Gene expression profiling of cell lines derived from T-cell malignancies

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Abstract The expression profiles of eight cell lines derived from T-cell malignancies were compared to CD4-positive T-cells using cDNA microarray technology. Unsupervised hierarchical clustering of 4364 genes demonstrated substantial heterogeneity resulting in four distinct groups. While no genes were found to be uniformly up- or down-regulated across all cell lines, we observed 111 over-expressed genes (greater than two-fold) and 1118 down-regulated genes (greater than two-fold) in the lymphomas as a group when compared to CD4-positive T-cells. These included genes involved in cytokine signaling, cell adhesion, cytoskeletal elements, nuclear transcription factors, and known oncogenes and tumor suppressor genes. Quantitative fluorescent reverse transcription-polymerase chain reaction analysis demonstrated 70% concordance with the microarray results. While freshly isolated malignant cells may differ in their individual expression patterns relative to established cell lines from the same diagnoses, we feel that the variety of different lymphocytic cell lines that we examined provides a representative picture of the molecular pathogenesis of T-cell malignancies.

Key words: T-cell; Microarray; Expression profiling

1. Introduction

T-cell malignancies comprise approximately 10–20% of non-Hodgkin's lymphomas in the USA and Europe [1,2] and can develop at different stages in the pathway of normal T-cell differentiation. Precursor lymphoblastic lymphomas are derived from immature (thymus-based) lymphocytes, while peripheral T-cell lymphomas arise from mature T-lymphocytes in peripheral nodal and extranodal sites [3,4]. The mechanisms involved in T-cell lymphomagenesis and progression are not well defined, in part due to the infrequent nature of these neoplasms. In contrast to B-cell lymphomas, characteristic chromosomal translocations are found in a small subset of peripheral T-cell lymphomas. Chromosomal translocations or inversions of the TCL1 locus at chromosome 14q21.1 are seen in a subset of T-prolymphocytic leukemias [5], and the t(2;5) translocation involving the NPM-ALK gene is seen in anaplastic large cell lymphomas (ALCLs) [6,7]. The specific molecular abnormalities underlying many of the other classes of T-cell lymphoma are currently unknown.

Complementary DNA (cDNA) microarrays are now widely used to analyze global gene expression patterns [8–10]. This technology permits the identification of pathologically relevant gene expression patterns on a genomic scale, and also allows a simultaneous comparison of the relative expression levels for several thousand genes between different cell types or tissue samples. DNA microarrays have been used to identify gene expression profiles associated with specific pathological processes and malignancies [11–14]. Analysis of differentially expressed genes identified by this technology may further our understanding of the possible role these genes might play in the progression of various diseases and malignancies. Additionally, the expression patterns of particular genes may serve as molecular markers for the diagnosis of specific disease states.

In this study, we have compared expression patterns of genes from eight cell lines derived from T-cell malignancies to those of CD4-positive peripheral blood T-lymphocytes using cDNA derived from a 'composite' group of five lymphoma cell lines as a reference sample. Our results demonstrate patterns of differential gene expression distinct for each sample, which may be associated with characteristics specific to each category of malignancy. We found genes uniquely upregulated within each class of lymphoma, which may serve to distinguish molecular changes related to the development of each type of T-cell lymphoma. Additionally, we found a number of unknown genes (indicated by expressed sequence tags, ESTs) exhibiting differential expression in each of the various lymphoma cell lines.

2. Materials and methods

2.1. Cell lines

Cell lines established from various T-cell malignancies were maintained in culture, in RPMI 1640 medium (Novatech, Inc., Grand Island, NE, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum and an antibiotic/antimycotic mixture (Gibco BRL, Rockville, MD, USA). Each cell line was grown to confluency, and samples (consisting of approximately 10^7 cells) from each cell type were used as 'test sample' sources for mRNA.

The Jurkat and CCRF-CEM lines are immature TCR$\alpha\beta^+$ T-lymphocytes, derived from acute lymphoblastic leukemias (ALLs) [15,16]. The Hut 78 and HH lines are derived from mature peripheral T-lymphocytes, established from two different stages of cutaneous T-cell lymphoma (CTCL) [17,18]. Mac 2A was also derived from a CTCL, but represents a cutaneous CD30-positive ALCL without the t(2;5) translocation [19,20]. SUDHL-1 and Karpas 299 were derived from ALCLs, which have a t(2;5)(p23;q35) translocation, producing an

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NPM-ALK fusion gene [21,22]. The SKW-3 line was established from a mature peripheral T-cell chronic lymphocytic lymphoma (T-CLL) [23,24].

Samples from each of the T-cell lymphoma cell lines and from peripheral blood lymphocyte (PBL) T-cells (activated or control) were compared to our reference sample, consisting of cDNAs derived from total RNA from the Jurkat, SKW-3, NCEB (a mantle cell lymphoma-derived cell line), Raji (a mature B-cell t(8;14)-positive Burkitt's lymphoma line), and L-428 (a Hodgkin lymphoma) cell lines [25–27].

2.2. Isolation and activation of PBL T-cells

PBLs from healthy donors were isolated by density-dependent cell separation using Ficoll–Paque™ PLUS reagent (Amersham Pharmacia Biotech, Piscataway, NJ, USA). T-lymphocytes were phenotypically purified with anti-CD4 antibodies conjugated to magnetic beads (Dynal, Inc., Lake Success, NY, USA).

2.3. mRNA preparation

TRIzol reagent (Gibco BRL) was used to extract total RNA 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 total RNA was phenol/chloroform-extracted, ethanol-precipitated and resuspended in DEPC-treated RNase-free distilled water. Messenger RNA was obtained using the Qiagen Oligotex mRNA purification kit as described by the manufacturer (Qiagen, Inc., Valencia, CA, USA).

2.4. Microarray analysis

Microarray analysis was performed in the core facility in the Huntsman Cancer Institute, University of Utah. This facility maintains a sequence-verified 4364-clone collection supplied by Research Genetics (Huntsville, AL, USA). The instrumentation used to print and scan microarray slides has been developed by Molecular Dynamics (Sunnyvale, CA, USA) following principles previously described [8]. In addition to these cDNA clones, we have customized our slides to include a curated list of genes previously shown to be expressed in subsets of lymphoid cells [28]. All hybridizations were performed in quadruplicate. Differential gene expression was investigated using a simultaneous two-color hybridization scheme. Fluorescently labeled cDNA was prepared from RNA by oligo dT-primed polymerization using SuperScript II reverse transcriptase (Gibco BRL) in a 20 μl reaction. The pool of nucleotides in the labeling reaction consisted of 50 μM dGTP, dATP, and dTTP, and 5 μM dCTP, in the presence of 12.5 μM of the fluorescent nucleotides, Cy5 or Cy3 dCTP (Amersham Pharmacia Biotech). The composite sample was labeled with Cy3 dCTP (green fluorescence), while the test sample was labeled with Cy5 dCTP (red fluorescence). After incubation at 42°C for 2.5 h, the labeling reaction was stopped by the addition of 1.0 μl of 5 M NaOH, with incubation at 37°C for 10 min. The resulting alkaline solution was partially neutralized with 2.5 μl of 2 M Tris–HCl (pH 7.5) and 1 μl of 5 M HCl, and unincorporated nucleotides were removed using the Qiagen polymerase chain reaction (PCR) clean-up kit (Qiagen, Inc.). The two fluorescently labeled samples were mixed, vacuum-concentrated, resuspended in hybridization buffer, and then hybridized to the target genes on the microarray slides. Four replicate samples for each cell line were added to separate slides and hybridization patterns were captured electronically using a two-color confocal laser microscope. Analysis of array images was performed both by visual inspection and electronic quantitation of the fluorescent intensities of the individual spots. Visual inspection of microarray images was performed with the assistance of the ImageQuant NT software program (Molecular Dynamics, Sunnyvale, CA, USA). Electronic spot quantification was achieved using the ArrayVision 4 software program from Imaging Research (Ontario, Canada), which permits assignment of numerical values indicative of the fluorescence intensities of the individual spots. Once captured, the data were exported to Microsoft Excel for further analysis.

2.5. Data analysis

Manipulation and interpretation of raw fluorescent signals into interpretable data was performed using GeneSpring™ software (Silicon Genetics, Redwood City, CA, USA). Data sets were loaded from Excel and the Cy5 (red)/Cy3 (green) ratios were normalized for each data set. Gene expression patterns were considered significant only when maintained across at least three of four replicate hybridizations [29]. The data were analyzed to identify those genes expressed at levels two-fold above or two-fold below the composite sample values. Gene lists were then compiled from the over- or under-expressed genes, and clustering analysis was used to construct dendrograms for comparison of the differentially expressed genes in the eight lymphoma cell lines and the $CD4^+$ PBL T-cells.

2.6. Quantitative fluorescent reverse transcription (RT)-PCR analysis

First-strand cDNA synthesis was performed using 1.0 μg of total RNA and SuperScript II Reverse Transcriptase (Gibco BRL) according to the manufacturer's instructions. Fluorescent PCR analysis was performed using the LightCycler™ (Roche Molecular Biochemicals, Indianapolis, IN, USA) and SYBR Green I™ (Molecular Probes, Eugene, OR, USA) as previously described [30,31]. Amplification reactions consisted of 40 cycles of denaturation at 94°C (0 s), annealing at 50°C (10 s), and extension at 72°C (15 s), using the primers listed in Table 1. Fluorescent signals were obtained once in each cycle by sequential-fluorescence monitoring of each sample tube at the end of extension. Crossing thresholds (CTs) for each gene were obtained using the second derivative maximum function of the Roche LightCycler™ quantitation software. Amplifications were repeated for each pair of primers for the cDNA derived from each cell line and the CT values were averaged. Normalization was accomplished by subtracting the CTs obtained for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the CTs of the genes of interest, for each sample. A further normalization was then performed for each primer set by subtracting the CTs derived from a composite cDNA sample (comprised of 100 ng of cDNA from each of the composite cell lines) from the CT values obtained for each test sample for the same primer pair. The resulting normalized values were used to determine the relative levels of up- or down-regulation for each gene.

Table 1
Primers used for quantitative fluorescent RT-PCR analysis

Gene	Primer	Sequence
GAPDH	Forward	5′-CGACCACTTTGTCAAGCTCA-3′
	Reverse	5′-AGGGGAGATTCAGTGTGGTG-3′
Akt kinase 1 (AKT1)	Forward	5′-GATGACAGATAGCTGGTG-3′
	Reverse	5′-GCTGGACGATAGCTGGTG-3′
Human alpha 1 (ALPHA1)	Forward	5′-GGTAACGATGGTGTCGAGGT-3′
	Reverse	5′-GTTCCCACCCAGCATTACAG-3′
Endoglin 1 (ENG1)	Forward	5′-CAACTGTGTGAGCCTGCTGT-3′
	Reverse	5′-GACAGGTCAGGGCTGATGAT-3′
Myeloblastosis oncogene (v-MYB)	Forward	5′-GTCGGAAACGTTGGTCTGTT-3′
	Reverse	5′-TTCGTCCAGGCAGTAGCTTT-3′
Nuclear factor kappa B1 (NFKB1)	Forward	5′-GCACGACAACATCTCATTGG-3′
	Reverse	5′-TCCCAAGAGTCATCCAGGTC-3′
Prothymosin a28 (PTMA)	Forward	5′-GCAAATCACCACCAAGGACT-3′
	Reverse	5′-CTCCATCCTCTTCCTCACCA-3′

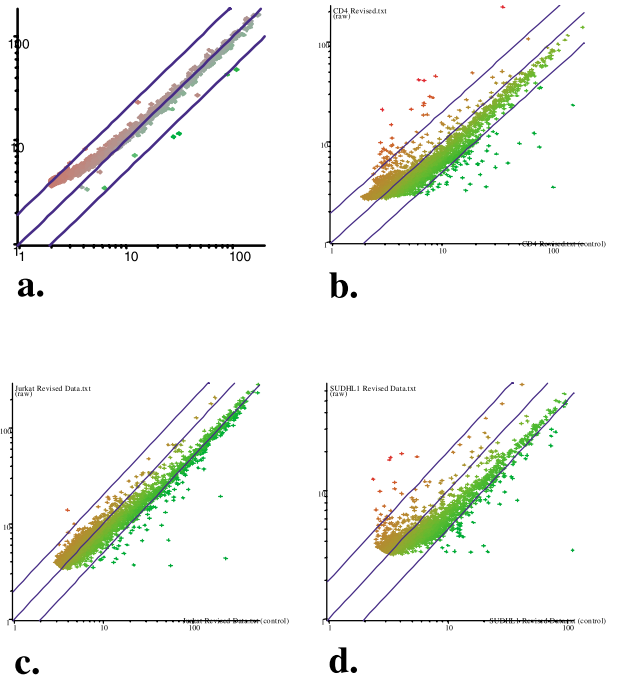

Fig. 1. Scatter plots of cDNA samples hybridized to the composite reference sample. The cDNA samples from different cell types were labeled with Cy5 and mixed with the composite samples labeled with Cy3. Labeled samples were hybridized to slides containing 4364 different cDNA sequences and fluorescent signals were measured. Each data point represents a ratio of Cy5 (vertical axis) to Cy3 (horizontal axis) fluorescent signals. Red data points represent up-regulation and green data points represent down-regulation relative to the mean signal ratio of the entire array slide. The blue lines represent two-fold differences from the ($y = x$) axis. a: The composite reference sample was hybridized vs. itself (the correlation coefficient for the plot is $r = 0.994$). b: CD4-positive T-cells were hybridized vs. the composite sample. c: The Jurkat lymphoma cell line was hybridized vs. the composite sample. d: The SUDHL-1 lymphoma cell line was hybridized vs. the composite sample.

3. Results

3.1. Microarray analysis

Gene expression profiles for eight cell lines derived from T-cell malignancies and phenotypically purified CD4-positive PB T-cells were compared, relative to the expression patterns found within a reference sample (composite) consisting of a mixture of five lymphoma cell lines. Use of a composite sample as a reference for microarray analysis permitted the identification of a large number of differentially expressed genes and also permitted comparisons of gene expression patterns among the various cell lines. Since most of the T-lymphomas we analyzed were derived from CD4-positive T-cells [32,33], we used CD4-positive PB T-cells to provide a 'baseline' gene expression profile for normal T-cells for comparison.

Fig. 1a demonstrates the reproducibility of our microarray analysis system. It shows a scatter plot of the composite reference sample cDNA labeled with Cy5 hybridized with the same cDNA labeled with Cy3. Since the mRNA levels should be virtually identical in the two cDNAs assayed, we would expect little difference in the expression levels between the two samples. Indeed, the majority of data points lie within one standard deviation of the ($y = x$) axis, with a very high correlation coefficient ($r = 0.994$). Differential expression of a gene was considered to be present when the Cy5/Cy3 ratio was greater than or equal to two standard deviations from the mean signal ratio of the entire array slide, and very few genes showed fluorescent signal ratios outside of this limit, clearly establishing the reliability of our analysis system.

As shown in Fig. 1, scatter plots of $CD4^+$ T-cells (Fig. 1b), Jurkat (Fig. 1c) and SUDHL-1 (Fig. 1d) hybridized to the reference 'composite' cDNA show individual patterns of gene expression.

Unsupervised hierarchical clustering (Fig. 2), using all 4364 genes, grouped samples according to the similarities of their gene expression patterns. The CD4-positive PBL T-cells clus-

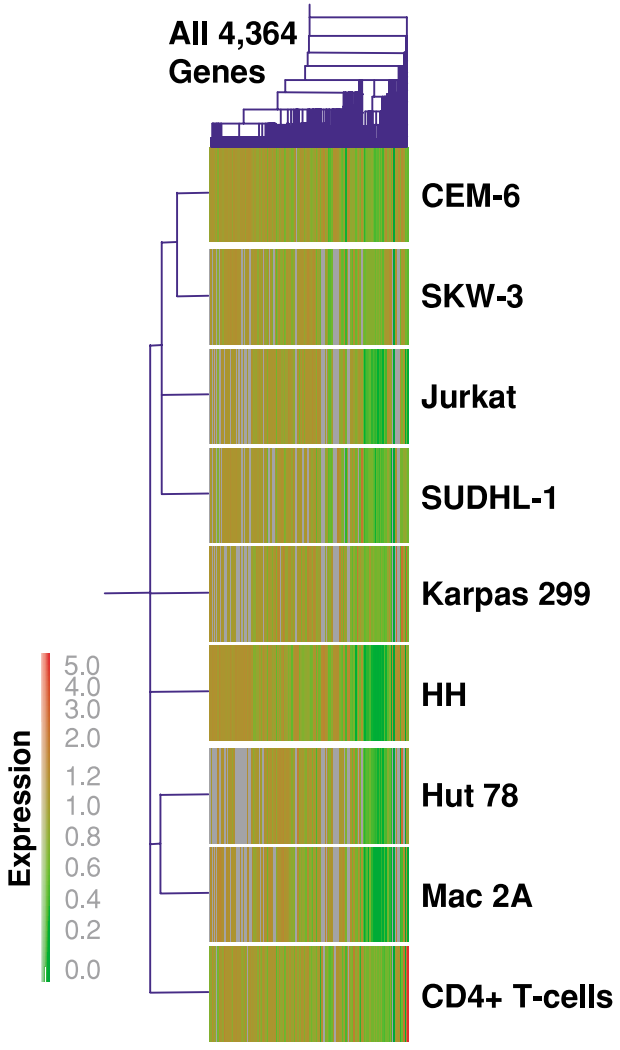

Fig. 2. Unsupervised hierarchical clustering of malignant T-cell lines and $CD4^+$ T-cells. Cell types were compared over the entire list of genes used for the microarray without restriction. Adjacent samples have the most similar expression profiles and the branching patterns designate clustered groups with high relative degrees of similarity. The top dendrogram represents a measure of the relatedness of the hybridization signals across each of the samples and the dendrogram to the left is a measure of the relatedness in each sample, so that samples exhibiting closely related expression profiles are located in adjacent columns. Gene expression profiles for each individual cell line or sample are designated on the right-hand side of the figure. The color bar indicates the expression levels for the genes shown, with red indicating up-regulation, green representing down-regulation, and gray signifying a lack of data for a specific gene.

Table 2
Genes up- or down-regulated in subgroups of T-lymphoma cell lines

Description	GenBank	Map	Description	GenBank	Map
Up two-fold in ALCLs relative to CD4$^+$ PBLs			**Down two-fold in ALCLs relative to CD4$^+$ PBLs**		
glycogenin[a]	AA411678	3q24–q25.1	lymphocyte-specific protein tyrosine kinase[a]	AA469965	1p35–p34.3
protease inhibitor 2[a] (anti-elastase), monocyte/neutrophil	R54664	!!6p25	myosin, light polypeptide 6	AA488346	12
interleukin 1 receptor, type I	AA464525	2q12	profilin 1	AA521431	17p13.3
interleukin 2 receptor, beta	AA057156	22q13.1	villin 2 (ezrin)	AA411440	6q25–q26
interleukin 1 receptor, type II	H78484	2q12–q22	tumor necrosis factor receptor superfamily, member 12	R34121	1p36.2
Homo sapiens protein tyrosine phosphatase type IVA	R61674		moesin	R22977	Xq11.2–q12
natural killer cell transcript 4	AA458965	16p13.3	filamin A, alpha (actin-binding protein-280)	AA478436	Xq28
neuronal cell adhesion molecule	R25521	7q31.1–q31.2	transcription factor 7 (T-cell-specific, HMG-box)	AA480071	5q31.1
hemopoietic cell kinase	AA149096	20q11–q12	human alpha 1	AF001540	11q13
integrin, beta 4	AA485668	17q11–qter	human HLA-DR alpha-chain mRNA	R47979	
+9 ESTs			+38 ESTs		
Up two-fold in ALLs			**Down two-fold in ALLs**		
ovarian granulosa cell protein (13 kDa)	R63543	X	major histocompatibility complex, class II, DQ beta 1[a]	AA442984	6p21.3
cold shock domain protein A	AA465019	12p13.1	human HLA-DR alpha-chain mRNA[a]	R47979	
natural killer cell transcript 4	AA458965	16p13.3	caspase 8, apoptosis-related cysteine protease	AA448468	2q33–q34
ubiquitin carrier protein E2-C	AA430504	20	S-phase kinase-associated protein 2 (p45)	R22239	5p13
annexin A1	H63077	9q11–q22	vinculin	AA486728	10q22.1–q23
major histocompatibility complex, class II, Y box-binding I	AA599175	1p34	interleukin 18 (interferon-gamma-inducing factor)	AI129421	11q22.2–q22.3
+2 ESTs			FKBP-associated protein	N95144	
			bone marrow stromal cell antigen 1	N52293	14q32.3
			tumor protein p53 (Li–Fraumeni syndrome)	R39356	17p13.1
			LIM protein	R92455	4q22
			human alpha 1	AF001540	11q13
			+141 ESTs		
Up two-fold in CTCLs			**Down two-fold in CTCLs**		
Homo sapiens mRNA for H-2K binding factor-2[a]	R19314		chaperonin containing TCP1, subunit 2 (beta)[a]	N38959	12
tumor rejection antigen (gp96) 1	AA598758	12q24.2–q24.3	adenylate cyclase 9[a]	H64280	16p13.3
interleukin 2 receptor, beta	AA057156	22q13.1	S-phase kinase-associated protein 2 (p45)[a]	R22239	5p13
human mRNA for lymphocyte glycoprotein T1/Leu-1	AA406027		clathrin, heavy polypeptide-like 2[a]	H54366	
CD4 antigen (p55)	AA451863	12pter–p12	protein phosphatase 2, regulatory subunit B″ (PR 72)[a]	N63863	
interleukin 15 receptor, alpha	AA053285	10p15–p14	LIM protein[a]	R92455	4q22
Homo sapiens TNF receptor-associated factor 1 (TRAF1)	R71725		tumor necrosis factor receptor superfamily, member 12[a]	R34121	1p36.2
protein phosphatase 3	W17217	8	CD36 antigen (collagen type I receptor)	H69048	7q11.2
natural killer cell transcript 4	AA458965	16p13.3	cathepsin H	AA487346	15q24–q25
tumor necrosis factor (ligand) superfamily, member 10	H54629	3q26	collagen, type XVI, alpha 1	R54778	1p35–p34
spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	T60235	9q33–q34	lymphocyte-specific protein tyrosine kinase	AA469965	1p35–p34.3
syndecan 4 (amphiglycan, ryudocan)	AA148736	20q12	filamin A, alpha (actin-binding protein-280)	AA478436	Xq28
apoptosis inhibitor 1	R19628	11q22	CD47 antigen (Rh-related antigen)	AA455448	3q13.1–q13.2
+4 ESTs			human alpha 1	AF001540	11q13
			+161 ESTs		
Up two-fold in miscellaneous TCLs			**Down two-fold in miscellaneous TCLs**		
filamin A, alpha (actin-binding protein-280)	AA478436	Xq28	transgelin 2[a]	H08564	1q21–q25
natural killer cell transcript 4	AA458965	16p13.3	colony stimulating factor 3 receptor (granulocyte)	AA443000	1p35–p34.3
S100 calcium-binding protein P	R32952	4p16	myosin, light polypeptide 1, alkali; skeletal, fast	T52894	2q33–q34

Table 2 (*Continued*).

Description	GenBank	Map	Description	GenBank	Map
lymphocyte-specific protein 1	T83159	11p15.5	caspase 8, apoptosis-related cysteine protease	AA448468	2q33–q34
interleukin 2 receptor, beta	AA057156	22q13.1	protein tyrosine phosphatase type IVA, member 2	AA504327	1p35
protease inhibitor 2 (anti-elastase), monocyte/neutrophil	R54664	6p25	human alpha 1	AF001540	11q13
interferon gamma receptor 2	AA448929	21q22.1	KIAA0182 protein	H05563	
+1 EST			adenylate cyclase 9	H64280	16p13.3
			+282 ESTs		

ALCL = anaplastic large cell lymphoma; ALL = acute lymphocytic leukemia; CTCL = cutaneous T-cell lymphoma; TCL = T-cell lymphoma; EST = expressed sequence tag.
[a]Both members of each class.

tered separately from the lymphoma cell lines, and many of the lymphoma cell lines clustered together in groups of histologically related T-cell lymphoma subtypes. For example, the cutaneous T-lymphomas, Mac 2A and Hut 78, grouped together on one branch, while the lower-grade, clinically indolent CTCL cell line HH aligned next to the other CTCLs, but on a separate branch. The T-CLL cell line SKW-3 aligned closely with the precursor T-ALL cell line CEM-6, and the Jurkat (T-ALL) and SUDHL-1 cell lines also clustered in this group. The two t(2;5) ALCLs, Karpas 299 and SUDHL-1, did cluster next to each other; however, their branching patterns were quite distinct.

3.2. Differential gene expression in lymphoma cell lines

Based upon two-fold differences in expression represented by genes lying outside the blue line in Fig. 1, we identified shared and distinct groups of up- and down-regulated genes in each of the lymphoma cell lines relative to the CD4-positive T-cells. Venn diagrams were used to construct lists of genes for each of four categories of T-cell lymphoma (precursor ALLs, t(2;5)-positive ALCLs, CTCLs, or miscellaneous T-cell lymphomas), relative to the CD4-positive T-cells (data not shown, see Fig. 3, Supplementary Data, http://www.elsevier.com/PII/S0014579302029149). Greater numbers of genes were found to be down-regulated than up-regulated in all of the cell lines, with 1118 down-regulated genes (greater than two-fold) and 111 up-regulated genes (greater than two-fold) in the lymphoma cell lines as a group, relative to normal CD4-positive T-cells. No genes were consistently up- or down-regulated in all of the lymphoma cell lines as a group, indicating significant molecular heterogeneity in the cell lines. Even within groups of clinicopathologically related lymphomas, very few genes were commonly over- or under-expressed. For example, Karpas 299 and SUDHL-1 both harbor the chromosomal translocation t(2;5) resulting in over-expression of the NPM-ALK oncoprotein. Surprisingly, only 15 genes were shared in the two cell lines, three of which were over-expressed and 12 of which were under-expressed compared to the composite sample. The HH and Hut 78 cell lines shared the largest number of commonly expressed genes with 135 genes that were under-expressed in both cell lines. Table 2 lists genes that were differentially expressed greater than two-fold in the four groups of cell lines. Many genes involved in cell cycle regulation, intracellular signaling, cytoskeletal regulation, cellular metabolism, and apoptosis were identified as commonly deregulated genes. Many ESTs were also found to be differentially expressed.

3.3. Confirmation of differential gene expression by quantitative fluorescent RT-PCR

In order to confirm the differential expression of genes revealed by microarray analysis of the cell lines derived from T-cell malignancies, we analyzed several genes by real-time quantitative fluorescent RT-PCR. We selected several genes differentially expressed in the different cell types for our quantitative RT-PCR analyses. Overall, there was approximately 70% agreement between the microarray results and the quantitative RT-PCR data for the six genes we examined (data not shown, see Table 3, Supplementary Data, http://www.elsevier.com/PII/S0014579302029149).

4. Discussion

The molecular aberrations underlying many subtypes of T-cell malignancies are largely unknown. In the current study, we examined the gene expression profiles of several diverse T-cell malignancies and compared them to those of PB T-cells. In this report, we have shown (using hierarchical clustering analysis, which groups samples based upon the similarity of gene expression profiles) that $CD4^+$ T-cells could be distinguished from T-cell malignancies. The clustering analysis revealed significant heterogeneity in most malignant T-cell lines, and the clustering patterns were consistent with our expectations for the different types of cell lines. Some of the clustering patterns, however, produced interesting and surprising patterns of relatedness between the malignant categories. For example, the two precursor T-ALLs, CEM-6 and Jurkat, along with the SKW-3 (T-CLL) cell line and SUDHL-1, all came off one major branch, with CEM-6 and SKW-3 forming the tightest cluster. Very little is known regarding the molecular pathogenesis of T-CLLs. Although they have been thought to represent the T-cell counterpart of B-chronic lymphocytic leukemia, T-CLLs are considered to be much more clinically aggressive [34]. In this regard, the clustering of the SKW-3 cell line with high-grade ALLs may reflect its high proliferative index and biological aggressiveness. Hut 78 is an aggressive transformed counterpart of mycosis fungoides, a low-grade CTCL, whereas Mac 2A represents lymph node metastasis of a $CD30^+$ cutaneous lymphoma. The close clustering of Hut 78 and Mac 2A, which are both aggressive lymphomas that transformed from two clinicopathologically distinct cutaneous lymphomas [35], is also of interest. The two t(2;5) ALCLs, Karpas 299 and SUDHL-1, also clustered next to each other; however, their branching patterns were quite distinct, with SUDHL-1 grouping with other aggressive neo-

plasms (Jurkat, SKW-3, and CEM-6) and Karpas 299 in its own distinct group. Despite the common expression of the NPM-ALK oncogene in these two cell lines, their gene expression profiles indicate significant molecular differences [36,37].

Comparison of gene expression to PB $CD4^+$ T-cells demonstrated numerous differentially expressed genes involved in proliferation, signaling pathways, apoptosis, and cytoskeletal regulation in each lymphoma cell line. There was no single gene that was differentially expressed in all of the lymphoma cell lines compared to the PB T-cells. This highlights the presence of significant molecular heterogeneity within the groups of lymphomas. It is possible that the level of differential expression of many commonly altered genes is lower than the plus or minus two-fold change that was used as a cutoff point. As many significant changes in gene expression occur via minute changes in the level of mRNA transcripts, these findings are not too surprising.

Relative to $CD4^+$ T-cells, very few genes were similarly expressed in the related groups of tumors. These observations suggest that there is significant molecular heterogeneity even within a group of clinicopathologically related entities. Recent studies have demonstrated the existence of molecular subgroups within other lymphomas, such as diffuse large B-cell lymphomas, using microarray technology [11].

Our RT-PCR validation of six genes revealed an overall concordance rate of approximately 70%. This is in keeping with those that have been reported in the literature [38]. The multiple, complex steps involved in cDNA preparation, labeling, hybridization, and image analysis may be possible reasons for the lack of perfect concordance.

Our current study demonstrates the utility of cDNA microarray analysis and quantitative real-time RT-PCR for the assessment of transcriptional expression patterns of T-cell malignancies. Very few reports have studied the expression profile of specific T-cell malignancies [39,40]. Our study represents a comprehensive global expression analysis of a diverse group of T-cell leukemic lymphomas with actual comparison to a normal T-cell population. Comparison of expression profiles of T-cell malignancies to those of non-neoplastic $CD4^+$ T-cells allowed the identification of a number of genes and unknown ESTs that may potentially contribute to the development of these neoplasms.

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