0023-6837/03/8306-765$03.00/0
LABORATORY INVESTIGATION

Vol. 83, No. 6, p. 765, 2003
Printed in U.S.A.

Comparative Microarray Analysis of Gene Expression During Activation of Human Peripheral Blood T Cells and Leukemic Jurkat T Cells

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SUMMARY: Activation of T cells involves a complex cascade of signal transduction pathways linking T-cell receptor engagement at the cell membrane to the transcription of multiple genes within the nucleus. The T-cell leukemia–derived cell line Jurkat has generally been used as a model system for the activation of T cells. However, genome-wide comprehensive studies investigating the activation status, and thus the appropriateness, of this cell line for this purpose have not been performed. We sought to compare the transcriptional profiles of phenotypically purified human $CD2^+$ T cells with those of Jurkat T cells during T-cell activation, using cDNA microarrays containing 6912 genes. About 300 genes were up-regulated by more than 2-fold during activation of both peripheral blood (PB) T cells and Jurkat T cells. The number of down-regulated genes was significantly lower than that of up-regulated genes. Only 79 genes in PB T cells and 37 genes in Jurkat T cells were down-regulated by more than 2-fold during activation. Comparison of gene expression during activation of Jurkat and PB T cells revealed a common set of genes that were up-regulated, such as *Rho GTPase-activating protein 1*, *SKP2*, *CDC25A*, *T-cell specific transcription factor 7*, cytoskeletal proteins, and signaling molecules. Genes that were commonly down-regulated in both PB T cells and Jurkat T cells included CDK inhibitors (*p16, p19, p27*), proapoptotic caspases, and the transcription factors *c-fos* and *jun-B*. After activation, 71 genes in PB T cells and only 3 genes in Jurkat T cells were up-regulated 4-fold or more. Of these up-regulated genes and expressed sequence tags, 44 were constitutively expressed at high levels in nonactivated Jurkat cells. Quantitative real-time RT-PCR analysis confirmed our microarray data. Our findings indicate that although there is significant overlap in the activation-associated transcriptional profiles in PB T cells compared with Jurkat T cells, there is a subset of genes showing differential expression patterns during the activation of the two cell types. (*Lab Invest 2003, 83:765–776*).

Many studies of T-cell activation have been performed using either the human leukemic Jurkat cell line (Black et al, 1997; Ghaffari-Tabrizi et al, 1999) or primary T cells (Cooper and Pellis, 1998; Ellisen et al, 2001). T-cell activation is associated with regulation of multiple intracellular signaling events, mediated by either protein tyrosine kinases and serine/threonine kinases (Rudd, 1999; Tsuchida et al, 1999). Several key processes have been well documented during T-cell activation, including the activation of phospholipase C-γ, which is required for TCR–dependent activation of IL-2 (Lindholm et al, 1999). Up-regulation of protein kinase C, a downstream target of phospholipase C-γ (Cooper and Pellis, 1998; Ghaffari-Tabrizi et al, 1999), results in the stimulation of AP-1 transcriptional activity, which activates the transcription of c-Jun-N-terminal kinase/stress-activated protein kinase (Ghaffari-Tabrizi et al, 1999). Other signaling molecules such as mitogen-activated/extracellular signal-regulated kinase and calcium ions are also involved in T-cell activation (Tsuchida et al, 1999).

Both the leukemic Jurkat T-cell line (Black et al, 1997; Ghaffari-Tabrizi et al, 1999; Tanaka et al, 1997; Whisler et al, 1994) and primary PB T cells (Cooper and Pellis, 1998; Ellisen et al, 2001; Whisler et al, 1993) are commonly used as a model for human T-cell activation. Genomic expression programs in primary PB T-cell activation have been reported (Diehn et al, 2002; Ellisen et al, 2001; Feske et al, 2001); however, no genomic-scale comparison has been performed to analyze the activation-induced gene expression profiles of these two T cell types.

The use of cDNA microarray technology is an effective approach for analysis of gene expression profiles in T-cell activation (Ellisen et al, 2001; Teague et al, 1999). The technology is based on two-color fluorescence hybridization in which two cDNA populations (from test and reference samples) are labeled separately with red or green fluorochrome and are subsequently hybridized to a microarray containing thousands of predeposited cDNAs (Schena et al, 1995). The ratio of fluorescence intensity (red/green) reflects

DOI: 10.1097/01.LAB.0000073130.58435.E5

Received March 13, 2003.
This work was supported by the ARUP Institute for Clinical and Experimental Pathology and National Institutes of Health grant CA 83984-01 (to KSJ-EJ).
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the relative expression level between the tested cDNA populations (such as the test sample and the control sample). The technology has been used in the analysis of gene expression patterns, signaling pathways, and identification of new functional genes on a genomic scale (Chtanova et al, 2001; Ellisen et al, 2001; Teague et al, 1999).

In this study we sought to compare the gene expression profiles of phenotypically purified human $CD2^+$ T cells with those of Jurkat T cells during PHA-induced activation. The results show that general patterns of gene expression profiles were similar during activation of Jurkat T cells and peripheral blood (PB) T cells. However, a subset of genes showed differential expression with varying degrees of gene regulation between the Jurkat and PB T cells. This is the first comparative analysis of transcription profiles on a genomic scale between primary T cells and leukemic Jurkat T cells during T-cell activation (Gonzales and Bowden, 2002; Rowan et al, 1995).

Results and Discussion

Overall Gene Expression Patterns Between Jurkat and PB T Cells During Activation

The baseline similarity of gene expression between Jurkat and PB T cells was evaluated by hybridizing cDNA from resting $CD2^+$ PB T cells with cDNA from nonactivated Jurkat T cells. The correlation coefficient between gene expression profiles of the two T cell types was 0.674 (Fig. 1), indicating that the two T cell types were significantly different. By contrast, cohybridization of PB T-cell mRNA with themselves revealed a correlation coefficient of 0.99 (data not shown).

To compare the transcriptional profiles of PB T cells and Jurkat T cells during activation, Cy-5-cDNAs from either activated Jurkat or PB T cells were hybridized with Cy-3-cDNAs from their nonactivated counterparts. Genes that were expressed greater than 2-fold (2-fold higher than control) after activation were considered overexpressed, whereas genes that were expressed less than 0.5 fold (2-fold lower than control) were considered underexpressed. After 24 hours of activation, approximately 300 genes (4.3% of 6912 genes tested) were overexpressed in each T cell type (Table 1). The number of underexpressed genes was much lower than that of the overexpressed genes in both T cell types, with only 79 genes (1.1% of 6912 genes tested) in PB T cells and 37 genes (0.5% of 6912 genes tested) in Jurkat T cells. The ratio of up-regulated genes, expressed sequence tags (ESTs), and unknown genes are also shown in Table 1; the accession numbers of these ESTs and unknown genes are listed in Tables 2 and 3. A significant portion of the up-regulated genes represents ESTs and unknown genes, highlighting the limited knowledge we have regarding the genes involved in T-cell activation. In PB T cells, the majority of the 6912 tested genes were expressed at similar levels (between the upper and lower blue lines in Fig. 1) during activation. This result is consistent with other reports that resting T cells are not in a quiescent stage; instead, there are many cellular transcriptional activities that are needed to maintain viability and mobility of unstimulated T cells (Boise et al, 1995; Teague et al, 1999; Vella et al, 1998). Jurkat T cells are derived from a T-precursor lymphoblastic lymphoma (Black et al, 1997). As a result, many of the genes were expected to be constitutively expressed before activation treatment; however, Jurkat T cells responded to activation treatment similar to resting T cells, as reflected by the similar numbers of up-regulated genes in both T cell types (Table 1).

Functional Groups of Similarly Expressed Genes in Both Jurkat and PB T Cells During Activation

Analysis of the 300 genes that were overexpressed in each cell type revealed that many were unknown genes and ESTs. This suggests the existence of many novel, as yet uncharacterized, genes that are involved

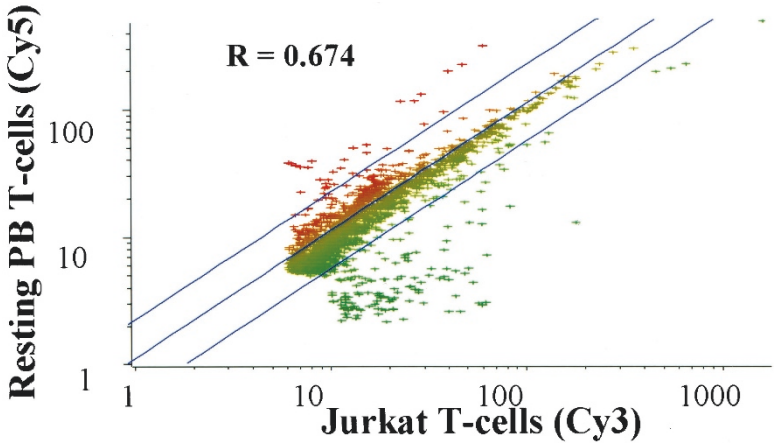

Figure 1.
Overall expression profile of 6912 genes between resting peripheral blood (PB) T cells and Jurkat T cells. Red and green data points indicate overexpressed and underexpressed genes, respectively. Genes above the upper blue lines represent those that were overexpressed by greater than 2-fold, whereas genes below the lower blue line represent those that were underexpressed by more than 2-fold.

Table 1. Numbers of Differentially Expressed Genes During Activation of Jurkat and PB T Cells[a]

Experiments (Cy5 vs Cy3)	≥4-fold up-regulated (total/known genes:EST:unknown genes)	≥2-fold up-regulated (total/known genes:EST:unknown genes)	≥2-fold down-regulated (total/known genes:EST:unknown genes)
Activated PB T cells vs Resting PB T cells	71/10:4:57	300/76:30:194	79/28:34:17
Activated Jurkat vs Jurkat T cells	3/0:0:3	301/62:97:142	37/19:8:10

[a] Fold changes shown represent the ratio of gene expression in activated vs nonactivated cells.

Table 2. GenBank Accession Numbers of Overexpressed ESTs and Unknown Genes (≥2-fold) During PB T-Cell Activation[a]

150221	23496	23157	17979	23384	19676
249856	23492	20784	23964	23380	13016
135527	9101	23162	15787	23375	13011
233365	7863	23159	20563	23364	20025
134753	23436	23245	22782	23363	21901
135094	23477	23216	13404	23366	18363
123065	23453	23256	20538	23365	23175
123817	23691	23250	20512	22265	20032
154789	11984	23207	9683	6660	12742
295866	21464	21943	12406	21150	300237
197637	21462	12602	20618	10586	23911
194704	23635	20826	21755	22246	23405
193617	20280	21978	22859	23388	12554
246449	18764	23132	22815	23385	11690
711857	15458	15871	23967	20236	23377
141627	23618	10260	18197	7049	23419
198694	11054	21845	17017	7039	
121275	20373	17148	14557	21388	
110582	20384	16911	15578	22495	
110987	20382	23049	23714	21332	
303048	8290	23031	21540	20228	
206816	19958	23096	22688	19836	
23616	19957	23059	23713	21173	
23533	18832	7536	15521	23188	
23114	19969	16099	16657	23181	
22355	7053	20766	23707	23396	
23398	23592	23122	23703	23393	
21634	23590	23105	7275	23362	
23121	9252	20716	20492	18493	
23454	23598	12530	22701	18457	
20394	23580	21893	1588	17373	
19745	23536	23280	7268	23520	
19739	23535	20981	21589	2355	
19776	22419	23349	20419	23524	
21269	23563	23358	18925	23523	
13083	19873	10512	21597	17573	
22388	12704	20952	23010	20150	
21228	22162	20900	21790	19787	
19734	20892	168	20620	2317	
21232	20891	20916	20640	20178	
20070	21003	66686	23007	22342	

[a] This list was generated using GeneSpring software and the database available when this manuscript was prepared. Some of the genes listed are now identified known genes.

in T-cell activation. Activation of T cells leads to expression of multiple genes that are involved in cellular events such as proliferation, cytoskeletal construction, apoptosis, and secretion of cytokines (Ellisen et al, 2001; Teague et al, 1999). To determine the functional groups of genes that were commonly up- or down-regulated in T-cell activation, we examined the identities of 93 overexpressed and 15 underexpressed genes in both Jurkat and PB T cells. Of the genes that were up- and down-regulated by 1.5 fold or more, the

Table 3. GenBank Accession Numbers of Overexpressed ESTs and Unknown Genes (≥2-fold) During Jurkat T-Cell Activation[a]

346134	293683	127185	23177	8745	23380
233365	129567	120631	8556	20716	10586
127408	129922	246143	21751	17148	22265
294916	292171	196109	22843	16099	23477
211202	144802	295492	21755	21887	23471
346299	132323	295594	9683	18239	21228
247901	128627	128118	510760	23096	9101
245426	130801	20308	241874	23049	23465
128735	138837	22594	17017	15871	19696
246073	194656	21462	17803	10260	20025
134753	234380	19945	7248	23188	7863
154789	194401	21434	7298	23181	13963
156023	194704	6957	17842	17265	66864
193617	193533	23675	21505	972	23362
155128	197637	19908	23713	23175	136801
340840	195358	21408	23700	23137	138168
67037	295866	18880	15578	23132	198961
207379	142397	8292	23714	23162	127542
21634	137760	16657	18132	23155	20280
23121	137797	17740	23911	23023	23592
144951	160730	8290	22806	21279	17373
292452	244299	19958	15787	20116	18457
203772	139189	19957	18109	20178	5546
196125	248535	18832	18009	20156	23399
209583	297102	21495	10043	21266	135094
199327	247710	11984	17979	19734	247082
196350	121521	21388	20584	19721	123817
122161	38465	19836	23256	21253	127710
23454	293417	19894	23246	19737	7053
292207	110904	8173	22115	23562	23606
198694	243770	9275	20892	23542	3210
135527	110582	9255	15069	15521	23297
276286	66390	9252	23222	23580	21173
711857	245485	19824	8789	23536	20014
128775	294133	9259	15068	11813	
292679	206816	23641	23224	2317	
196148	110987	15458	12742	23535	
201207	122913	23660	20906	23523	
66377	123196	23647	10512	21232	
135212	122126	23618	20952	20007	
135450	123065	18764	9984	21150	

[a] This list was generated using GeneSpring software and the database available when this manuscript was prepared. Some of the genes listed are now identified known genes.

genes with known functions were grouped together (Tables 4 and 5). Among the known up-regulated genes (Table 4), several genes involved in cellular proliferation, cell adhesion, and cell cycle progression were identified, including *CDC kinases*, *integrin*, and *Rho-activating protein-encoding genes* (Klekotka et al, 2001; Lew and Reed, 1993; Welsh and Assoian, 2000). Genes encoding for protein synthesis machinery (eukaryotic translational termination factor 1, ribosomal proteins L7a, L31, L21), cytoskeletal elements (myosin, actin, tubulin, calponin), and cell surface antigens (CD68, CD36, CD47) were also commonly up-regulated in both cell types during activation. Interestingly, a group of KIAA genes encoding proteins with potential signaling function, involved in cell migration (Chen et al, 2002) and metastasis (Lee et al, 2002), were also up-regulated upon activation.

The down-regulated genes in both activated Jurkat and PB T cells consisted mostly of genes encoding CDK inhibitors, proapoptotic caspases, and transcription factors C-Fos and Jun-B (Table 5). Down-regulation of CDK inhibitors is critical for inhibition of cell cycle progression. The stimulation of cell proliferation via PHA activation has been shown to induce the down-regulation of $p27^{Kip}$ and $p21^{Waf}$ CDK inhibitors, resulting in the release of cells into G2/S phase of the cell cycle (Polyak et al, 1994; Sawada et al, 1996). The fact that some cytokines such as IL-4 (Boise et al, 1995; Vella et al, 1998) can only induce activated T

Table 4. Functional Groups of Genes That Were Up-Regulated in Both Jurkat and PB T Cells During Activation[a]

Genban	Common name	Description	Fold change in expression: Activated PB T cells vs resting PB T cells	Fold change in expression: Activated Jurkat vs nontreated Jurkat T cells
Proliferation/cell cycles				
AA443506	ARHGAP1	Rho GTPase-activating protein 1	2.1	2.3
AA487634	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta	1.5	1.5
R22239	SKP2	S-phase kinase-associated protein 2 (p45)	1.5	1.8
H15504	ANXA7	Annexin A7	1.5	1.9
R48796	ITGAL	Integrin, alpha L [antigen CD11A (p180)]	1.5	1.8
W44701	IL3RA	Interleukin 3 receptor, alpha	1.5	1.6
R09063	CDC25A	Cell division cycle 25A	3.6	1.6
H75547	CLK1	CDC-like kinase 1	1.5	1.9
AA459292	CKS1	CDC28 protein kinase 1	1.5	1.5
Antiapoptosis				
H21071	NAIP	Neuronal apoptosis inhibitory protein	1.9	2.7
Transcription factors				
AA480071	TCF7	Transcription factor 7 (T-cell specific, HMG-box)	1.5	2.0
AA284693	TFAP4	Transcription factor AP-4	1.5	1.7
Protein synthesis				
AA496838	RPL5	Ribosomal protein L5	1.9	1.6
R01139	RPL10A	Ribosomal protein L10a	2.0	1.5
AA464034	RPL21	Ribosomal protein L21	3.2	1.7
H73727	RPS14	Ribosomal protein S14	3.6	1.8
Cytoskeleton				
AA284568	CNN2	Calponin 2	1.7	2.1
AA488346	MYL6	Myosin, light polypeptide 6	2.1	1.7
W32281	KRT8	Keratin 8	1.5	1.9
T60048	ACTG2	Actin, gamma 2	1.7	1.6
AA180912	TUBA1	Tubulin, alpha 1 (testis specific)	1.5	1.8
Proto-oncogenes				
AA436591	MERTK	c-mer proto-oncogene tyrosine kinase	1.5	1.5
H90415	BRCA1	Breast cancer 1, early onset	1.5	2.0
Surface antigens				
H69048	CD36	CD36 antigen (collagen type I receptor)	1.5	1.6
AA455448	CD47	CD47 antigen (Rh-related antigen)	2.1	2.1
AA421296	CD68	CD68 antigen	2.3	2.6
AA486653	CD81	CD81 antigen	1.9	1.6
Potential signaling molecules				
H05563	KIAA0182	KIAA0182 protein	1.6	2.3
AA456352	KIAA0224	KIAA0224 gene product	1.6	1.6
N22435	KIAA0229	KIAA0229 protein	1.7	1.6
T90374	KIAA0798	KIAA0798 gene product	1.7	2.5
R91264	KIAA0205	KIAA0205 gene product	1.7	2.0
N79669	KIAA0231	KIAA0231 protein	1.7	2.0
AA486524	KIAA0264	KIAA0264 protein	2.1	2.4
T74606	KIAA0057	KIAA0057 gene product	1.8	2.3

[a] Fold changes shown represent the ratio of gene expression in activated vs nonactivated cells.

cells (but not resting T cells) to proliferate also supports these data.

Caspases are a group of proteases that are generally associated with apoptosis. Activation of several caspases including caspase-1, -3, -8, and -9 has been reported to induce T-cell apoptosis (MacFarlane et al, 2000; Morley et al, 2000; Ruiz et al, 2001; Takahashi et al, 2001; Varghese et al, 2001). Our data showed that several caspases (caspase-1, -3, -4, -7, -8, -9) were down-regulated in both Jurkat and PB T cells after 24 hours of activation (Table 5). This occurred during the period of T-cell proliferation in which cell numbers

Table 5. Functional Groups of Genes That Were Down-Regulated During Activation of Both Jurkat and PB T Cells[a]

Gene name	Fold change in expression	
	Activated PB T cells vs resting PB T cells	Activated Jurkat vs nontreated Jurkat T cells
Cell growth and/or maintenance		
p16	−2.2	−3.0
p19	−2.1	−2.5
p27	−1.8	−2.1
CDKI 3	−2.2	−2.4
Death		
caspase 1	−1.7	−2.6
caspase 3	−1.5	−1.9
caspase 4	−1.9	−1.9
caspase 7	−1.5	−2.4
caspase 8	−1.5	−1.6
caspase 9	−1.9	−2.5
Transcription regulator		
C-fos	−1.8	−2.1
jun-B	−1.5	−1.9

[a] Fold changes shown represent the ratio of gene expression in activated vs nonactivated cells.

increased by 3-fold (data not shown). This is consistent with a previous report demonstrating that the activation of caspase 1 and 3 in $CD4^+$ and $CD8^+$ T cells was time dependent. The maximum activities of caspase-1 and -3 were observed after the proliferative period, at Days 7 and 10 (Ruiz et al, 2001).

The proto-oncogenes *jun-B* and *c-fos* are generally involved in proliferation-associated transcriptional activation (Sylvester et al, 1998; Wang et al, 1997); thus, our results of their down-regulation were unexpected. Our results are, however, in keeping with those of Ellisen et al (2001) who observed down-regulation of *jun-B* and *c-fos* in ConA-treated PB T cells. In our experiments, *jun-B* and *c-fos* were down-regulated in both Jurkat and PB T cells during activation; this is consistent with other reports (Kreuzer et al, 1997; Passegue and Wagner, 2000) that C-Fos and Jun-B may be involved in the transcriptional activation of antiproliferative genes such as $p16^{INK4a}$.

Diehn et al (2002) performed a study of gene expression profiles during PB T-cell activation. To compare our results with theirs, we used their web-based GeneXplorer software and Figure 1 (available at http://genome-www.stanford.edu/costimulation) to search for the expression levels of 22 randomly selected genes from our list of genes that were up- or down-regulated during T-cell activation. The expression levels of these genes showed a high level of concordance between the two studies (18 of 22). Among the consistently expressed genes, there were proliferation-related genes (four), CDK inhibitors (four), caspases (three), and transcription factors (two).

Differences in Gene Expression Between Jurkat and PB T Cells During Activation

Interestingly, many activation-induced genes in PB T cells were constitutively overexpressed to similar levels in the non-activated Jurkat T cells (Fig. 2, A and C). Of the 300 overexpressed (by 2-fold) genes and ESTs, more than one-third (118 genes and ESTs) were expressed at similar levels in nonactivated Jurkat cells (Fig. 2A, Fig. 3). Although the vast majority of genes were similarly expressed between Jurkat and PB T cells during activation, one of the most significant differences between the expression profiles of the two cell types was the total number of genes that were expressed greater than 4-fold after activation (Table 1). Although 71 genes and ESTs were expressed greater than 4-fold in PB T cells, only three genes were overexpressed to this degree in Jurkat T cells. Among the ~300 2-fold up-regulated genes during T-cell activation, there were only 93 genes (31%) that were common between the two T cell types (Fig. 3).

Among the 71 genes and ESTs overexpressed by 4-fold or higher in activated PB T cells, more than half were constitutively overexpressed in nontreated Jurkat cells (Fig. 2C). These data suggest that when using Jurkat cells as model of T-cell activation, the number of activated genes may be underrepresented because many are constitutively overexpressed. The constitutively overexpressed genes (greater than 2-fold) in Jurkat cells (Table 6) most likely reflect the activated status of these leukemic T cells and probably are important in the pathogenesis of the neoplasm. Similar observations have been made when expression of phosphoinositide (PI)3-kinase was compared between PB T cells and Jurkat cells (Astoul et al, 2001). The finding that many of the constitutively overexpressed genes in Jurkat cells were also overexpressed in activated PB T cells suggests that genes induced during activation of T cells may be involved in leukemogenesis. Indeed, growth factors such as transforming growth factor-β and TNF activate several signaling pathways including the PI3-kinase pathway (Kim et al, 2002) and the nuclear factor-κB pathway (Natoli et al,

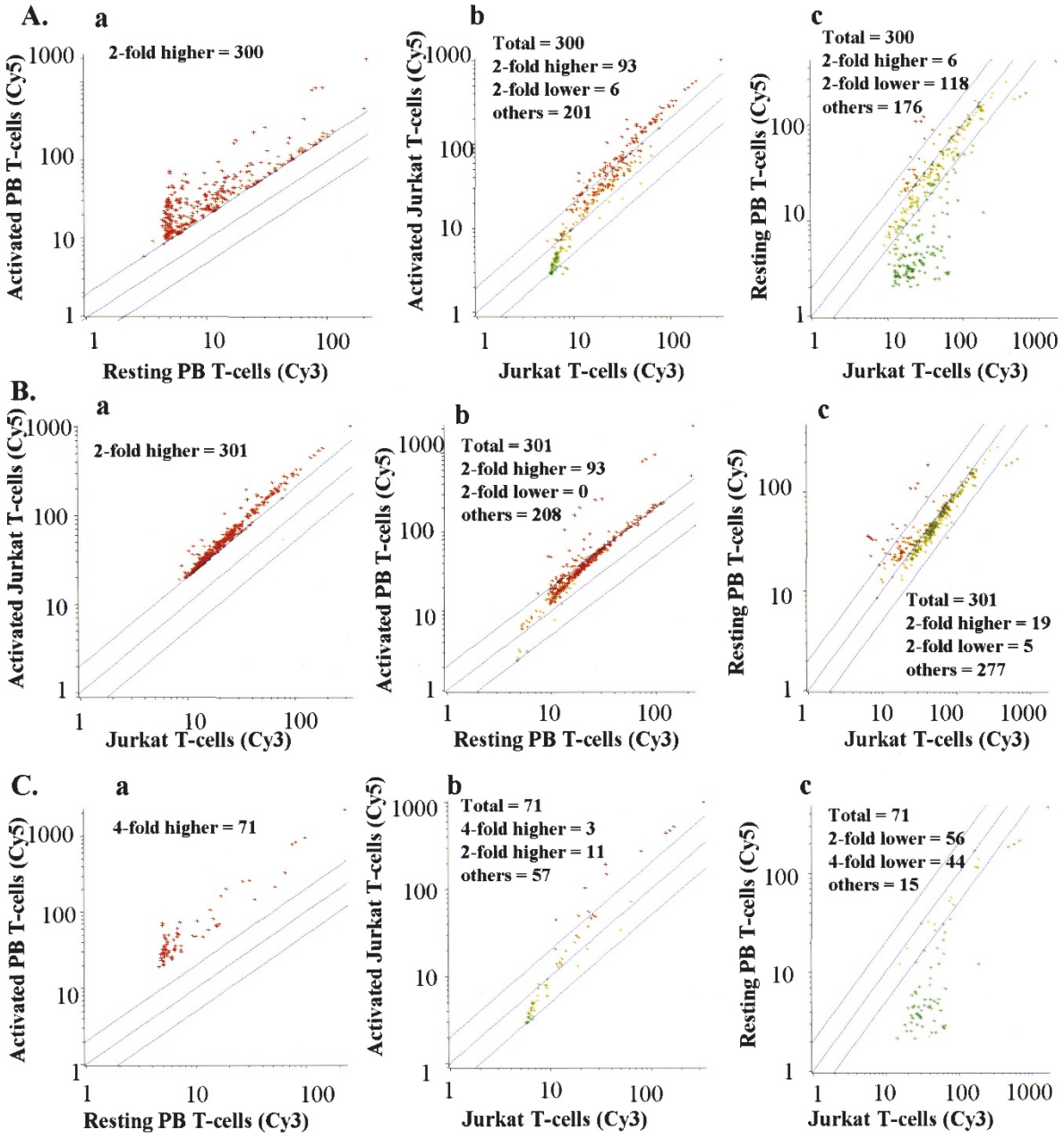

Figure 2.

Distribution of genes overexpressed greater than 2-fold during activation. A, Of the 6912 genes tested, 300 were overexpressed greater than 2-fold in PB T cells (a). Among these 300 overexpressed genes, 93 genes were also overexpressed in Jurkat T cells during activation (b), and 118 genes were expressed more than 2-fold higher in nonactivated Jurkat than in resting PB T cells (c). B, Profile of 301 genes overexpressed more than 2-fold in Jurkat T cells (a) during activation, in PB T cells (b) during activation, and in resting PB T cells compared with nonactivated Jurkat T cells (c). C, Profile of 71 genes up-regulated by 4-fold in PB T cells (a) during activation, in Jurkat T cells (b) during activation, and in resting PB T cells compared with nonactivated Jurkat T cells (c). Red points indicate overexpressed genes and green points represent underexpressed genes. Genes above the upper blue line were overexpressed by more than 2-fold, whereas genes below the lower blue line were underexpressed by more than 2-fold.

1998) to ultimately induce cell survival and inhibit apoptosis. The expression of transcription factors such as jun and fos are also activated by PI3-kinase and AKT (Duan et al, 2002; Walsh et al, 2002) and contribute to T-cell survival. Cross-linking of cell surface proteins such as CDW52 (CAMPATH-1 antigen), a glycopeptide antigen expressed on T and B lymphocytes, by anti-CD52 antibody mediates effective growth inhibition, supporting its important role in T-cell transformation (Rowan et al, 1998). Another cell surface antigen, endoglin (a recently recognized component of the transforming growth factor-β receptor complex), is a strong marker of neovascularization and may thus play a role in Jurkat T-cell leukemogenesis (Calabro et al, 2003). Furthermore, several other genes in this list (Table 6) may be influenced by the persistent activation of the PI3-kinase pathway. For example, matrix metalloproteinase secretion in some cell types occurs via the PI3 kinase-AKT pathway (Ellerbroek et al, 2001). Matrix metalloproteinases are critical for cell

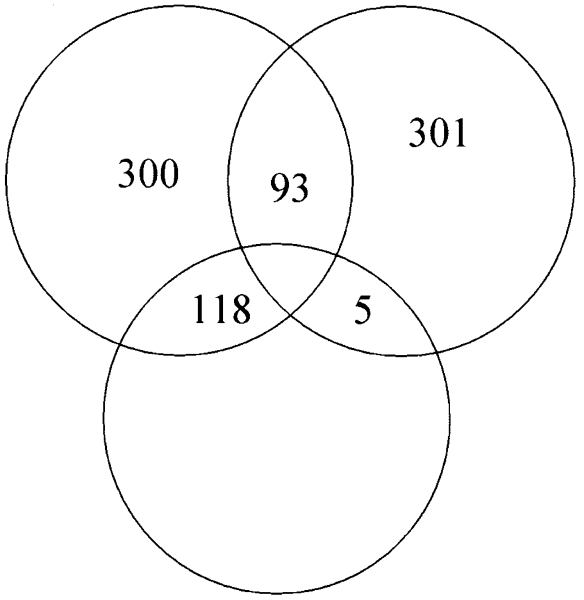

Figure 3.
A Venn diagram showing the overlaps of up-regulated genes during T-cell activation. Among the 300 genes that were up-regulated more than 2-fold (*left circle*) during PB T-cell activation, 93 of them were also overexpressed more than 2-fold during activation of Jurkat T cells (*right circle*). Approximately 118 genes (*bottom circle*) up-regulated during PB T cell activation were constitutively up-regulated in nonstimulated Jurkat T cells compared with resting PB T cells.

adhesive and invasive properties of malignant cells including leukemic T cells (Stetler-Stevenson et al, 1997) and may contribute to the neoplastic phenotype of Jurkat T cells. Further functional studies of these differentially expressed genes may lead to identification of novel leukemia-specific genes.

Validation of Gene Expression by Real-Time RT-PCR

To validate the gene expression patterns observed in our microarray experiments, we used the same source of mRNAs to perform quantitative fluorescent real-time RT-PCR (Elenitoba-Johnson et al, 2002). The RT-PCR quantitation of nine selected gene transcripts (Table 7) showed that the trend of gene expression during activation of both Jurkat and PB T cells was consistent between the microarray and RT-PCR data. The overall correlation coefficient between relative expression levels from the microarray analysis and from RT-PCR was 0.81. Using this methodology, our previous validation of microarray data has shown approximately 70% concordance (Fillmore et al, 2002; Robetorye et al, 2002).

In summary, comparative microarray studies of PHA-induced activation of PB T cells and Jurkat cells reveal that genes associated with proliferation, protein synthesis, cytoskeletal construction, and signaling were similarly up-regulated, whereas genes associated with cell cycle inhibition and apoptosis were down-regulated at similar levels. A subset of genes induced during PB T-cell activation, however, were constitutively expressed at high levels in nonactivated Jurkat cells, suggesting a role for these genes in the neoplastic transformation and/or biology of T-ALL. Finally, with the exception of a small subset of genes, the high degree of overlap between the gene expression profiles of the two cell types suggests that Jurkat T cells remain a suitable model for T-cell activation.

Materials and Methods

Isolation of Human PB T Cells

Fresh PB was obtained from normal donors. To isolate T cells, buffy coat samples were collected from blood using Ficoll-Paque (Amersham Pharmacia, Piscataway, New Jersey), following the manufacturer's instructions. $CD2^+$ T cells were then isolated by Dynal beads coated with CD2-specific mAbs (Dynal CD2 CELLection Kit; DYNAL Biotech, Oslo, Norway), following the manufacturer's protocol.

T-Cell Activation

Both $CD2^+$ T cells and leukemic Jurkat T cells were cultured in RPMI-1640 (Life Technologies, Rockville, Maryland) containing 10% fetal bovine serum and activated in the same medium supplemented with 1% (v/v) PHA (Life Technologies, Carlsbad, California) for 24 hours at 37° C in 5% CO_2.

Separation of Total RNA and mRNA

Total RNA was extracted by using the TRIZol reagent (Life Technologies, Carlsbad, California). Messenger RNA was purified from total RNA using poly(T)-coated beads (Oligotex mRNA Isolation Kit; Qiagen, Valencia, California), according to the manufacturer's protocol.

Preparation and Hybridization of Fluorescent-Labeled cDNA

For each comparative array hybridization, fluorescent cDNA was synthesized by reverse transcription from a test sample (activated T-cell mRNA or activated Jurkat mRNA) in the presence of Cy5-dUTP and from a reference sample (nonactivated T-cell mRNA or Jurkat mRNA) with Cy3-dUTP, using the Superscript II RT kit (Life Technologies, Carlsbad, California) with several modifications. The RT reaction mixture contained 0.5 mM dATP, dCTP, and dGTP, 0.2 mM dTTP, and 0.1 mM Cy5- or Cy3-dUTP (Amersham Pharmacia). The labeling reaction was stopped by the addition of 5 μl of 500 mM EDTA. The mRNA was hydrolyzed by adding 10 μl of 1 N NaOH and heating to 65° C for 1 hour. The resulting alkaline solution was neutralized with 1 M Tris-HCl (pH 7.5). Unincorporated nucleotides were removed using a Biospin 6 column (Bio-Rad Laboratories, Hercules, California). The two fluorescently labeled cDNA samples were mixed, concentrated using a Microcon 30 microconcentrator (Amicon Inc., Beverly, Massachusetts), and hybridized to an arrayed slide (Research Genetics, Huntsville, Alabama) containing 6912 clones as described previously (Manos

Table 6. Constitutively Overexpressed Genes and ESTs in Nontreated Jurkat T cells[a] in Comparison to Nonactivated PB T Cells

GenBank/Unigene	Description
Cell growth and/or maintenance	
Hs.153889	Transforming growth factor-β precursor
Hs.2017	Latent transforming growth factor-β binding protein 1
Hs.2206	TNF type 2 receptor-associated protein
Transcription regulator	
Hs.75889	V-jun avian sarcoma virus 17 oncogene homolog
Hs.83428	Nuclear factor of kappa light polypeptide gene enhancer in B cells
Hs.44450	Sp3 transcription factor
Hs.169465	FOS-related antigen 1
Cellular component	
AA278759	Proteoglycan 1, secretory granule
AA410265	Lysosomal-associated multispanning membrane protein-5
Hs.1908	Hematopoetic proteoglycan core protein
Others	
R38270	*Homo sapiens* clone 24734 mRNA sequence
AA410517	Protease inhibitor 6 (placental thrombin inhibitor)
N73222	ESTs, moderately similar to hypothetical protein (*H. sapiens*)
R38105	ESTs
H70017	Mannosidase, alpha, class 2A, member 1
H17882	Kallmann syndrome 1 sequence
R47979	Human HLA-DR α-chain mRNA
AA446108	Endoglin
Hs.2248	Interferon γ-induced protein 10
Hs.75462	Human BTG2
Hs.100000	S100 calcium-binding protein A8 (calgranulin A)
Hs.112405	S100 calcium-binding protein A9 (calgranulin B)
Hs.59847	ESTs
Hs.29748	ESTs
Hs.28102	ESTs
Hs.3452	ESTs
Hs.151031	Human Aac11
Hs.77961	Major histocompatibility complex, class I, C
Hs.159120	CDW52 antigen (CAMPATH-1 antigen)
Hs.3842	*Homo sapiens* ATPase homolog
Hs.10590	ESTs
Hs.155609	CAMP-dependent protein kinase regulatory subunit type I
Hs.15953	ESTs
Hs.76807	HLA class II histocompatibility antigen
Hs.21278	ESTs
Hs.180029	Human guanine nucleotide exchange factor
Hs.62954	Enolase 1 (alpha)
Hs.347	Lactotransferrin
Hs.155485	Huntingtin-interacting protein 2
Hs.151738	Matrix metalloproteinase 2
Hs.8249	ESTs
Hs.16291	ESTs
Hs.8248	Ubiquinone oxidoreductase precursor
Hs.13059	ESTs
R39288	ESTs

[a] These genes were overexpressed greater than 4-fold during PB T-cell activation.

and Jones, 2001). Hybridization patterns were captured electronically using a two-color confocal laser microscope (Molecular Dynamics, Sunnyvale, California).

Microarray Experimental Design and Data Analysis

To directly analyze the changes in transcriptional profile induced by activation, mRNA from PHA-

Table 7. Validation of Microarray Gene Expression Data by Real-Time RT-PCR

Gene name	Activated PB T cells vs resting T cells		Activated Jurkat vs nontreated Jurkat T cells	
	Microarray[a]	RT-PCR[a,b]	Microarray[a]	RT-PCR[a,b]
NFKB1	3.1	1.4	−1.5	−3.1
Fos B	1.5	1.7	−2.3	−1.1
Jun D	1.6	1.7	−2.0	−1.2
Jun B	−1.5	−3.5	−1.9	−3.9
p16	−2.2	−1.5	−3.0	−1.7
p27	−1.8	−2.2	−2.1	−1.3
RPS14	3.6	3.2	1.8	1.7
CDC 25A	3.6	3.5	1.6	1.5
CD 68	2.3	1.8	2.6	1.8

[a] The numbers represent the ratio of gene expression level between activated and nonactivated T cells.
[b] Gene expression levels were normalized to the housekeeping gene GAPDH.

stimulated cells were hybridized directly with their nonactivated counterpart. As a control, Cy5-labeled cDNA from $CD2^+$ T cells was also hybridized with Cy3-cDNA from Jurkat cells. Each hybridization was performed in duplicate and repeated twice. Microarray analysis was performed using GeneSpring 4.0.4 (Silicon Genetics, Redwood City, California). Relative levels of transcripts between test and reference samples were shown as ratio of Cy5 signal (red fluorescence) versus Cy3 (green fluorescence) signal. Two-fold overexpression of a gene was indicated when the Cy5 signal intensity from the test sample was twice that of Cy3 (from the reference sample), and 2-fold underexpression was indicated by a Cy3 signal intensity twice as high as that of Cy5. To ensure that the reported fold changes in gene expression were significant, $p < 0.05$ was used as a cut-off value so that only those fold changes with p values < 0.05 were presented.

Quantitative Fluorescent Real-Time RT-PCR

RNA was reverse transcribed using the First-Strand cDNA Kit (Life Technologies, Carlsbad, California). After cDNA synthesis, 1 μl of cDNA template was used for each PCR reaction. The forward and reverse primers used for PCR reactions were as follows: 5'-CGACCACTTTGTCAAGCTCA-3' (forward, GAPDH), 5'-AGGGGAGATTCAGTGTGGTG-3' (reverse, GAPDH); 5'-CAAGGGTCTTGGGTCCTA-3' (forward, RPS 14), 5'-TGCCCTTGTGAACTGGTG-3' (reverse, RPS 14); 5'-GCACGACAACATCTCATTGG-3' (forward, NFKB1), 5'-TCCCAAGAGTCATCCAGGTC-3' (reverse, NFKB1); and 5'-TCTCTCAAGCTCGCCTCTTC-3' (forward, Jun B), 5'-ACGTGGTTCATCTTGTGCAG-3' (reverse, JunB). Real-time PCR was conducted using a LightCycler DNA Master SYBR Green I Kit (Roche Molecular Biochemicals, Indianapolis, Indiana), following the manufacturer's instructions with the following thermal cycling conditions: 95° C for 30 seconds, followed by 45 cycles of 95° C for 0 seconds, 55° C for 5 seconds, and 72° C for 10 seconds. Each gene was normalized to a housekeeping gene (GAPDH) before fold change was calculated based on the signal intensities at the midexponential stages of the real-time amplification curves.

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