Chapter 1

mRNA MICROARRAY ANALYSIS IN LYMPHOMA AND LEUKEMIA

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1. INTRODUCTION

The analysis of gene expression in lymphoma and leukemia has been previously characterized by the analysis of single genes or proteins, whether by Western or Northern blots, reverse transcriptase-polymerase chain reaction (RT-PCR), or in situ hybridization. Advances in the last decade have led to the ability to analyze the expression of thousands of known and unknown genes by the use of microarrays on filters, glass slides, or other substrate devices. These analyses have been pursued to aid in the identification of unique expression profiles of the tumors, the molecular subclassification of hematologic cancers, the discovery of new proteins to diagnose lymphoma and predict prognosis, and the cloning of new genes involved in the pathogenesis of lymphoma/leukemia.

2. CLUSTERIN: A DIAGNOSTIC PROTEIN DISCOVERED BY MICROARRAY

After using a small filter based microarray with 588 probes, Wellman demonstrated the first novel protein finding that now assists in the diagnosis of a subtype of lymphoma. Clusterin was found to be solely expressed in anaplastic large cell lymphoma cell lines, and secondly was shown by immunohistochemistry to be expressed almost exclusively in clinical cases
of anaplastic large cell lymphoma. This observation has been confirmed in two immunohistology studies on paraffin embedded tissue where the highest frequency of clusterin expression was seen in anaplastic large cell lymphoma.

3. LANDMARK STUDIES IN LEUKEMIA AND LYMPHOMA

The reports by Golub on leukemia and Alizadeh on lymphoma illustrated the potential for high density gene expression arrays to contribute to the understanding of hematologic malignancies. While two different microarray platforms were chosen in the two studies, powerful results were obtained by both methods. Golub et al demonstrated that the major subtype of acute myeloid leukemia (AML) could be distinguished from acute lymphoid leukemia (ALL) while using an oligoprobe microarray designed by Affymetrix (Figure 1-1). In Alizadeh’s study, a spotted cDNA microarray Lymphochip slide was utilized to separate diffuse large cell lymphoma into two subgroups, germinal center B-cell and activated B-cell (Figure 1-2). Since 1999, there have been a number of microarray studies analyzing subtypes of leukemias and lymphomas.

4. VARIABLES IN MICROARRAY STUDIES IN LYMPHOMA

A comparison of the different studies of microarray analysis in lymphoma will demonstrate that there are variables in the type of platform used, the number of probe targets present, the control (if any) RNA source utilized, the number of cases studied, and the type of software used to analyze the data (Table 1-1). Variables in tissue preservation, percent of tumor cells, and normalization of the data also likely exist, but will not be further detailed here as these data were variably reported. The two major platforms are cDNA microarrays, popularized by Pat Brown, and the oligoprobe microarray developed by Affymetrix. The number of targets in the published studies in lymphoma have ranged from 588 to approximately 18,000. The source of control RNA included cell lines, reactive lymph nodes, isolated germinal centers, sorted cells from tonsils, or another subtype of lymphoma. Software analysis methods are characterized into two broad categories of unsupervised and supervised clustering, including ratio ranking, hierarchal clustering, self-organized mapping and others. The