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The Diagnostic Significance of Immunoglobulin Light Chain Gene Expression Signatures by Gene Expression Profiling for B-Cell Lymphoma

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Abstract

Gene expression profiling (GEP) has been shown to have great potential to become a powerful diagnostic tool for lymphoma classification. However, it is not clear whether the expression pattern of immunoglobulin light chain genes defined by GEP can be used as a diagnostic signature to differentiate B-cell lymphoma from B-cell hyperplasia. To assess the difference in expression signatures of kappa and lambda genes among samples from reactive tonsils, normal peripheral bloods, chronic lymphocytic leukemias (CLL), follicular lymphomas (FL) and diffuse large B-cell lymphomas (DLBCL), GEP data available from the Lymphoma/Leukemia Molecular Profiling Project (LLMPP) were reanalyzed. A normal reference range of kappa and lambda light chain gene expression was defined by analysis of polyclonal B-cells from normal peripheral bloods and tonsils. Restricted light chain gene expression was detected in 100% (11/11) of CLL, in 83% (5/6) of FL, and 67% (198 of 296) of DLBCL. Our findings provide the evidence for the concept that immunoglobulin light chain restriction can be determined at the gene transcription level by GEP microarray in most of B-cell malignancies. This will be important in future clinical application of this technology to add in the differentiation of B-cell lymphomas from reactive hyperplasias.

Keywords: Diffuse large B-cell lymphoma; Microarray; Gene expression profiling; Immunoglobulin light chain

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Introduction

Diagnosis of mature B-cell lymphoma is confirmed by the evidence of clonal proliferation of B-cells, with restricted immunoglobulin light chain protein expression. Absence of light chain restriction in mature B-cells argues against the diagnosis of B-cell lymphoma. In clinically practice, flow cytometry has been a method of choice for assessing the light chain restriction in B-cells for several decades [1,2]. As an emerging diagnostic tool, microarray gene expression profiling (GEP) uses solid platforms (typically glass arrays) containing probes for thousands of transcripts yielding a global pattern of gene expression at the transcription level [3,4]. Several gene expression signatures with prognostic value for B-cell non-Hodgkin lymphoma (B-NHL) have been identified with GEP [5-8]. However, it is not clear whether immunoglobulin light chain gene expression signature in the gene expression profile can be used to differentiate polyclonal B-cells (reactive) from monoclonal B-cells (neoplastic). Therefore, we re-analyzed two microarray data sets which were previously published but the immunoglobulin light chain gene expression patterns have not yet been interrogated among these lymphoid tissue samples [5,7]. In this report, we describe a protocol to define light chain restriction by GEP microarray which has not been previously reported. We also identify a subset of diffuse large B-cell lymphomas (DLBCL) without detectable light chain gene expression which appears due to impaired transcription.

Materials and Methods

GEP databases

The two GEP data sets previously published by the Lymphoma/Leukemia Molecular Profiling Project (LLMPP) were downloaded from its web site companions [5,7]:

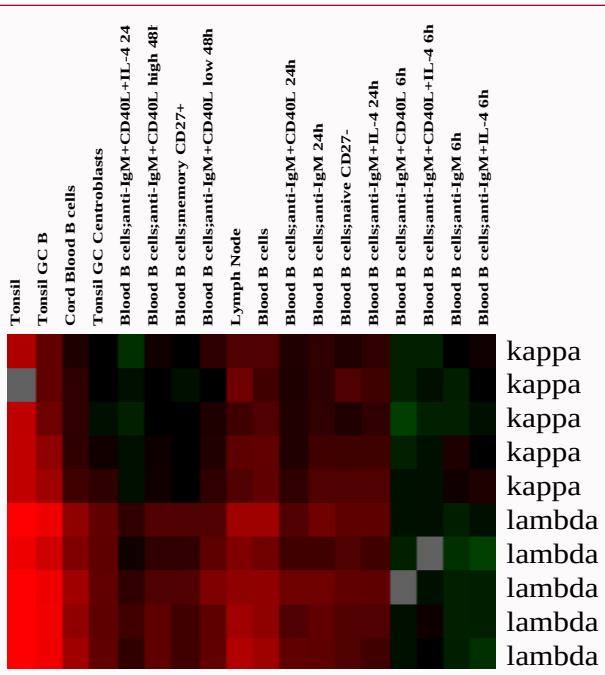

Figure 1: Light chain gene expression polyclonal B-cells. Depicted are the measurements of the transcripts of kappa and lambda light chain genes. Each row represents a separate cDNA clone on the microarray and each column a separate mRNA sample. The results presented represent the ratio of hybridization of fluorescent cDNA probes prepared from each experimental mRNA samples to a reference mRNA sample. These ratios are a measure of relative gene expression in each experimental sample and were depicted according to the colour scale shown at the bottom. As indicated, the scale extends from fluorescence ratios of 0.25 to 4 (-2 to +2 in log base 2 units) Green indicated decreased expression. Red indicates increased expression. Grey indicates missing or excluded data as previously described [1].

Data set 1 (http://llmpp.nih.gov/lymphoma/data/figure1/) includes samples of benign germinal center B-cells, benign centroblasts from human tonsils, normal peripheral blood B-cells, normal peripheral blood T-cells, reactive lymph nodes as well as cryopreserved tumor tissues from patients with DLBCL, FL and CLL, and lymphoma cell lines (SUDHL-6, OCILy3 and OCILy10). As previously described, the reference mRNA sample was made from a pool of nine lymphoma cell lines (Raji, Jurkat, L428, OCI-Ly3, OCI-Ly8, OCI-Ly1, SUDHL5, SUDHL6, and WSU1). The expression level of each gene was measured ratio to the mRNA in the reference pool. The cDNA probes for immunoglobulin include clone 293425, 293425, 1240813, 840451, and 725263. The cDNA probes for immunoglobulin lambda light chain include clone 1240959, 1240590, 1241741, and 771065.

Data set 2 (http://llmpp.nih.gov/DLBCL/NEJM_Web_Fig1data) is the gene expression profile of trimmed frozen biopsies from 254 cases of DLBCL. Same reference sample was used. The cDNA probes for immunoglobulin kappa include 5 expressed sequence tags (ESTs): Genbank accession numbers are AA291844, AA485725-2 ESTs, N68865, and R76324. The cDNA probes for immunoglobulin lambda light chain include 4 ESTs: Genbank accession numbers are AA427851-2 ESTs, AA714226, and AI225241).

Statistic analysis

The benign lymphoid tissue samples (n =18) included peripheral blood, cord blood, lymph nodes and tonsils in data set 1 were analyzed to define the GEP of kappa and lambda light chain gene expression in polyclonal B-cells. A reference range at the 95% confidence level was calculated for the relative expression levels of kappa and lambda genes expressed as the difference between the log red fluorescence/green fluorescence for the kappa gene and the log red fluorescence/green fluorescence for the lambda gene. The relative expression levels of the kappa and lambda genes in lymphoma samples were considered monoclonal if they were outside of the reference range. U937 and samples of T-cell lines or normal T-cells will be used as negative controls for kappa and lambda light chain expression. The Student *t* test was used to compare the microarray measurements of gene expression levels between the light chain gene transcript -positive and -negative subgroups of DLBCL. Clustering analysis was performed using the CLUSTER program (M. Eisen: http://www.microarrays.org/software).

Results

Kappa and lambda light chain GEP in normal/reactive samples or cell lines

The quality of the probes for kappa and lambda were first assessed based on negative and positive control samples. No kappa or lambda light chain transcripts were detected in T-cells, providing a negative validation. Both kappa and lambda transcripts were detected in polyclonal B-cell samples, serving as a positive validation. The expression of light chain transcripts with different cDNA probes was very consistent (r 0.88- 0.99).

There were no kappa or lambda transcripts detected in U937, OCI Ly13.2, OCI Ly1, OCI Ly12, Jurkat, samples of neonatal or adult T-cells (stimulated or unstimulated) or fetal thymic T-cells (stimulated or unstimulated). In log scale of signal of fluorescence intensity by the microarray, kappa showed a mean of -4.67 and standard deviation (SD) of 0.96. Lambda showed a mean of -2.86 and SD of 0.75. Therefore, a level less than a mean minus 2SD was considered as no significant expression of kappa or lambda light chain transcripts.

There were 18 samples of polyclonal B-cells include tonsils, tonsil germinal center B-cells, cord blood B-cells, and peripheral blood B-cells analyzed by the microarray in data set 1. The visual pattern is polyclonal (Figure 1). Of interest, blood B-cells with 6 hours of mitogen stimulation (anti-IgM, CD40L and IL-4) showed a low level of kappa or lambda light chain expression compared with samples with longer incubation with mitogen. The signal intensity of lambda appeared to be higher compared with kappa, but this does not necessarily suggest that lambda messages are more abundant than kappa. The measurements by microassay was relative to the kappa or lambda mRNA in the reference cDNA made from a pool of nine lymphoma cell lines. The information of relative amount of kappa and lambda in the reference pool was not available.

To quantify the difference of kappa and lambda light chain expression, we calculated the net difference of kappa and lambda signals in log scale as described before. The range was -1.82 to 0.68 for kappa-lambda with a mean of -0.79 and SD of 0.69. Therefore,

Table 1: Sensitivity of gene expression in clonality determination.

Samples	Positive for light chain restriction
CLL	100% (11/11)
FL	86% (6/7)
DLBCL-1	62% (26/42)
DLBCL-2	68% (172/254)

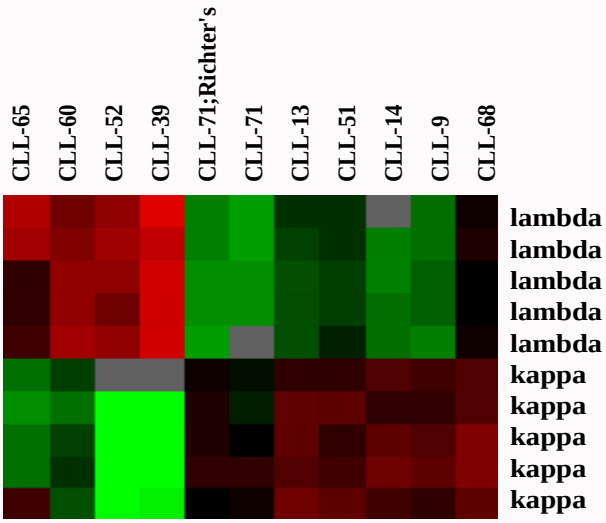

Figure 2: Light chain gene expression by B-CLL. The data is displayed as described for Figure 1.

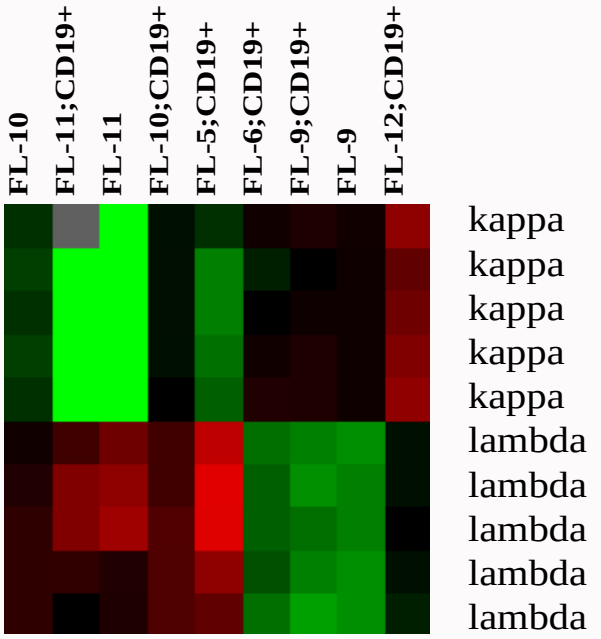

Figure 3: Light chain gene expression FL. The data is displayed as described for Figure 1.

a polyclonal reference range was defined as -2.17 to 0.60 (mean+/-2SD).

Kappa and lambda light chain expression signature measured by GEP in B-NHL

CLL: All 11 samples were defined as monoclonal based on the reference range described above (Table 1). As depicted in Figure 2, four of them were lambda restricted and seven of them were kappa restricted. CLL-71 and CLL-71 after Richter's transformation showed that same light chain restriction. Both kappa and lambda transcripts appeared abundant in CLL-68, suggesting possible residual polyclonal B-cells in the background.

FL: Five of six cases showed light chain restriction (Table 1 and Figure 3). FL-10 was classified as polyclonal, likely due to presence of significant polyclonal B-cell in the background admixed with

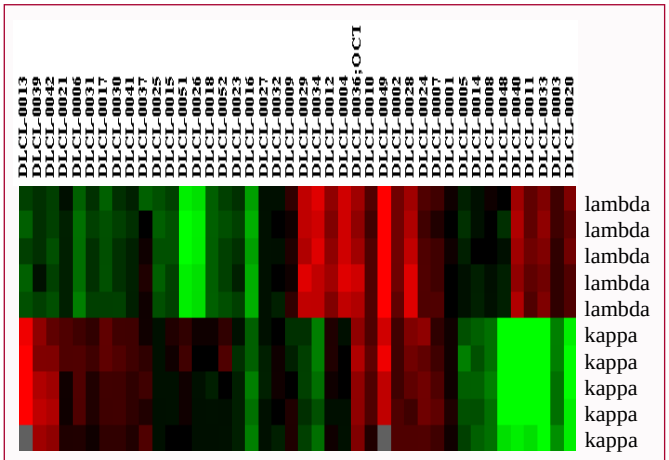

Figure 4: Light chain gene expression in DLBCL. The data is displayed as described for Figure 1.

lymphoma cells producing no or little light china mRNA. The CD19 positive B-cell fractions yield same expression patterns of kappa and lambda lights compared with whole tissue preparation in the cases FL-11 and FL-10.

DLBCL: Of 42 DLBCL samples in data set 1, 26 (62%) were defined showing light chain restriction (Table 1 and Figure 4). It was obvious that a subset of samples (DLCL-0007, 0024, 0010, 0002, 0012, 0036, 0028, 0049) contained relatively more abundant transcripts of both kappa and lambda light chains while other had low levels of transcripts of kappa and lambda light chains (DLCL-0023, 0005, 0014, 0027, 0032, 0008, 0001, 0009). Of 254 DLBCL samples in data set 2, 172 (68%) samples were defined as light chain restricted by GEP (Table 1). The pattern of light chain expression was very similar to data set 1.

Diffuse large B-cell lymphomas with low transcriptions of light chain genes

Of 82 cases without detectable light chain restriction by GEP (DLBCL data set 2), seven (63, 204, 172, 65, 69, 273, 234) showed kappa light chain transcripts less than the lowest amount of kappa in samples of polyclonal B-cells and lambda light chain transcripts less than the lowest amount of lambda in samples of polyclonal B-cells. Five of these seven cases were totally negative for both kappa and lambda transcripts, at the level comparable to that of T-cell samples. The remaining cases with no clear light chain restriction had abundant kappa and lambda transcripts in a polyclonal pattern.

To identify other unique gene expression signatures in cases of DLBCL with impaired light chain expression, seven cases with very low level of transcripts of kappa and lambda were compared with 172 cases DLBCL with light chain restriction. 473 genes were differentially expressed based on student *t* test ($p < 0.005$, data not shown). Three genes: immunoglobulin heavy constant mu (IGHM), immunoglobulin lambda-like polypeptide 1(IGLLP1), and major histocompatibility complex, class II, DQ beta 1(MHCII DQB1) showed expression at least 1 log less than that seen in the cases with detectable light chain restriction difference with at least three cDNA probes (Figure 5).

Discussion

Clonal B-cell proliferation based on restricted immunoglobulin light chain protein expression has been widely used as a diagnostic marker for mature B-cell non-Hodgkin lymphoma (B-NHL) over

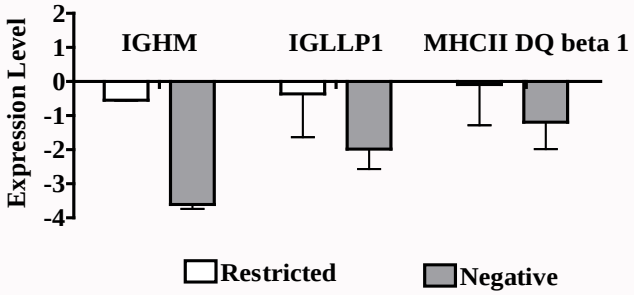

Figure 5: Three genes with markedly decreased transcripts in DLBCL without light chain transcripts from data set two. Gene expression is in depicted in mean and standard error in a log base 2 units. Restricted, DLBCL with light chain restriction by GEP (n=172). Negative, DLBCL with marked reduced light chain transcripts (n=7). The Gene expression level is expressed in log scale of signal of fluorescence intensity by the microarray. The p values are calculated by comparing both groups with student *t* test: p=0.00002 for IGHM, 0.0001 for IGLLP1, and 0.009 for MHCII DQ beta 1.

several decades. For the first time, we demonstrate that B-cell clonality can be defined by GEP with cDNA microarray in CLL, FL and DLBCL. Our protocol demonstrated a sensitivity of 100% for CLL, 86% for FL, 62-68% for DLBCL in clonality determination based on light chain gene expression signature. The sensitivity of clonality determination by microarray GEP in CLL, FL and DLBCL seems to be more or less at least comparable to flow cytometry for light chain restriction or molecular analysis for IGH gene rearrangements. With multicolor flow cytometry, light chain protein is not always detected in mature B-NHL. Horna P et al. [9] reported that lack of surface light chain protein was observed in 42 (7.4%) of 564 cases of B-NHL and was most commonly observed in cases of DLBCL (22%), less commonly in FL (7%, 5 of 74 cases) and CLL/SLL (5.5%, 13 of 235 cases). Clonality determination based evaluation of Ig gene rearrangement by PCR has variable sensitivities, with well recognized false negativity rate depending on the PCR strategy and the lymphoma subtype [10,11]. Catherwood MA et al. [12] reported positive clonal results were detected in 73% for FL and 69% for DLBCL using consensus primers. The sensitivity was further improved using BIOMED-2 family-specific primers. We anticipate the sensitivity in clonality determination based on light chain gene expression signature could be further improved in future generation of microarray platform. Our finding is conceptually important for diagnostic microarray chip design and further exploring the clinical application of microarray GEP in lymphoma diagnosis and classification. For construction of cDNA microarray, the pool of cell lines used for reference cDNAs should be constructed to maintain a relatively even ratio of kappa and lambda transcripts allowing reference range applicable to any given unknown sample in diagnostic practice. Since GEP relies on tissue homogenization and mRNA extraction, thus focusing the analysis of kappa: lambda, and aberrant gene expression on the lesional B-cells is a challenge. A potential solution is to have cell populations purified or sorted for GEP.

With DLBCL, we found that light chain restriction is not detected in about one third of cases with GEP. The causes appear to be one of following four: 1. Polyclonal B-cells or plasma cells admixed to tumor cells with low levels of light chain expression as the analysis was performed on extracts of biopsy samples, not pure tumor cells; this represents the most significant diagnostic challenge if the diagnosis is solely based on gene expression profile. Identification of additional gene expression abnormalities and correlation with clinical history and histopathology are critically important. 2. Absence of light chain gene transcripts due to impaired transcription of kappa or lambda light chain genes, which may provide a molecular explanation for a subset of mature B-cell lymphoma without surface and cytoplasmic light chain proteins. 3. Biclonal lymphoma cells with one clone of kappa restricted and the other lambda restricted. 4. Lymphoma cells may co-express both kappa and lambda light chains. Unfortunately flow cytometry data was not available for further correlation between protein expression and gene expression in these samples. At the protein levels, these situations have been encountered in practice or documented in the literature [13-16]. From diagnostic point of view, our findings suggest same diagnostic rule is applicable to both gene and protein expression data, absence of detectable light chain restriction does not rule out the possibility of lymphoma.

It has been well documented that a subset of mature B-cell lymphoma are immunoglobulin light chain negative, ranging from 3.4% to 12.2% depending on the criteria [17]. About 30 to 40% of light chain negative lymphomas were DLBCL and FL [18]. About one fourth of DLBCL were reported to be negative for light restriction based on flow cytometry evaluation [19]. The mechanisms accounting for the absence of light chain transcripts in the subset of DLBCL are not fully understood. Biologically, the absence of light chain gene expression is either due to gene deletion or impaired transcription. We discovered significantly decreased transcripts of three genes, IGHM, IGLLP1, and MHC class II, DQB1, very a striking common feature for this group of lymphomas with impaired light chain gene transcription. IGHM is an important gene in B-cell development and maturation. Mutations in IGHM have been reported to be associated with B cell deficiency and agammaglobulinemia [20]. The deletions involving IGHM or IGH intronic enhancer have been was detected in MALT lymphoma suggesting lymphoma cells may undergo aberrant isotype switch recombination in the development or progression of malignancy [21]. Further studies are necessary to find out whether such deletions indeed occur in a subset of DLBCL. IGLL1 encodes omega polypeptide chains associate with the immunoglobulin (Ig) mu chain to form a molecular complex that is expressed on the surface of B cells [22]. This complex presumably regulates Ig gene rearrangements in the early steps of B-cell differentiation. Mutations affecting IGHM and IGLL1 genes involved in the composition of the pre-B cell receptor (pre-BCR) has been implicated in pre-BCR signal transduction. It has been shown that B cell blockage at the pro-B to pre-B cell transition can be caused by a large homologous deletion in the IGH locus encompassing the IGHM gene leading to the inability to form a functional pre-BCR in autosomal recessive agammaglobulinemia [23]. Mutations in the HLA class II genes have been reported in diffuse large B-cell lymphoma [24,25]. The homozygous deletions and hemizygous deletions or mitotic recombination with mutations of the remaining allele may lead to loss of expression of HLA-DR and HLA-DQ. Loss of expression of human leukocyte antigen (HLA) class II molecules on tumor cells may affects the onset and modulation of the immune response, associated with poor survival. Further studies are necessary to confirm such findings and to explore the possible mechanisms for impairment of light chain transcription and to identify possible additional diagnostic markers for such cases.

In conclusion, we demonstrated for the first time that simultaneous analysis of kappa and lambda light chain gene expression patterns of lymphoid tissues with microarray gene expression profiling may provide evidence of B-cell clonality to differentiate polyclonal

(reactive) from monoclonal (neoplastic) lymphoid proliferation. Correlation of light chain gene expression patterns with other gene expression signatures detected simultaneously in the same lymphoma tissue is potentially a powerful tool to identify underlying molecular mechanisms associated abnormal light chain expression in neoplastic B-cells.

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