Genomic Analysis of Lymphoma: Potential for Clinical Application

Wing C. Chan, MD, and James O. Armitage, MD, Omaha, Nebraska

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Lymphoma, gene expression profiling, personalized medicine

Abstract
The application of gene expression profiling to the study of lymphomas will significantly influence the way these tumors are diagnosed and treated. Diffuse large B-cell lymphoma is now known to consist of several different genetic entities with different clinical presentations and therapeutic outcomes. In both follicular and diffuse large B-cell lymphoma, these studies have shown that host–tumor interactions have a major impact on the clinical course. Findings of gene expression profiling in diffuse large B-cell lymphoma has indicated the frequent up-regulation of the nuclear factor-κB and B-cell receptor signaling pathways in the activated B-cell type. Drugs targeting these pathways may be effective in the treatment of these cases and clinical trials have been initiated based on these findings. Gene expression profiling may assist in the selection of treatments based on specific metabolic pathways shown to be active in a particular lymphoma. These techniques offer the promise of truly personalized medicine for patients with lymphoma. (JNCCN 2010;8:353–360)

The ability to measure gene expression patterns in lymphomas provides the opportunity to revolutionize the way these tumors are grouped and treated. The first reports of applying gene expression profiling to patients with diffuse large B-cell lymphoma (DLBCL) showed the existence of multiple subtypes of what had been believed to be a reasonably homogenous entity. The application of these techniques to other histologic subtypes of lymphoma have improved diagnostic precision, provided direction in drug development, and shown the potential to improve treatment outcome by directing choice of therapy.

Methods of Genomic Analysis
Gene expression profiling studies can be performed using various techniques, but the most widely used has been the microarray platform. A microarray consists of thousands of regularly spaced DNA probes immobilized on a solid support. The probes on the array may be prepared as cDNA or oligonucleotide libraries that are then spotted robotically on the solid surface. They may also be synthesized in situ using photolithographic technique or inkjet synthesis. Prefabricated microarrays can be obtained from various companies and used directly for experiments. The mRNA in the sample is labeled with a fluorescent dye with or without prior amplification and hybridized to the microarray. The fluorescent signal on each probe is a function of the concentration of the corresponding transcript.

Currently available commercial arrays are close to whole transcriptome coverage, and arrays that cover all the expressed exons are also available to determine the expression of alternatively spliced forms of the gene. Each microarray experiment, therefore, generates a vast amount of data and requires sophisticated data management and analytical tools, many of which are now publicly available (e.g., http://linus.nci.nih.gov/BRB-ArrayTools.html). No single tool is best for all purposes, and the appropriate tools for an experiment depend on the experimental design, type of analysis needed, and questions being addressed.

Because a large discrepancy in dimensionality generally exists between the number of cases studied and the parameters measured, the importance of validation must be stressed. Validation may be performed computationally as...
in leave-one-out cross-validation. Important findings can be validated through specific measurements, such as quantitative reverse transcriptase polymerase chain reaction and correlation with certain biologic (e.g., genetic alterations) or clinical variables in the cases studied. Of course having specific conclusions validated by independent studies is useful.

Questions have been raised regarding the reliability and reproducibility of microarray experiments and the feasibility of cross-platform comparison of results. With properly manufactured microarrays, carefully performed studies with meticulously followed experimental procedures are highly reproducible using the same array platform. Cross-platform comparison is more difficult, but a recent large multicenter study found that microarray results are reasonably comparable even across different platforms. It is now generally recognized that although substantial variations may exist in the measurement of individual transcripts, the comparison of different signatures represented by large groups of transcripts is much more robust and reproducible.

Gene expression profiling through sequencing of the transcriptome was not a practical approach even a few years ago. However, with the development of high-throughput sequencing and the marked reduction in cost and time, expression profiling can be performed through whole transcriptome sequencing. This approach has several advantages, such as the unbiased survey of all transcripts, including different spliced isoforms; the accuracy of the measurement; the ability to quantitate low-level transcripts; and detection of mutations or polymorphisms that may have significant biologic implications.

**Clinical Applications**

Improved understanding of the genomics of lymphomas has already led to important observations in many diagnostic subgroups of lymphoma (Table 1). These are described according to WHO-defined subgroup.

**Diffuse Large B-Cell Lymphoma**

The initial observation that DLBCLs could be divided into at least 2 groups characterized as those of germinal-center origin (GCB) and those seeming to originate in activated B-cells (ABC) was quickly confirmed and has been incorporated into the most recent WHO classification. Patients with the GCB subtype have a survival approximately twice that of those with the ABC subtype when patients were treated with regimens such as CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone). The addition of rituximab has improved the outcome in both groups, although patients with the GCB subtype still have superior survival. An unusual subtype of diffuse large B-cell lymphoma presents with a mediastinal mass and occurs disproportionately in young women. When gene expression profiling was studied in these patients, the pattern was found to be different from that in other DLBCL, and in many ways similar to the pattern of gene expression seen in cell lines derived from classical Hodgkin lymphoma. Diffuse large B-cell lymphoma is one of the common subtypes of lymphomas seen in children, and the outcome of therapy has been much better than typically seen in adult patients. Most pediatric patients with DLBCL have the GCB subtype, at least partially explaining the better outcome seen in these patients. DLBCL presenting in the skin can be divided into 2 clinical subtypes: cutaneous follicle center lymphoma, which has a good prognosis with local therapy, and primary cutaneous large B-cell lymphoma, leg type, which has a poor prognosis. Genomic analysis suggests that these have different patterns of gene expression that might underlie the different clinical course, with the latter having an ABC-like profile.

DLBCL is the most frequent lymphoma to originate in the central nervous system, and primary central nervous system DLBCL has a very poor prognosis that has improved significantly with recent therapeutic regimens containing high-dose methotrexate plus cytosine arabinoside. Attempts to classify primary central nervous system DLBCL into the GCB and ABC subgroups have not been simple. Recent reports using microarrays suggest that these tumors have a gene expression pattern most closely related to later germinal-center B cells, with some called GCB and other ABC subtypes. Gene expression profiling of DLBCLs originating in certain other unique extranodal sites or clinical situations may have unique patterns of gene expression or a highly skewed presence of a certain subtype.

Although much of the efforts in genomic studies in DLBCL have focused on gene expression of the tumor cells, a recent report described an impact of the gene expression signature of stromal cells on treatment outcome in uniformly treated patients. The authors found that patients whose tumors had high expression of what was termed a stromal-1 pattern that reflected extracellular matrix deposition and histolytic infiltration and low expression of what was called the stro-
mal-2 pattern, reflecting angiogenesis and tumor blood vessel density had a better outcome than those whose tumor expressed the reverse pattern.

Tumor microvascular density and angiogenic factors and receptors, such as vascular-endothelial growth factors and vascular-endothelial growth factor receptor 1, were recently examined immunohistochemically in patients treated with CHOP, with somewhat conflicting findings.\(^{21,22}\) As previously described in other B-cell malignancies, the relationship between the tumor and host may have an important impact on eventual treatment outcome. It is possible that current treatments achieve part of their benefit from targeting the tumor stroma; this area may be exploited to the patients’ benefit. Another observation regarding patients treated with CHOP that also indicates the importance of tumor–host interaction is the poor outcome of patients with low expression of major histocompatibility complex class 1 and 2 signatures.\(^2\) Whether this observation is still valid in patients treated with R-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone with rituximab) has not yet been shown.

Studying gene expression profiles in lymphomas provides the opportunity to identify pathways on which the tumor is dependent and to target the pathways for the development of new drugs. The nuclear factor-κB (NF-κB) pathway seems to be particularly important in the ABC type of DLBCL\(^{23}\) and mediastinal large B-cell lymphomas,\(^4\) and several recent studies have shown several frequent abnormalities that affect this pathway, especially in the ABC type. These include mutations affecting one of several upstream regulators of the NF-κB pathways, such as CARD11, TNFAIP3 (A20), TNFRSF11A (RANK), TRAF5, and TRAF2.\(^{24}\) Mutations of the CD79B and -A molecules amplify BCR signaling (#2427) and lead to downstream activation of not only the NF-κB pathway, but also phosphatidylinositol 3-kinase (PI-3K), extracellular regulated kinase,

<table>
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<th>Disease</th>
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| Diffuse large B-cell lymphoma | • Recognition of germinal center B, activated B, and mediastinal B subtypes\(^{1,2,67}\)  
• Importance of host–tumor interaction in predicting treatment outcome\(^{20}\)  
• Recognition of the importance of the nuclear factor-κB pathway in activated B-cell and mediastinal large B-cell subtypes, and performance or design of early pathway-directed clinical trials\(^{23,25}\)  
• Finding that children usually have the germinal center B subtype\(^{11,14}\)  
• Demonstration of a biologic difference between diffuse large B-cell lymphoma leg type and other cutaneous large B-cell lymphomas\(^{19}\) |
| Mantle cell lymphoma          | • Finding that cyclin D1-negative and -positive cases express the same genomic signature\(^{26}\)  
• Identification of patients with grade 3b follicular lymphoma whose gene expression profile is different from that of patients with grades 1, 2, and 3a follicular lymphoma and whose clinical course is similar to that of patients with diffuse large B-cell lymphoma\(^{10}\) |
| Follicular lymphoma           | • Finding that the type of tumor stromal response has a major impact on treatment outcome\(^{11}\)  
• Identification of patients with grade 3b follicular lymphoma whose gene expression profile is different from that of patients with grades 1, 2, and 3a follicular lymphoma and whose clinical course is similar to that of patients with diffuse large B-cell lymphoma\(^{10}\) |
| Burkitt lymphoma              | • Demonstration that molecularly defined Burkitt lymphoma requires very intensive regimens for optimal treatment outcome\(^{26}\)  
• Finding that angioimmunoblastic T-cell lymphoma seems to arise in a follicular helper T cell\(^{29,40}\)  
• Recognition that some peripheral T-cell lymphoma not otherwise specified can be reclassified into angioimmunoblastic T-cell lymphoma and a spectrum of cytotoxic T-cell lymphomas\(^{39}\) |
| Peripheral T-cell lymphoma    | • Support for ALK-positive and -negative anaplastic large cell lymphomas being 2 different biologic entities\(^{58,59}\)  
• Finding that angioimmunoblastic T-cell lymphoma seems to arise in a follicular helper T cell\(^{29,40}\)  
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| Waldenstrom's macroglobulinemia | • Finding that gene expression more closely resembles chronic lymphoma leukemia than multiple myeloma\(^{46}\) |

Abbreviation: ALK, anaplastic lymphoma kinase.
mitogen-activated protein kinases, and nuclear factor of activated T cells pathways.

Therapeutic interventions targeting the different molecular mechanisms may now be considered. For example, inhibitors of the NF-κB pathway may be more effective on the former group of molecular lesions, whereas dasatinib or SYK inhibitors may be effective in targeting activation through the BCR signaling pathway. The PKC-β inhibitor, enzastaurine, may contribute by attenuating BCR signaling, including the phosphorylation of CARD11, which is important for forming the CBM complex that activates IKK. Interestingly, in a recent study on relapsed/refractory DLBCL, the ABC subtype had a better outcome than the GCB type when treated with bortezomib and chemotherapy. Furthermore, enzastaurine showed some clinical activity when used as a single agent; a phase III trial is underway using enzastaurine as a maintenance treatment after initial remission induced with R-CHOP. Selecting and combining agents that target appropriate pathways may produce significant synergistic effects.

**Mantle Cell Lymphoma**
Mantle cell lymphoma (MCL) is a well-defined entity characterized by a translocation involving the CCND1 locus that puts the cyclin D1 gene into the proximity of the immunoglobulin heavy chain enhancers, leading to overexpression of cyclin D1. This lymphoma has a very well-defined gene expression signature. Rare cases resemble MCL but without overexpression or translocation of the cyclin D1 gene. Further investigations, including genetic and clinical studies, indicate that these cases are similar to cyclin D1-positive MCL and can be defined by the MCL gene expression signature. Recently, researchers found that most classical MCL can be defined by immunohistochemical staining for SOX11, the protein product of one of the classifier genes for MCL. SOX11 is also expressed at a high level in the cyclin D1-negative MCL, thus the proliferation signature average with cyclin D1 level and deletion of the INK4A/ARF locus. These observations suggest that the proliferation signature average may be an integrator of multiple factors that contribute to a poor prognosis.

A gene expression profiling study also showed that the major determinant of prognosis is the proliferation signature that can be represented by the average expression of a set of proliferation-associated genes (the proliferation signature average). The higher the proliferation signature average, the poorer the overall survival. A positive correlation was also observed between the proliferation signature average with cyclin D1 level and deletion of the INK4A/ARF locus. These observations suggest that the proliferation signature average may be an integrator of multiple factors that contribute to a poor prognosis.

**Follicular Lymphoma**
The second most common lymphoma is follicular lymphoma. This group of lymphoma is divided into grades 1, 2, and 3a based on the average number of large centroblasts in the tumors. Grade 3b tumors have aggregates of large cells without centrocytes and frequently contain diffuse areas. Patients with grades 1 and 2 follicular lymphoma usually follow a relatively indolent clinical course, whereas those with grade 3b have an illness more similar to DLBCL. A recent report found that patients with grade 3b follicular lymphoma clustered separately from those with grades 1, 2, and 3a based on gene expression profiles, supporting the concept that it should be classified separately.

A gene expression profiling study has shown that the pattern of host response has a major impact on the outcome of patients with follicular lymphoma. High expression of the immune response 1 signature, reflecting a prominent T-cell infiltration of the tumor, and low expression of the immune response 2 signature, in which the infiltrating cells represented a predominance of macrophages and dendritic cells, was associated with a much better survival than the reverse pattern. Although these complex signatures cannot be reproduced with immunohistochemistry, the important impact of the microenvironment has been confirmed with immunohistochemical studies. However, some conflicts in specific findings exist, probably related to the methodological approaches, patient population studied, and treatment regimens used.

A hint that the impact of therapy might, at least partially, relate to the effect of drugs on the tumor microenvironment was provided in a study from France and Belgium. The investigators found that infiltrating macrophages in large numbers were associated with a poorer prognosis when an anthracycline-based chemotherapy regimen was administered. When rituximab was added to the regimen, the poorer outcome associated with a high number of infiltrating macrophages disappeared.
Good evidence shows that multiple clonal populations with different complements of genetic abnormalities frequently exist in follicular lymphoma. The tumor biopsied and studied may not represent the clone that ultimately determines the prognosis of the patient, thus compromising studies that attempt to find predictors of outcome based on the initial biopsy. The host response to the tumor may be more consistent, and therefore may be a more reliable prognosticator.

**Burkitt Lymphoma**

A major problem for pathologists for many years has been the distinction between Burkitt lymphoma and DLBCL. Although most tumors can be placed definitively into one or the other category, a subgroup of highly proliferative lymphomas have been variously categorized as “Burkitt-like lymphoma”, “high-grade B-cell lymphoma,” or “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma.”

Because most modern treatments for Burkitt lymphoma are very different from those for DLBCL, this distinction is important. Two recent reports have shown that gene expression profiling can improve, but not entirely resolve, the ability to make this distinction.

The clinical importance of being able to distinguish between molecularly defined Burkitt lymphoma and DLBCL was illustrated in a series of patients who had molecular Burkitt lymphoma but were treated with CHOP-like regimens rather than the very intensive regimens favored for Burkitt lymphoma. Patients with molecularly defined Burkitt lymphoma who were treated with very intensive regimens had an 80% 5-year survival, whereas those treated with CHOP-like regimens had an approximately 20% 5-year survival.

**Peripheral T-Cell Lymphoma**

Peripheral T-cell lymphomas are tumors of postthymic T-lymphocytes and represent approximately 10% of lymphomas worldwide, although their relative frequency reflects a very striking geographic variation. Although representing a minority of all lymphomas, a wide variety of peripheral T-cell lymphomas exist, including a few with an indolent course (e.g., mycosis fungoides), but most are aggressive lymphomas. Most reports of gene expression patterns have addressed peripheral T-cell lymphomas of nodal origin rather than those that begin in extranodal sites. T-cell anaplastic large-cell lymphoma can be subdivided into tumors that overexpress anaplastic lymphoma kinase (ALK) and have a better prognosis and those that are ALK-negative and have a poorer prognosis. These subtypes also seem to have different gene expression patterns.

Angioimmunoblastic T-cell lymphoma (AITL) is a clinical entity characterized by systemic symptoms, widespread lymphadenopathy and splenomegaly, and immunologic abnormalities. Gene expression profiling of AITL suggests that the tumor arises from a follicular helper T cell. The most common subtype of peripheral T-cell lymphoma (i.e., peripheral T-cell lymphoma, not otherwise specified [PTCL-NOS]) is a heterogeneous disease with variable gene expression patterns. Better molecular characterization of this group of tumors will allow some cases to be reclassified into other better-defined entities, such as AITL and a spectrum of cytotoxic T-cell lymphomas. Gene expression profiling also shows promise in identifying novel targets for therapy, such as platelet-derived growth factor receptor alpha for some PTCL-NOS; NF-kB and possibly interleukin-6 for AITL; and ALK kinase/STAT3 for ALK-positive AITL.

**Future Directions**

The many gene expression profiling studies in the past decade have clearly shown the potential of this approach in improving understanding of the biology of different types of lymphoma and the oncogenic pathways that are activated, and in applying this knowledge to diagnosis and management. One major impediment is that most of the information has been obtained using frozen materials, whereas in most current clinical practice settings, only formalin-fixed paraffin-embedded (FFPE) materials are available. Several attempts have been made to translate the gene expression profiling data into a platform that is applicable to FFPE tissues, such as real-time quantitative reverse transcription polymerase chain reaction (#2426), RNA protection-based assay, and immunohistochemistry. The many gene expression profiling studies in the past decade have clearly shown the potential of this approach in improving understanding of the biology of different types of lymphoma and the oncogenic pathways that are activated, and in applying this knowledge to diagnosis and management. One major impediment is that most of the information has been obtained using frozen materials, whereas in most current clinical practice settings, only formalin-fixed paraffin-embedded (FFPE) materials are available. Several attempts have been made to translate the gene expression profiling data into a platform that is applicable to FFPE tissues, such as real-time quantitative reverse transcription polymerase chain reaction (#2426), RNA protection-based assay, and immunohistochemistry. Since the recent discovery of a class of small regulatory RNA called microRNA, intense research interest has been focused on this group of small noncoding RNAs. Several studies have found that the pattern of expression of these microRNAs is related to the cell of origin of a tumor, and hence could be used in tumor classification. Furthermore, some of the microRNAs have been found to be associated with tumor outcome. Because microRNAs are small, they are well preserved in FFPE tissue and may be a novel platform for biomarker discovery.
for diagnosis and prognostication using routinely processed materials.

Other global genomic analyses hold promise for significantly improving understanding of the biology and clinical behavior of non-Hodgkin’s lymphoma. This includes the global analysis of copy number changes in the genome using high-resolution array comparative genomic hybridization technologies.56,57 Several studies using this technology have shown its ability to finely dissect the cancer genome.56 Combining this technology with gene expression profiling further facilitates the discovery of target genes in the abnormal loci.59,61 Aside from specific target genes in regions of gain or loss, these aberrations seemed to have a global effect on tumor cell proliferation and growth from changes in expression of functional groups of genes.60

The cancer genome is well known to have abnormal patterns of DNA methylation.62,63 Inappropriate hypomethylation may contribute to the abnormal activation of oncogenes and genomic instability, whereas abnormal hypermethylation may inactivate tumor suppressor genes. Global methylation profiles can now be investigated that may significantly improve understanding of gene regulation in lymphoma. This effort will be further enhanced if gene expression data are also available, so that the methylation pattern can be correlated with the corresponding gene expression profile.64

With the availability of high-throughput sequencing, mutational changes in large groups of genes can now be investigated. Preliminary data have shown mutations in both positive and negative regulators of important cellular pathways65 that may lead to abnormal activation. The NF-κB pathway was examined recently in B-cell lymphoma with similar findings.24,66 Massive sequencing analysis will lead to the discovery of numerous mutations, some of which may actually be uncommon polymorphisms, and the availability of nontumor DNA will help make this distinction. Furthermore, “passenger mutations” that may not have any biologic significance are important to distinguish from “driver mutations,” which are important in tumor pathogenesis and progression. In B-cell lymphoma, the somatic hypermutation mechanism will certainly introduce a higher frequency of mutational events than in other malignancies, making this an even more important area of investigation in lymphomas.

Availability of these large complementary data sets, especially on the same well-annotated series of patients, will vastly improve knowledge of lymphoma. It will lead to improvement in not only diagnosis and prognostication, but also the identification of important pathways that can be manipulated therapeutically in the management of patients. With the availability of new agents specific for different oncogenic pathways, non-Hodgkin’s lymphoma may conceivably be treated in the future with drugs directed against selected path-

**Figure 1** Goal of genomic investigations. Abbreviation: aCGH, array comparative genomic hybridization.
The era of personalized medicine for lymphoma has begun.

References


