Brief report

Gene expression profiling of peripheral T-cell lymphoma including γδ T-cell lymphoma

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The gene expression profile of peripheral γδ T-cell lymphoma (γδTCL) has not been investigated. Using oligonucleotide microarrays, we analyzed total RNA from 7 patients with γδTCL (4 hepatosplenic, 1 cutaneous, 1 intestinal, and 1 thyroidal) and 27 patients with αβTCL (11 peripheral TCL-unspecified, 15 angioimmunoblastic TCL, and 1 hepatosplenic). Unsupervised microarray analyses classified all hepatosplenic γδTCLs into a single cluster, whereas other γδTCLs were scattered within the αβTCL distribution. We identified a T-cell receptor signature gene set, which accurately classified γδTCL and αβTCL. A classifier based on gene expression under supervised analysis correctly identified γδTCL. One case of hepatosplenic αβTCL was placed in the γδTCL grouping. γδTCL signature genes included genes encoding killer cell immunoglobulin-like receptors and killer cell lectin-like receptors. Our results indicate that hepatosplenic γδTCL is a distinct form of peripheral TCL and suggest that nonhepatosplenic γδTCLs are heterogeneous in gene expression. (Blood. 2009;113:1071-1074)

Introduction

T cells expressing the γδ T-cell receptor (TCR) heterodimer comprise only a small fraction of the peripheral blood T-cell population and differ from those expressing the αβ TCR in terms of development, tissue distribution, and function.[1,2] Mature T-cell lymphomas (TCLs) with the γδ T-cell immunophenotype can be divided into hepatosplenic γδTCL[3] and nonhepatosplenic γδTCL.[4] The third World Health Organization (WHO) classification system describes hepatosplenic γδTCLs and hepatosplenic αβTCLs as a single disease entity (hepatosplenic TCL) as they exhibit nearly identical clinicopathologic and cytogenetic features.[4-6]

In contrast, nonhepatosplenic γδTCL occurs in only a limited number of anatomic sites, including cutaneous, nasopharyngeal, gastrointestinal, pulmonary, and thyroidal regions.[7-10] This disease has also been called mucocutaneous γδTCL because the majority of patients show some involvement of mucocutaneous sites. Among nonhepatosplenic γδTCLs, the cutaneous form is most common and overlaps with subcutaneous panniculitis-like TCL.[11,12] Whereas primary cutaneous γδTCL is categorized as a single disease entity in the new WHO scheme,[13] other nonhepatosplenic γδTCLs remains an enigma.

γδTCLs are rare lymphoid malignancies and are difficult to diagnose, resulting from the lack of available monoclonal antibodies against γδTCR for use with paraffin-embedded tissue. Several studies have elucidated the gene expression profile of peripheral TCLs (PTCLs)[14-17] but did not evaluate γδTCL. In our current study, we performed gene expression profiling in 34 PTCLs, including 7 cases of γδTCL.

Methods

Patients/samples

Our present study assessed 34 cases of PTCL, including 11 PTCL-unspecified with αβ T-cell immunophenotype, 15 angioimmunoblastic TCLs, 1 hepatosplenic αβTCL, 4 hepatosplenic γδTCLs, 1 cutaneous γδTCL, 1 intestinal γδTCL, and 1 thyroidal γδTCL. All specimens were collected between 1987 and 2002 at Mie University Hospital and diagnosed according to the third WHO classification.[6] Tumor cell expression of cell-surface antigens and TCR heterodimer (αβ or γδ) was confirmed by immunohistochemistry using frozen sections as described previously.[9] DNA microarray studies using specimens from patients with hematopoietic malignancies were approved by the Institutional Review Committee of Mie University Graduate School of Medicine. Informed consent was obtained from these patients in accordance with the Declaration of Helsinki. The clinicopathologic features of 6 of 7 cases of γδTCL have been reported previously.[8,9] The single patient we examined with thyroidal γδTCL remains alive with no evidence of disease 13 years after diagnosis. Clinical data for all cases examined are presented in Table S1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

Gene expression profiling and analysis

Gene expression profiles were generated and analyzed as previously reported.[18] We used the Agilent 44K human oligonucleotide microarray (Agilent 4112F; Agilent Technologies, Palo Alto, CA), and raw gene expression data are available at the Gene Expression Omnibus (accession number GSE11946).[19] For gene expression profiling supervised by TCR heterodimer phenotype, we selected genes with an average differential expression level of more than 3.0-fold and used a one-sample *t* test with a

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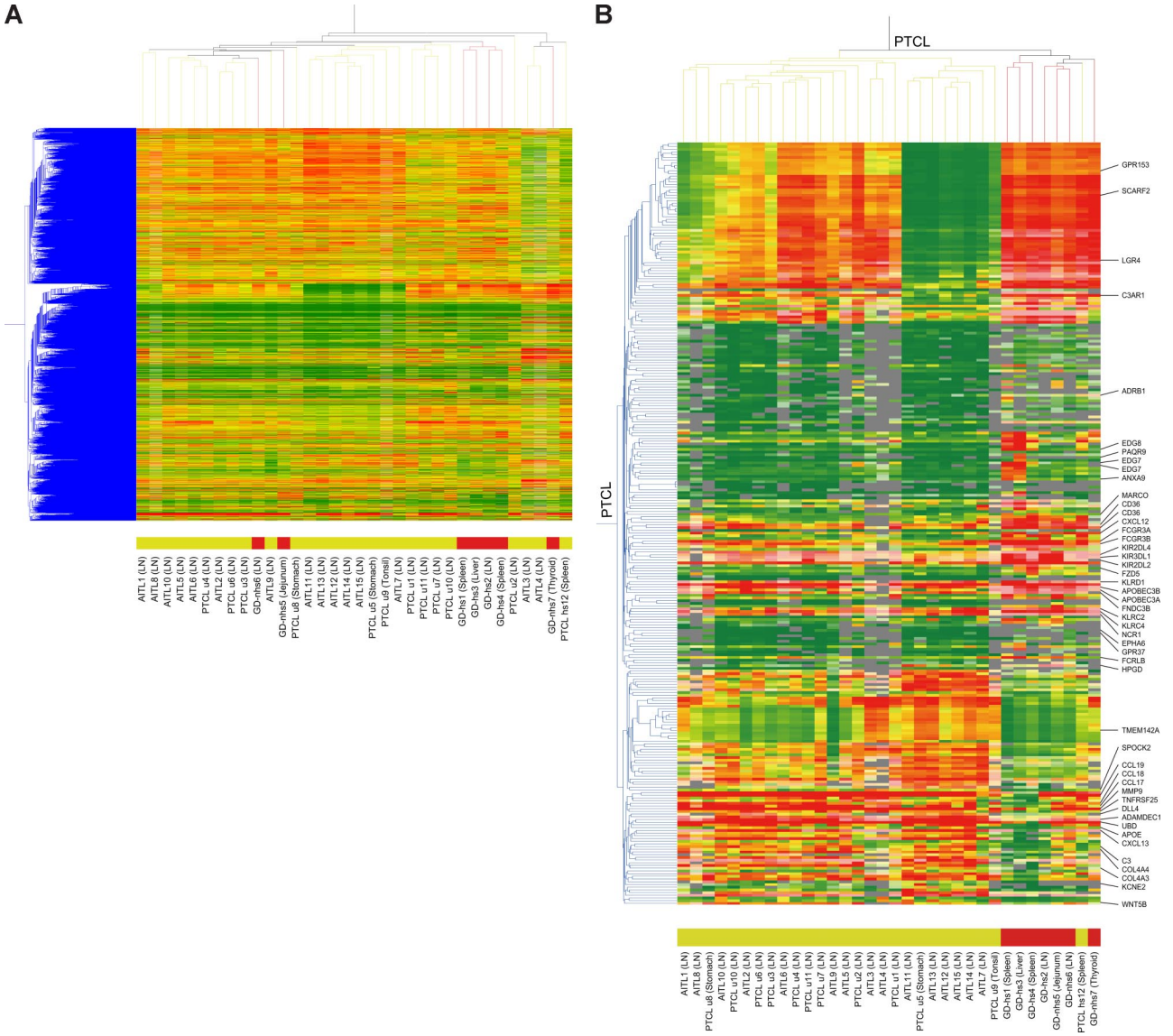

Figure 1. Hierarchical clustering of gene expression data. (A) Hierarchical clustering of 34 cases of PTCL using unsupervised analysis. Color blocks indicate the characteristics of the PTCLs; red blocks, γδTCL cases; amber blocks, αβTCL cases. The anatomic site of each specimen is shown in parentheses. AITL indicates angioimmunoblastic TCL; GD-hs, hepatosplenic γδTCL; GD-nhs, nonhepatosplenic γδTCL; LN, lymph node; PTCL hs, hepatosplenic αβTCL; PTCL u, PTCL-unspecified. Four cases of hepatosplenic γδTCL (GD-hs1-4) were classified into a single cluster. Nonhepatosplenic γδTCLs (GD-nhs5, intestinal; GD-nhs6, cutaneous; GD-nhs7, thyroidal) were scattered within the αβTCL distribution. (B) Hierarchical clustering of 34 cases of PTCL-based TCR signature gene expression. Genes also listed in Table 1 are shown on the right. γδTCL cases were correctly identified by the gene set, although one case of hepatosplenic αβTCL (PTCL hs12) was grouped with the γδTCLs.

P value cutoff of .005. Hierarchical clustering of genes was performed using the Pearson correlation, and hierarchical clustering of cases was obtained using an average linkage algorithm.

We chose WebGestalt using Gene Ontology (GO) hierarchies[20,21] for categorization of functional gene groups and the Kyoto Encyclopedia of Genes and Genomes (KEGG)[22,23] pathway for identification of signaling pathways. In both analyses, we performed a separate hypergeometric test with a *P* value cutoff of .001.

Results and discussion

Gene expression profiling is a powerful tool for establishing a molecular basis for lymphoma subtypes.[24] Unsupervised analysis of our PTCL cases classified hepatosplenic γδTCL as a single cluster, whereas other γδTCLs were scattered within the αβTCL distribution (Figure 1A). Since the 1980s, cumulative clinicopathologic evidence has demonstrated that hepatosplenic γδTCL is a distinct clinicopathologic disease entity. Our gene expression results also confirm that hepatosplenic γδTCL is distinct from other PTCLs. Conversely, nonhepatosplenic γδTCLs appeared to be more heterogeneous. The possibility that tissue differences were responsible for these data was excluded by our observation that hepatosplenic γδTCL was classified as a single cluster, irrespective of the specimen examined (Figure 1). Angioimmunoblastic TCL cases were not classified as a single cluster, consistent with prior gene expression studies.[14-17]

We next compared the gene expression profiles of γδTCL and αβTCL using 291 genes showing a greater than 3.0-fold average expression difference, which we designated the TCR signature gene set (Table S2). Of note, a classifier from supervised analysis based on gene expression identified γδTCL and hepatosplenic αβTCL (Figure 1B). This finding is supported by the notion that γδ

Table 1. GO category analysis and KEGG pathway analysis in the TCR signature gene set

Analytical tool	Gene no.	Gene	*P*
GO category			
γδTCL			
Cellular defense response	5	*KIR2DL4*, *NCR1*, *C3AR1*, *KLRC2*, *KLRC4*	1.17×10^{-4}
Signal transduction activity	29	*KLRD1*, *ANXA9*, *FNDC3B*, *GPR37*, *KIR2DL4*, *MARCO*, *EDG7*, *NCR1*, *MS4A5*, *HPGD*, *FCGR3A*, *LGR4*, *FCRLB*, *C3AR1*, *CXCL12*, *RTN4R*, *PAQR9*, *KLRC2*, *GPR153*, *FCGR3B*, *KIR2DL2*, *EDG8*, *ADRB1*, *CD36*, *FZD5*, *SCARF2*, *KIR3DL1*, *EPHA6*, *KLRC4*	5.09×10^{-6}
Receptor activity	28	*GPR37*, *KIR2DL4*, *ANXA9*, *KLRD1*, *FNDC3B*, *EDG7*, *HPGD*, *MARCO*, *LGR4*, *MS4A5*, *FCGR3A*, *NCR1*, *C3AR1*, *FCRLB*, *KLRC2*, *PAQR9*, *RTN4R*, *GPR153*, *EDG8*, *FCGR3B*, *KIR2DL2*, *ADRB1*, *FZD5*, *CD36*, *SCARF2*, *KIR3DL1*, *EPHA6*, *KLRC4*	7.10×10^{-8}
Transmembrane receptor activity	16	*ANXA9*, *GPR37*, *KIR2DL4*, *KLRD1*, *LGR4*, *HPGD*, *MARCO*, *EDG7*, *C3AR1*, *KLRC2*, *EDG8*, *GPR153*, *ADRB1*, *FZD5*, *EPHA6*, *KIR3DL1*	4.46×10^{-4}
IgG binding	2	*FCGR3A*, *FCGR3B*	3.07×10^{-4}
αβTCL			
Organismal physiologic process	14	*UBD*, *CXCL13*, *COL4A4*, *CCL18*, *KCNE2*, *CCL17*, *C3*, *TMEM142A*, *DLL4*, *APOE*, *COL4A3*, *TNFRSF25*, *MMP9*, *CCL19*	3.24×10^{-4}
Regulation of organismal physiologic process	4	*COL4A4*, *KCNE2*, *C3*, *APOE*	5.87×10^{-4}
Circulation	4	*KCNE2*, *DLL4*, *COL4A3*, *APOE*	3.14×10^{-4}
Regulation of neurophysiologic process	2	*COL4A4*, *APOE*	7.55×10^{-4}
Regulation of transmission of nerve impulse	2	*COL4A4*, *APOE*	7.55×10^{-4}
Regulation of synapse structure and function	2	*COL4A4*, *APOE*	8.69×10^{-4}
Regulation of synaptic transmission	2	*COL4A4*, *APOE*	7.55×10^{-4}
Inflammatory response	5	*CXCL13*, *CCL18*, *CCL17*, *C3*, *CCL19*	6.05×10^{-4}
Behavior	5	*CCL18*, *CXCL13*, *CCL17*, *APOE*, *CCL19*	2.47×10^{-4}
Locomotory behavior	4	*CXCL13*, *CCL18*, *CCL17*, *CCL19*	4.92×10^{-4}
Taxis	4	*CXCL13*, *CCL18*, *CCL17*, *CCL19*	4.22×10^{-4}
Chemotaxis	4	*CXCL13*, *CCL18*, *CCL17*, *CCL19*	4.22×10^{-4}
Receptor binding	9	*ADAMDEC1*, *CCL18*, *CXCL13*, *CCL17*, *C3*, *DLL4*, *APOE*, *COL4A3*, *CCL19*	2.76×10^{-5}
G-protein–coupled receptor binding	4	*CCL18*, *CXCL13*, *CCL17*, *CCL19*	1.57×10^{-5}
Chemokine receptor binding	4	*CCL18*, *CXCL13*, *CCL17*, *CCL19*	6.97×10^{-6}
Chemokine activity	4	*CCL18*, *CXCL13*, *CCL17*, *CCL19*	6.37×10^{-6}
Extracellular region	11	*CCL18*, *COL4A4*, *CXCL13*, *CCL17*, *C3*, *WNT5B*, *MMP9*, *COL4A3*, *APOE*, *SPOCK2*, *CCL19*	7.97×10^{-5}
Extracellular region part	9	*CCL18*, *COL4A4*, *CXCL13*, *CCL17*, *SPOCK2*, *APOE*, *COL4A3*, *MMP9*, *CCL19*	7.33×10^{-5}
Sheet-forming collagen	2	*COL4A4*, *COL4A3*	1.70×10^{-4}
Collagen type IV	2	*COL4A4*, *COL4A3*	1.21×10^{-4}
KEGG pathway			
γδTCL			
Natural killer cell–mediated cytotoxicity	5	*FCGR3A*, *KIR3DL1*, *KLRC2*, *KLRD1*, *NCR1*	8.10×10^{-4}
Antigen processing and presentation	4	*KIR3DL1*, *KLRC2*, *KLRD1*, *KLRC4*	7.53×10^{-4}
Atrazine degradation	2	*APOBEC3A*, *APOBEC3B*	4.42×10^{-4}
αβTCL			
Cytokine–cytokine receptor interaction	5	*CXCL13*, *CCL17*, *CCL18*, *CCL19*, *TNFRSF25*	2.65×10^{-4}

T cells partially share a cytotoxic immunophenotype with cytotoxic αβ T cells.[1,2] Among 30 patients for whom survival data were available, the prognosis for 8 cases with a γδTCL gene profile (7 γδTCLs and 1 hepatosplenic αβTCL) was not significantly poorer than that of 22 patients with an αβTCL gene profile ($P = .152$; Figure S1). The unusual case of thyroidal γδTCL in the γδTCL gene profile group may affect the result because the *P* value was .009 when we excluded this patient from the survival analysis (data not shown). Future analyses will probably reveal the relationship between our TCR signature gene set and prognostic indicators.

In γδTCL, genes of natural killer (NK) cell–associated molecules, such as killer cell immunoglobulin (Ig)–like receptor (KIR) genes (*KIR3DL1*, *KIR2DL4*, and *KIR2DL2*), and killer cell lectin-like receptors (*KLRC4*, *KLRD1*, and *KLRC2*) were found to be overexpressed (Figure 1; Table S2). These NK receptors are expressed by a subset of NK cells, γδ T cells, and $CD8^+$ αβ T cells.[25] KIR3DL1 and KIR2DL2 exhibit inhibitory functions, and KIR2DL4 has potentially both inhibitory and activating roles.[25] KIR3DL1, KIR2DL2, and KLRD1 are reported to be expressed in some cases of hepatosplenic γδTCL.[26,27] Although *KLRC4*, a top 10 feature gene that characterizes γδTCL and its protein, NKG2F, is expressed in human NK cells,[28] its expression in normal γδ T cells has not been determined. CD16 is also frequently expressed in cases of hepatosplenic γδTCL,[4,27] and its genes (*FCGR3B* and *FCGR3A*) were among the γδTCL signature genes identified in this study.

To search for functionally important genes overexpressed in γδTCL, we performed GO and pathway analysis using 139 of 204 and 53 of 87 known genes in the γδTCL and αβTCL groups, respectively. By WebGestalt, 5 and 20 GO categories were enriched in γδTCL and αβTCL, respectively (Table 1). The enriched GO categories in γδTCL were cellular defense response, signal transduction activity, receptor activity, transmembrane receptor activity, and IgG binding. Three γδTCL pathways and 1 αβTCL pathway were found to be altered in KEGG-signaling analyses (Table 1). No γδTCL and αβTCL signature genes were

shared in a GO category or KEGG pathway, indicating different functional profiles between γδTCL and αβTCL. Four of the 5 γδTCL-enriched GO categories and 2 of the 3 KEGG-signaling pathways altered in γδTCL contained genes encoding KIRs and killer cell lectin-like receptors, a finding that suggests that the expression of these genes may be important for the differential diagnosis of γδTCL and αβTCL.

In conclusion, our current gene expression data confirm that hepatosplenic γδTCL is a distinct lymphoma entity in PTCLs and reveal differences in the gene expression profiles of αβTCL and γδTCL. Further investigations of our newly identified TCR signature genes are warranted to identify novel therapeutic targets and facilitate the diagnosis of γδTCL.

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Authorship

Contribution: K.M. and M. Yamaguchi designed and performed the study, collected data and samples, interpreted data, and wrote the paper; H.I. and S.T. performed the study and collected samples; M. Yuda and H.S. contributed analytical tools and supervised the research; K.N. collected samples and wrote the paper; and T.K. and N.K. supervised the research and wrote the paper.

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