase 5 family, member A1 (succinate-semialdehyde dehydrogenase)	ALDH5A1	44505	H06675	6p22	1.6
Hexosaminidase B (beta polypeptide)	HEXB	80095	T63321	5q13	1.7
UDP-N-acetylglucosamine pyrophosphorylase 1; sperm associated antigen 2	UAP1	292515	N68465		1.8
Reticulocalbin 2, EF-hand calcium binding domain	RCN2	898253	AA598676	15q22.33-q24.1	1.6
Calumenin	CALU	144881	R78585	7q32	1.8
Glycogenin	GYG	753285	AA411678	3q24-q25.1	1.9
Group 3: Miscellaneous					
Membrane protein, palmitoylated 1 (55kD)	MPP1	296880	W01240	Xq28	1.7
Lysosomal-associated membrane protein 2	LAMP2	289615	N77754	Xq24	1.7
Adaptor-related protein complex 3, mu 2 subunit	CLA20	28823	R14443	8p11.2	1.9
Human DNA from overlapping chromosome 19 cosmid containing COX6B and UPKA		771000	AA429281		1.6
KIAA0286 protein	KIAA0286	109221	T81399		1.6
Integrin, alpha 8	ITGA8	165878	R87964		1.9
Breakpoint cluster region	BCR	756163	AA419342	22q11.23	1.7
RD RNA-binding protein	RDBP	509484	AA056390	6p21.3	1.6
Glycoprotein M6B	GPM6B	713660	AA284329	Xp22.2	1.9
Nuclear matrix protein p84	P84	711450	AA280748	18	1.8
Tryptophan rich basic protein	WRB	758329	AA401236	21q22.3	1.7
Metallothionein 1L	MT1L	297392	N80129	16q13	1.7
Novel human gene mapping to chromosome 13		841695	AA488718		2.6
Homo sapiens mRNA; cDNA DKFZp564H2416 (from clone DKFZp564H2416)		294487	W01536		1.8
DKFZP586D2223 protein	DKFZP586D2223	199158	H83178		1.6
EST 1		195051	R91137		1.6
EST 2		308478	W24883		1.9
EST 3		346545	W74293		2.2
22 ESTs					

cell lines, we analyzed a number of up- and down-regulated genes by real-time quantitative fluorescence RT-PCR (Figures 3 and 4, Table 5). Using this approach, we consistently observed a greater dynamic range of expression in the RT-PCR data relative to the corresponding cDNA microarray expression data (Figure 4). We were able to validate microarray expression data for the vast majority of the genes reported here (Table 5; 11 different genes, 47 of 52 separate RT-PCR reactions), with the

exception of two cell lines for neurotrophic tyrosine kinase expression (Figures 3C and 4C, Table 5), and one cell line for FosB expression (Figures 3K and 4K, Table 5). We also confirmed the differential regulation of three different ESTs (EST 1, EST 2, and EST 3) in the four t(14;18) cell lines (Figures 3, F-H and 4, F-H, Table 5). Similarly, the over-expression of cyclin D1 observed in the t(11;14)-positive mantle-cell lymphoma cell lines was also validated by real-time fluorescence RT-PCR (data

Table 3. Genes Overexpressed in Both Mantle Cell Lines and at Least Three t(14;18) Cell Lines

Gene name	Gene symbol	GenBank no.	Locus
Lysosomal-associated membrane protein 2	LAMP2	N77754	Xq24
Adaptor-related protein complex 3, mu 2 subunit	CLA20	R14443	8p11.2
Human DNA from overlapping chromosome 19 cosmids containing COX6B and UPKA		AA429281	
Stearoyl-CoA desaturase (delta-9-desaturase)	SCD	R00707	10
KIAA0286 protein	KIAA0286	T81399	
Integrin, alpha 8	ITGA8	R87964	
Glycine cleavage system protein H (aminomethyl carrier)	GCSH	R28294	
Erythropoietin receptor	EPOR	H15634	19p13.3-p13.2
RYK receptor-like tyrosine kinase (H-Ryk)	RYK	T77810	3q22
Glycoprotein M6B	GPM6B	AA284329	Xp22.2
Primase, polypeptide 1 (49kD)	PRIM1	AA025937	12q13
Cytochrome c-1	CYC1	AA447774	8q24.3
Cyclin-dependent kinase 8	CDK8	R59697	13q12
Metallothionein 1L	MT1L	N80129	16q13
Homo sapiens mRNA; cDNA DKFZp564H2416 (from clone DKFZp564H2416)		W01536	
Calumenin	CALU	R78585	7q32
Hexosaminidase B (beta polypeptide)	HEXB	T63321	5q13
Bcl-2	BCL2	H74208	18q21.3
v-Myb	MYB	N49526	6q22-q23
7 ESTs			

not shown).³⁶ Overall agreement with the microarray data, including additional RT-PCR data not shown here, was approximately 80% (15 different genes, 53 of 66 separate RT-PCR reactions). These results are in agreement with a recent study by Rajeevan et al³⁷ in which they were able to verify the expression levels of approximately 70% of differentially regulated genes identified by microarray analysis using a similar RT-PCR approach that also used the LightCycler. These results also underscore the importance of validation of microarray data by additional methods such as RT-PCR or Northern blot analysis.

Confirmation of BCL-2 Protein Expression by Immunoblotting

All six of the lymphoma cell lines in our study also over-expressed bcl-2 mRNA by microarray analysis (Tables 2 and 3). We confirmed the expression BCL-2 protein in each of our t(14;18)-positive cell lines and mantle-cell lines by immunoblotting (data not shown).

Discussion

In this study, we analyzed the gene expression profiles of four B-cell lymphoma cell lines containing the t(14;18) and two mantle-cell lymphoma-derived cell lines containing the t(11;14) using cDNA microarrays consisting of almost 4500 genes. We found that we could readily discriminate the t(14;18)-positive cell lines from those derived from another aggressive B-cell lymphoma, mantle-cell lymphoma. The t(14;18)-positive cell lines also exhibited a transcriptional profile that included a number of differentially expressed genes that could contribute to the development of NHLs harboring the t(14;18), including several novel genes with no known function. Up-regulated genes identified by microarray analysis included a number of genes involved in the promotion of

cellular proliferation and survival, as well as cell metabolism. Many of these genes have also been found to be over-expressed in a variety of carcinomas and lymphomas. For example, stearoyl-CoA desaturase (0 δ 9-desaturase) (Table 2, Group 2), a key enzyme involved in the biosynthesis of unsaturated fatty acids,³⁸ has previously been shown to be over-expressed in colonic and esophageal carcinomas, as well as in hepatocellular adenomas.³⁹ A number of human lymphoma and leukemia cell lines express this gene,⁴⁰ and it has also been found to be over-expressed in experimentally induced mammary carcinogenesis in rats.⁴¹

Another interesting over-expressed gene identified by microarray analysis in the t(14;18) cell lines was cyclin-dependent kinase 8 (CDK8) (Table 2, Group 1). CDK8 and its cyclin partner, cyclin C,⁴² are components of the RNA polymerase II holoenzyme complex, functioning as a protein kinase that phosphorylates the carboxy-terminal domain of the largest subunit of RNA polymerase II, thereby regulating its transcriptional activity.^{43,44} CDK8 can also function as a transcriptional activator when fused to the DNA binding domain of GAL4, and this activity is independent of the kinase activity of the protein.⁴³ Interestingly, CDK8's cyclin partner, cyclin C, is frequently mutated in acute lymphoblastic leukemias.^{45,46}

Our studies also revealed that the human receptor-like tyrosine kinase (H-Ryk) was over-expressed in the t(14;18)-positive cell lines (Table 2, Group 1). This gene was originally identified as a PCR-amplified cDNA fragment from a chronic myelogenous leukemia cell line (K-562)⁴⁷ and subsequently found to be expressed in a wide variety of human tissues.^{48,49} H-Ryk over-expression can induce anchorage-independent growth in the mouse fibroblast NIH3T3 cell line and also produces tumors when H-Ryk-transfected fibroblasts are inoculated into nude mice.⁵⁰ H-Ryk is also over-expressed in malignant ovarian tumors,⁵¹ and over-expression of this gene has been

Table 4. Genes Underexpressed in All Four t(14;18) Cell Lines

Gene name	Gene symbol	Clone ID no.	GenBank no.	Locus	Mean fold decrease
Group 1: Putative negative regulators of cell activation and growth					
Protein tyrosine phosphatase, receptor type, alpha polypeptide	PTPRA	240099	H82419	20p13	2.6
Protein tyrosine phosphatase, receptor type, gamma polypeptide	PTPRG	137531	R38343	3p21-p14	3.7
Protein tyrosine phosphatase, receptor type, c polypeptide	PTPRC	229365	H74265	1q31-q32	3.8
Transforming growth factor, beta 1	TGFB1	136821	R36467	19q13.1	3.4
Endoglin (Osler-Rendu-Weber syndrome 1)	ENG	774409	AA446108	9q33-q34.1	3.4
Phosphodiesterase 1/nucleotide pyrophosphatase 1 (homologous to mouse Ly-41 antigen)	PDNP1	82991	T70503	6q22-q23	2.7
JunB	JUNB	122428	T99236	19p13.2	7.5
JunD	JUND	767784	AA418670	19p13.2	6.1
Group 2: Putative apoptosis related genes					
CD53 antigen	CD53	504226	AA132090	1p31-p12	9.1
Lysosomal-associated multispinning membrane protein-5	LAPTM5	753313	AA410265	1p34	5.9
Group 3: Mediators of cell adhesion and cytoskeletal genes					
Villin 2 (ezrin)	VIL2	755145	AA411440	6q25-q26	2.1
Actin related protein 2/3 complex, subunit 5 (16 kD)	ARPC5	340558	W55964		2.6
Profilin 1	PFN1	826173	AA521431	17p13.3	3.9
L-Selectin (lymphocyte adhesion molecule 1)	SELL	149910	H00756	1q23-q25	6.0
Actin, gamma 2, smooth muscle, enteric	ACTG2	81289	T60048	2p13.1	7.5
CD44 antigen (homing function and Indian blood group system)	CD44	713145	T69168	11p13	3.8
Kallmann syndrome 1 sequence	KAL1	50182	H17882	Xp22.32	11.0
Group 4: Major histocompatibility complex					
Major histocompatibility complex, class II, DM alpha	HLA-DMA	183337	H42679	6p21.3	4.6
Major histocompatibility complex, class II, DQ alpha 1	HLA-DQA1	80109	T63324	6p21.3	5.9
Human HLA-DR alpha-chain mRNA		153411	R47979		6.3
Major histocompatibility complex, class II, DP beta 1	HLA-DPB1	840942	AA486627	6p21.3	7.4
Major histocompatibility complex, class II, DQ beta 1	HLA-DQB1	809598	AA442984	6p21.3	8.0
Group 5: Miscellaneous					
Eukaryotic translation termination factor 1	ETF1	811999	AA456664	5	2.0
Interleukin 4 receptor	IL4R	714453	AA292025	16p11.2-12.1	2.1
Poly(A)-binding protein, cytoplasmic 1	PABPC1	840940	AA486626	8q22.2-q23	2.3
Mel transforming oncogene (derived from cell line NK14)-RAB8 homolog	MEL	525566	AA064715	19p13.1	2.3
Chemokine (C-X-C motif), receptor 4 (fusin)	CXCR4	79629	T62491	2q21	2.3
Interferon regulatory factor 1	IRF1	740476	AA478043	5q31.1	2.4
Phosphofructokinase, platelet	PFKP	950682	AA608558	10p15.3-p15.2	2.5
Myxovirus (influenza) resistance 1, homolog of murine (interferon-inducible protein p78)	MX1	815542	AA456886	21q22.3	2.5
Platelet-derived growth factor receptor-like	PDGFR	810010	AA455210	8p22-p21.3	2.5
DNA segment on chromosome X and Y (unique) 155 expressed sequence	DXYS155E	897619	AA496863	Xp22.32	2.6
Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	NFKBIA	340734	W55872	14q13	2.6
KIAA0220 protein	KIAA0220	785605	AA448998		2.6
Phosphoglucosyltransferase 1	PGM1	843174	AA488504	1p31	2.6
Poly(rC)-binding protein 1	PCBP1	839890	AA490047	2p12-p13	2.7
Ribosomal protein, mitochondrial, L3	RPML3	44255	H06113		2.8
Growth factor receptor-bound protein 2	GRB2	788654	AA449831	17q24-q25	3.0
Mannose-binding lectin (protein C) 2, soluble (opsonic defect)	MBL2	82879	T69359	10q11.2	3.0
Hematopoietic cell-specific Lyn substrate 1	HCLS1	767183	AA424575	3q13	3.1
Proteoglycan 1, secretory granule	PRG1	703581	AA278759	10q22.1	3.1
Nuclear receptor subfamily 4, group A, member 2	NR4A2	898221	AA598611	2q22-q23	3.2
Mannosidase, alpha, class 2A, member 1	MAN2A1	212496	H70017	5q21-q22	3.2
Guanine nucleotide exchange factor; 115-kD; mouse Lsc homolog	SUB1.5	815239	AA481277	19q13.13	3.3
GDP dissociation inhibitor 2	GDI2	197176	R92806	10p15	3.4
High-mobility group (nonhistone chromosomal) protein 17	HMG17	241826	H93087	1p36.1	3.4
Matrix metalloproteinase 16 (membrane-inserted)	MMP16	46916	H09997	8q21	3.5
CD69 antigen (p60, early T-cell activation antigen)	CD69	704459	AA279883	12p13-p12	3.6
KIAA0058 gene product	KIAA0058	34795	R19889		3.6
LIM domain only 2 (rhombotin-like 1)	LMO2	810521	AA464644	11p13	3.8
KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2	KDEL2	123117	T98559	7p	3.8
Human Alpha1		382773	AF001540	11q13	4.0
Tumor necrosis factor receptor superfamily, member 5	TNFRSF5	261519	H98636	20q12-q13.2	4.6
Myristoylated alanine-rich protein kinase C substrate (MARCKS, 80K-L)	MACS	840865	AA482328	6q22.2	5.6
Human germline IgD chain gene, C-region, C-delta-1 domain		814124	AA465378		6.1
NAD(P)H menadiene oxidoreductase 2, dioxin-inducible	NMOR2	824024	AA491124	6pter-q12	6.9
Ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	RAC2	827132	AA521232	22q13.1	7.2
Human 1.1 kb mRNA upregulated in retinoic acid treated HL-60 neutrophilic cells		810734	AA480820		7.5
CD79A antigen (immunoglobulin-associated alpha)	CD79A	115281	T87012	19q13.2	14.4
Colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	CSF2RA	289337	N92646	Xp22.32	15.9
FosB	FOSB	79022	T62179	19q13.3	7.8
Non-histone chromosomal protein	NHC	31873	R17124	6p21.3	2.0
Ras homolog gene family, member H	ARHH	302591	W38571	4p13	2.0
Interferon, gamma-inducible protein 16	IFI16	824602	AA491191	1q22	2.5
Dopachrome tautomerase (dopachrome delta-isomerase, tyrosine-related protein 2)	DCT	753104	AA478553	13q32	2.9
4 ESTs

Figure 3. Quantitative fluorescence RT-PCR analysis of selected differentially expressed genes identified by microarray analysis in the four t(14;18)-positive cell lines. Expression of the housekeeping gene GAPDH was used as a control for input cDNA in each RT-PCR reaction. Once the level of GAPDH expression was determined for each cDNA sample, it was used to normalize all other genes tested from the same cDNA samples as described in the Materials and Methods. RT-PCR results are depicted as amplification profiles with relative fluorescence on the y axis and cycle number on the x axis. Cell lines with the highest expression of a given gene will have a lower crossing threshold (CT), and therefore, will show an earlier onset of the exponential phase of the amplification curve for that gene. Amplification profiles are included for: (A) stearoyl-CoA desaturase, (B) v-Myb, (C) neurotrophic tyrosine kinase, receptor-related 1, (D) cyclin-dependent kinase 8, (E) human receptor-like tyrosine kinase (H-Ryk), (F) EST 1, (G) EST 2, (H) EST 3, (I) JunB, (J) JunD, (K) FosB, and (L) glyceraldehyde-3-phosphate dehydrogenase control.

correlated with significantly decreased overall survival and progression-free survival in patients with these tumors.⁵²

Interestingly, one of the over-expressed genes identified in our study, neurotrophic tyrosine kinase, receptor-

related 1 (NTRKR1 or Ror1) (Table 2, Group 1), had previously been found to be up-regulated in some DL-BCL tissue samples by another recent microarray study by Alizadeh et al.²¹ NTRKR1/Ror1 was originally identified in a neuroblastoma cell line⁵³ and was subsequently

Figure 2. Hierarchical clustering of gene expression data from the four t(14;18)-positive cell lines using the list of 137 previously identified differentially expressed genes contained in Tables 2 and 4. These genes include all genes over-expressed by approximately twofold or more in at least three of the four t(14;18) cell lines and under-expressed by twofold or more in all four t(14;18) cell lines relative to tonsillar B-cells. The genes corresponding to each horizontal row of the expression matrices are listed on the right side of the figure. Genes that were validated by quantitative fluorescence PCR are denoted by asterisks. The gene expression scale is the same as for Figure 1.

Figure 4. Comparison of cDNA microarray expression data and quantitative fluorescence RT-PCR data for selected differentially expressed genes identified by microarray analysis in the four t(14;18)-positive cell lines. RT-PCR results were calculated as described in the Materials and Methods. We have consistently observed a larger dynamic range of expression for our RT-PCR data relative to the corresponding cDNA microarray expression data. Results are included for: (A) stearyl-CoA desaturase (SCD), (B) v-Myb, (C) neurotrophic tyrosine kinase, receptor-related 1 (NTRKR1), (D) cyclin-dependent kinase 8 (CDK8), (E) human receptor-like tyrosine kinase (H-RYK), (F) EST 1, (G) EST 2, (H) EST 3, (I) JunB, (J) JunD, and (K) FosB. Note that no microarray data were available for SCD/Karpas 422 (A), SUDHL-6/CDK8 (D), and SUDHL-4/H-RYK (E).

found to be expressed in a wide variety of human adult and fetal tissues and a number of additional human tumor cell lines derived from neuroblastomas, medulloblastomas, melanomas, small cell lung carcinomas, leukemias, and lymphomas.⁵⁴

The two mantle-cell lymphoma lines in our study also over-expressed a small proportion of the same genes as the t(14;18)-containing DLBCL cell lines (Table 3). However, the global gene expression profiles in these two cell lines permitted them to be readily discriminated from the four t(14;18)-positive cell lines (Figure 1). Interestingly, the genes over-expressed in both the t(14;18)- and t(11;14)-positive cell lines also included the stearyl-CoA desaturase, CDK8, and H-Ryk genes mentioned above. As expected, both mantle-cell lymphoma cell lines also over-expressed cyclin D1 and other genes in the cyclin D1

pathway, including cyclin-dependent kinase 4 (CDK4), activating transcription factor 3 (ATF3), and the E2F transcription factor dimerization partner DP-2 (data not shown). In addition to these genes, we also found that a number of the over-expressed genes in our mantle-cell lymphoma lines were reported to be up-regulated in a recent microarray study of mantle-cell lymphomas,⁵⁵ including bcl-2, v-myb, the human homolog of mouse double minute 2 (MDM2), and H1 histone, family member 0 (data not shown).

Down-regulated genes identified by microarray analysis in the t(14;18)-positive cell lines included negative regulators of cell activation and growth, mediators of cell adhesion, major histocompatibility complex genes, genes possibly involved in the apoptosis process, and a large group of miscellaneous genes that could not be

Table 5. Quantitative Fluorescence RT-PCR Analysis of Selected Up- and Down-Regulated Genes Identified by Microarray Analysis

Gene	Cell line/RNA source	Test CT	GAPDH CT	Fold increase or decrease	Overall concordance with microarray data
Up-regulated genes by microarray analysis (in at least 3 of 4 cell lines)					
SCD	SUDHL-4	15.78	14.78	66.8	Yes
SCD	SUDHL-6	18.36	15.83	23.2	Yes
SCD	Karpas 422	21.22	15.64	2.8	NMDA
SCD	OCI-LY1	20.02	17.26	19.5	Yes
SCD	Tonsillar B-cell	22.79	15.73	N/A	Yes
v-Myb	SUDHL-4	22.05	14.78	0.9	Yes
v-Myb	SUDHL-6	15.54	15.83	166	Yes
v-Myb	Karpas 422	17.94	15.64	27.6	Yes
v-Myb	OCI-LY1	19.11	17.26	19.5	Yes
v-Myb	Tonsillar B-cell	22.81	15.73	N/A	Yes
NTRKR1	SUDHL-4	31.17	14.78	0.02	No
NTRKR1	SUDHL-6	31.29	15.83	0.04	No
NTRKR1	Karpas 422	19.91	15.64	94.1	Yes
NTRKR1	OCI-LY1	20.56	17.26	181.6	Yes
NTRKR1	Tonsillar B-cell	26.55	15.73	N/A	No
CDK8	SUDHL-4	21.36	14.78	4.2	Yes
CDK8	SUDHL-6	23.31	15.83	2.3	NMDA
CDK8	Karpas 422	22.07	15.64	4.7	Yes
CDK8	OCI-LY1	22.81	17.26	8.5	Yes
CDK8	Tonsillar B-cell	24.38	15.73	N/A	Yes
HRYK	SUDHL-4	25.26	16.25	0.5	NMDA
HRYK	SUDHL-6	22.87	16.96	5.8	Yes
HRYK	Karpas 422	22.24	16.14	7.9	Yes
HRYK	OCI-LY1	21.39	18.95	43	Yes
HRYK	Tonsillar B-cell	25.3	17.45	N/A	Yes
EST 1	SUDHL-4	44.29	16.25	0.0004	Yes
EST 1	SUDHL-6	31.36	16.96	6.3	Yes
EST 1	Karpas 422	28.64	16.14	36.2	Yes
EST 1	OCI-LY1	30.64	18.95	27.4	Yes
EST 1	Tonsillar B-cell	33.9	17.45	N/A	Yes
EST 2	SUDHL-4	25.53	16.25	7.2	Yes
EST 2	SUDHL-6	25.46	16.96	15.6	Yes
EST 2	Karpas 422	26.48	16.14	6.8	Yes
EST 2	OCI-LY1	27.14	18.95	13	Yes
EST 2	Tonsillar B-cell	29.32	17.45	N/A	Yes
EST 3	SUDHL-4	21.45	16.25	3.3	Yes
EST 3	SUDHL-6	22.3	16.96	3.8	Yes
EST 3	Karpas 422	20.93	16.14	8.7	Yes
EST 3	OCI-LY1	21.32	18.95	20	Yes
EST 3	Tonsillar B-cell	24.13	17.45	N/A	Yes
Down-regulated genes by microarray analysis (in all 4 cell lines)					
JunB	SUDHL-4	21.91	14.78	0.02	Yes
JunB	SUDHL-6	23.18	15.83	0.02	Yes
JunB	Karpas 422	22.95	15.84	0.02	Yes
JunB	OCI-LY1	22	17.26	0.1	Yes
JunB	Tonsillar B-cell	17.25	15.73	N/A	Yes
JunD	SUDHL-4	28.66	14.78	0.07	Yes
JunD	SUDHL-6	28.66	15.83	0.2	Yes
JunD	Karpas 422	28.95	15.64	0.1	Yes
JunD	OCI-LY1	28.17	17.26	0.55	Yes
JunD	Tonsillar B-cell	25.8	15.73	N/A	Yes
FosB	SUDHL-4	31.65	14.78	0.2	Yes
FosB	SUDHL-6	32.24	15.83	0.3	Yes
FosB	Karpas 422	31.2	15.64	0.54	Yes
FosB	OCI-LY1	31.89	17.26	0.98	No
FosB	Tonsillar B-cell	30.4	15.73	N/A	No

CT, Crossing Threshold; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SCD, stearoyl-CoA desaturase; NTRKR1, kinase, receptor-related 1; CDK8, cyclin-dependent kinase 8; HRYK, human receptor-like tyrosine kinase; EST = expre, N/A, not applicable; NMDA, no microarray data available; RT-PCR, Reverse transcriptase-polymerase chain reaction.

further categorized (Table 4, Groups 1–5). The genes in Group 1 included a number of protein phosphatases that may act to down-regulate signal transduction pathways that promote cell growth,⁵⁶ TGF- β 1, a potent inhibitor of

cellular proliferation,⁵⁷ and endoglin, a member of the TGF- β superfamily of receptors.⁵⁸ The genes in Group 1 of Table 4 also include two members of the AP-1 (activating protein-1) family of transcription factors that con-

sists of homodimers and heterodimers of Jun (v-Jun, c-Jun, JunB, JunD), Fos (v-Fos, c-Fos, FosB, Fra1, Fra2), Jun dimerization partners (JDP1 and JDP2), or activating transcription factor (ATF2, ATF3, B-ATF) proteins.⁵⁹ In contrast to c-Jun, a well-known promoter of cell proliferation, JunB and JunD are both potent negative regulators of proliferation. c-Jun expression results in up-regulation of cyclin D1 expression and down-regulation of the cyclin-dependent kinase inhibitor p16, while JunB expression has the opposite effect on these genes, resulting in inhibition of cell growth.⁶⁰ Another member of the Jun family of proteins, JunD, causes slowing of cellular proliferation and accumulation of cells in the G1 phase of the cell cycle when it is over-expressed in immortalized fibroblasts.⁶¹ JunD-deficient immortalized fibroblasts also display higher proliferation rates than their wild-type counterparts.⁶² Interestingly, TGF- β regulates the transcription (induction) of both JunB and FosB (another AP-1 family member in Group 5 of Table 4),⁵⁷ suggesting that low TGF- β expression may also contribute to the low levels of JunB and FosB observed in our cell lines. Presumably, reduced expression of the phosphatases and other members of this category might confer an increased proliferative advantage on these tumor cell lines.

The two under-expressed genes in Group 2 (Table 4), CD53 and lysosomal-associated multi-spanning membrane protein-5 (LAPTM5), have been recently implicated in the process of apoptosis. The expression of CD53 (a tetraspanin) is elevated in both apoptotic human neutrophils⁶³ and in an apoptosis-sensitive B-cell mouse lymphoma cell line.⁶⁴ The lysosome-associated protein LAPTM5 was originally identified as a gene that was preferentially expressed by adult human hematopoietic tissues,⁶⁵ but a rat homologue of this gene was recently found to be up-regulated in response to neuronal apoptosis.⁶⁶

A number of the down-regulated genes in Group 3 (Table 4) are cell adhesion molecules involved in cell-cell and cell-matrix interactions. Cell adhesion molecules have a significant role in lymphoma dissemination and may also contribute to lymphoma aggressiveness.⁶⁷ For example, studies of L-selectin expression in NHL have shown that the majority of nodal lymphomas express this protein, whereas aggressive disseminated B-cell and T-cell lymphomas are generally L-selectin negative.⁶⁷ Several experimental and clinical studies also suggest an important role for CD44 in the biological behavior of NHL and suggest that CD44 may be a useful marker for predicting disease outcome.⁶⁷ Thus, down-regulation of adhesion molecules may be related to the propensity of high-grade lymphomas to metastasize.^{68–70}

The under-expressed genes in Group 4 (Table 4) consists of a number of major histocompatibility complex genes. Interestingly, loss of human leukocyte antigen (HLA) class I and class II expression has been described in a subset of aggressive B-cell lymphomas (consisting primarily of DLBCLs), and this loss correlated with extranodal disease⁷¹ and poor survival.^{72–74} Similarly, a recent study of 258 DLBCLs found extensive loss of heterozygosity within the HLA region on the p arm of chromosome 6, primarily in extranodal lymphomas.⁷⁵ The

authors of this study also speculated that a structural loss of HLA class I and II gene expression might contribute to escape of aggressive B-cell lymphomas from immune surveillance. All four of the DLBCL cell lines used for this study were derived from extranodal lymphomas,^{25,27} and one of the cell lines (SUDHL-6) has been reported to have a 6p deletion.³⁰

In summary, this study demonstrates the utility of the combination of cDNA microarray analysis and quantitative real-time RT-PCR for rapid assessment of the transcriptional changes that characterize t(14;18)-positive lymphoma cell lines, and also for the identification of novel genes that could act in concert with deregulated BCL-2 in the genesis and progression of t(14;18)-positive NHLs. A comparison of the gene expression profiles of the t(14;18) cell lines to that of a reactive tonsillar B-lymphocyte population also allowed the identification of a number of novel genes that could potentially contribute to the genesis of t(14;18)-positive DLBCL. However, we have not ruled out the possibility that some of the differentially expressed genes identified by this study might simply be due to the microenvironment of the cell lines in culture (especially for genes involved in cellular metabolism). Confirmation of the utility of our approach is evidenced by the fact that we were able to validate approximately 80% of the gene expression trends observed by microarray analysis by real-time quantitative fluorescence RT-PCR. This method allows rapid confirmation of microarray expression data with very little starting material (1 μ g of total RNA or less), making it extremely useful for RT-PCR confirmation testing of microarray data obtained from small clinical specimens such as lymph node biopsies.

Although the results of this study are preliminary in nature, they provide a number of potential targets for further analysis in primary patient tumor samples that might provide additional insights into the process of lymphomagenesis. Indeed, we have noted that a number of the differentially expressed genes identified in the t(14;18)-positive cell lines also exhibited differential regulation in a cDNA microarray analysis of various B-cell lymphomas by Alizadeh et al.²¹ For example, myb and BCL-2 were over-expressed in the follicular lymphomas in the Alizadeh et al.²¹ study, and interleukin regulatory factor 1 (IRF-1) was under-expressed in these lymphomas. In our cDNA microarray study, we also observed over-expression of myb and BCL-2 and under-expression of IRF-1 in all four of the t(14;18)-positive cell lines (Figure 2). Collectively, these data suggest a potential relationship between follicular lymphomas and t(14;18)-positive DLBCLs. Furthermore, as a result of genomic-scale microarray-based analyses, new targets for diagnostic refinement and novel therapeutic approaches may emerge from the analysis of newly identified differentially expressed genes.⁷⁶

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