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DIFFUSE LARGE B-CELL LYMPHOMA: FROM GENE EXPRESSION PROFILING TO PREDICTION OF OUTCOME

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Abstract

Diffuse large B cell lymphoma (DLBCL) is a subtype of non-Hodgkin lymphoma characterized by a markedly heterogeneous clinical course and response to therapy that is not appreciated with standard histopathologic and immunophenotypic evaluations. Recent studies have focused on the use of genome-scale expression profiles that provide a snap fingerprint of the tumor and identifying tumors with similar genetic alterations and clinical features. Gene expression studies have the ability to recognize distinct subgroups of patients based on similar molecular characteristics and markedly different outcomes that were independent of the International Prognostic Index (IPI). Further, DNA microarray studies also allow identification of new prognostic biomarkers in DLBCL. However, new methods for immunohistochemical analysis of tissue microarray and RNA extraction from paraffin-embedded blocks are required to overcome the major pitfall of this technology - the requirement for fresh tissue. Herein, we summarize the progress made in better prediction of prognosis of DLBCL patients as a result of gene expression profiling.

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) it the most common adult Non-Hodgkin's lymphoma with an annual incidence of more than 25,000 cases in the United States[1]. Although DLBCL has characteristic morphology, marked immunophenotypic, cytogenetic and molecular heterogeneity underlies the variable clinical outcome of DLBCL patients. Clinical surrogates, such as the International Prognostic Index (IPI)[2], while highly useful, do not adequately capture the molecular and cellular variability that affects clinical behavior of DLBCL. Biologic mechanisms underlying DLBCL pathogenesis are complex and involve intricate relationships between multiple genes, signaling pathways and regulatory processes[3]. Elucidation of DLBCL pathogenesis is necessary to allow recognition of new molecular therapeutic targets, discovery of DLBCL subgroups with distinct clinical outcomes and identification of molecular prognostic markers that may more accurately predict DLBCL outcomes. Accomplishment of these goals is of paramount importance and may form the basis for future risk-adapted treatments. Historically, attempts to elucidate DLBCL pathogenesis or identify new prognostic markers

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utilized a single gene approach. However the latter cannot account for the complex multigene processes underlying DLBCL pathogenesis and thus do not accurately reflect the complex changes observed in these tumors. Consequently, new investigational tools enabling simultaneous evaluation of multiple components of these biologic processes might further advance our understanding of DLBCL and potentially lead to specific molecularly targeted and patient-tailored therapies.

DNA microarrays are a new technology used to measure the expression of tens of thousands of genes simultaneously, enabling a more comprehensive evaluation of gene expression. This technique allows the comprehensive analysis of messenger RNA (mRNA) expression in tumor samples. The clinical characteristics and behavior of a tumor are determined by the specific genetic changes present in the tumor cells that are reflected in their pattern of mRNA expression creating a “molecular signature” or “fingerprint” for the tumor. The full potential of microarrays has not yet been realized, however they may a) identify previously unrecognized disease entities with distinct biological and clinical features; b) elucidate the key genetic profiles and lesions that define each of these new nosologic entities; c) discover new molecular targets for future therapeutic intervention; d) identify genes that play a potential role in determining prognosis; e) discover previously unknown genes of major clinical relevance from numerous EST clones present on the arrays, and f) identify gene expression signatures correlated with response to specific therapeutic agents. Herein, we briefly review the contribution of gene expression profiling and its role in prediction of outcome of DLBCL patients.

Less than half of patients with DLBCL will be cured with conventional chemotherapy regimens[4,5]. Improvement in disease-free and overall survival may be obtained with the addition of monoclonal antibodies, such as rituximab[5]. While standard pathologic techniques do not reliably predict sensitivity to chemotherapy or outcome for individual patients, gene expression profiling has provided important insights into the biology of DLBCL allowing a better molecular classification of tumors that are more homogeneous in pathogenesis and clinical behavior.

The pivotal microarray study was performed by Alizadeh *et al* with the use of a cDNA Lymphochip array.[6] The evaluation of tumors from 42 DLBCL patients treated with anthracycline-based chemotherapy led to the identification of two distinct subgroups based on the expression of genes characteristic of germinal center B cells (GC) or *in vitro* activated peripheral blood cells (ABC). Patients with GC subtype had a significantly better overall 5-year survival (76% versus 16%, $P < 0.01$), independent of the IPI score. These findings were further confirmed by the larger Lymphoma and Leukemia Molecular Profile Project (LLMPP) study.[7] Using similar cDNA Lymphochip array platform, analysis of tumor samples from 240 DLBCL patients treated with anthracycline-based chemotherapy demonstrated a significant difference in the 5-year overall survival between the GC-like and ABC-like subgroups (60% versus 35% respectively). Although the early microarray expression profile studies were able to identify the presence of biologically distinct subgroups of DLBCL, they were unable to identify the relative contribution of individual genes, therefore making it difficult to build clinically useful prognostic models based on a relatively small number of genes. To address this question, both the Rosenwald[7] and Shipp groups [8] applied supervised analytical methodologies to the Lymphochip and Affymetrix-derived gene expression profiles of 240 and 58 DLBCL patients, respectively. This approach led to construction of outcome predictors based on expression of 17 and 13 genes, respectively. However, there was no overlap between the lists of genes comprising these two outcome prediction models. This disparity between large genome-scale expression profile models has been attributed to patient selection, technical differences, arrays composition and variable analytical approaches. Wright *et al* designed a method based on Bayes’ rule that could be used to translate experimental results across different

microarray platforms.[9] Expression data from 14 genes identified by the LLMPP[7] and analyzed by Shipp *et al* was able to subdivide patients into GC-like and ABC-like, with significant different outcomes[8]. Nevertheless, despite the positive results, this model may not be clinically useful because of complex manipulations with shifting and scaling of gene expression from Affymetrix data to match the mean and variance of the corresponding expression values in the cDNA microarray dataset.

In an attempt to devise a technically simple method that could be applicable for routine clinical use, we evaluated the mRNA expression of 36 genes previously reported to predict survival[10] in tumor specimens from 66 DLCBL patients treated with anthracycline-based therapy. The top six genes ranked according to their predictive power on univariate analysis were used to construct a model based on their relative individual contribution into a multivariate analysis. Among the selected genes, *LMO2*, *BCL-6* and *FN1* predicted longer survival whereas *CCND2*, *SCYA3*, and *BCL-2* predicted shorter survival. Based on the expression of these 6 genes, patients could be subdivided into IPI-independent low, intermediate and high-risk groups with significantly different 5-year survival rates ranging from 65% in the low-risk to 15% in the high-risk subgroups. This model was subsequently validated in the data sets available from previously reported studies[7,8].

However, gene expression arrays are not widely available, require fresh tumor specimens, and are labor-intensive and expensive. Therefore, researchers have tried to use the information derived from RNA profiling studies to create prediction models based on more amenable techniques such as immunohistochemistry (IHC). However, multiple IHC studies have led to contradictory results[11,12] suggesting the lack of an ideal set of IHC markers for outcome prediction in DLBCL. Hans *et al*, complimented cDNA microarrays with immunohistochemistry (IHC) staining[13]. They proposed an IHC model based on 3 markers: CD10, *BCL-6* and MUM1 for determination of GC-like and ABC-like DLBCL subtypes. This model demonstrated positive predictive values of 87% and 73% for correctly identifying GC-like and ABC-like DLBCL subtypes and could predict patient survival: 76% of IHC-defined GC-like DLBCL survived at 5 years compared to 34% of non-GC patients. However, comparison of this IHC model with the gold- standard gene expression profiling revealed a 20% misclassification rate, suggesting the need for incorporation of additional IHC markers to improve the predictive value of this model. Since antibodies are not available for many of the GC-specific genes, novel monoclonal antibodies directed to newly identified RNA-based prognostic biomarkers need to be generated and assessed in the future IHC-based prediction models[14,15]. Furthermore, although IHC is used routinely in diagnostic laboratories, its applicability for outcome prediction requires standard methods for tissue fixation, antigen retrieval protocols and staining methodologies, a uniform use of the same antibodies directed to specific epitope on the target protein and application of identical pre-determined thresholds to define positivity for specific antibodies. This information, however, is currently unavailable.

Alternatively, it is possible to construct predictive models based on RNA-based gene expression profiling in formalin-fixed paraffin-embedded tissues, which are used routinely for IHC and thus are widely available. Unfortunately, the process of formalin fixation may contribute to RNA degradation and modification that limits the extractability of high-quality RNA by routine methods. Recent improvements in RNA extraction protocols have allowed the extraction of short informative RNA fragments from paraffin blocks, with potential use in RNA quantification[16]. We have recently developed an optimized methodology for RNA extraction from formalin fixed, paraffin-embedded lymphoid tissues[17]. Applicability of this new methodology in DLBCL patients is currently under investigation.

In addition, it is important to recognize that the usefulness of prognostic factors or models may depend on the specific clinical setting and therapeutic approach. Almost all of the previous

studies were performed in newly diagnosed DLBCL patients in the pre-rituximab era. Improved survival with the addition of rituximab to chemotherapy might be associated with a change in the predictive value of clinical and/or biological markers resulting in the loss of prognostic power of previously established markers or the discovery of new, previously unidentified predictors[18,19]. Therefore, the predictive value of the previously established risk factors should be re-evaluated and new factors identified for patients treated with rituximab-based chemotherapy. Further, there are no well established prognostic biomarkers that can reliably predict survival of DLBCL patients following hematopoietic cell transplantation (HCT). About 40% of primary resistant or relapsed DLBCL patients may be cured with autologous HCT but gene expression profiling studies or biomarker studies were not performed in these patients. Recently, Moskowitz *et al*[20] evaluated whether the cell of origin in repeat biopsies before autologous HCT may predict patient survival. An IHC model proposed by Hans *et al*[13] was applied to biopsies from 88 transplantation-eligible patients with relapsed or primary refractory DLBCL undergoing ifosfamide, carboplatin, etoposide (ICE) second-line chemotherapy (SLT) followed by high-dose therapy (HDT) and autologous HCT. There was no significant difference in event-free or overall survival based on the cell of origin or any of the common pathologic markers examined. Whether these findings suggest that distinct cell of origin is not associated with outcome in patients undergoing HDT and autologous HCT or simply was due to poor reproducibility of this model in newly diagnosed untreated DLBCL patients (Natkunam and Lossos-unpublished observations), is presently unknown. Further studies are needed in this patient population. Of note, the IHC-defined GC phenotype was correlated with improved survival in high-risk DLBCL patients treated with autologous HCT as first-line therapy[21].

SUMMARY

In conclusion, microarrays are powerful tools for discovery and hypothesis generation, allowing researchers to obtain an unbiased survey of gene expression in lymphoma samples. These studies allowed sub-classification of DLBCL into distinct subtypes with different pathogenesis and prognosis. Furthermore, these studies also enabled identification of new prognostic biomarkers and models in these tumors. However, the “prime-time” for their incorporation into routine clinical practice has not arrived yet. Continuous research will address the remaining hurdles to allow future routine use of prognostic biomarkers in daily Oncology practice. These advances will have significant implications for design of clinical trials and development of new therapeutic approaches.

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