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# Gene Expression Profiling and Outcome Prediction in Non-Hodgkin Lymphoma

*Sandeep Dave*

Correspondence and reprint requests: Sandeep Dave, MD, MS, National Cancer Institute, 9000 Rockville Pike, Bethesda, MD 20892 (e-mail: daves@mail.nih.gov).

### ABSTRACT

Gene expression profiling with microarrays has provided new insights into the molecular biology of tumors can that underlie differences in responses to therapy and patient outcomes. In diffuse large B-cell lymphoma, gene expression profiling has revealed at least 2 diseases that are strikingly different in their response to chemotherapy and the inhibition of critical oncogenic pathways. In follicular lymphoma, gene expression profiling showed that the host immune response to tumors is an important determinant of outcome and can strongly predict survival at the time of diagnosis. The application of immunologic therapies that modify the host immune response could have a major effect on survival in patients with follicular lymphoma. Thus, the application of gene expression profiling in non-Hodgkin lymphoma provides important prognostic information at the time of diagnosis and can be translated into therapeutic options that improve patient outcomes.
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Initially described a decade ago [1], gene expression profiling with microarrays has emerged as a rapidly adopted technique for the characterization of malignancies. Microarrays rely on the fact that although each somatic cell in the organism carries the same complement of DNA, only a fraction of the genes encoded by the DNA are transcribed into messenger RNA. The genes selected for transcription depend on the lineage of the cell and the influence of the intracellular and extracellular signaling pathways. The transcribed genes thus reflect the state of differentiation of the cell, as well as whether a cell is normal or malignant. Microarrays provide a powerful window into the underlying biology by measuring, in parallel, the thousands of expressed genes that constitute the gene expression profile of the normal or malignant cells being studied [2].

Microarrays have provided a new means of understanding the observed variability in clinical outcomes for patients with the same diagnosis. For instance, in diffuse large B-cell lymphoma (DLBCL), gene expression profiling revealed that what was once considered a single disease is in fact at least 2 morphologically indistinguishable diseases with strikingly different clinical outcomes [3]. When treated with anthracycline-containing chemotherapy, patients with germinal center B cell–like DLBCL have an expected 5-year survival of approximately 60%. This is in contrast to the activated B cell–like DLBCL subgroup, which carries a dismal prognosis, with an expected 5-year survival of <30%. The gene expression patterns that underlie the differences in the subtypes of DLBCL can be quite instructive in their ability to identify pathways that provide therapeutic targets in a disease. For example, activated B cell–like DLBCL expresses nuclear factor-κB constitutively and is dependent on its activity for survival. Hence, selective inhibitors of this pathway are toxic in activated B cell–like DLBCL [4] but not germinal center B cell–like DLBCL. Such inhibitors are being explored in clinical trials. The clinical application of microarrays might help to risk-stratify such patients for appropriate treatment while providing valuable prognostic information.

In a recent study of follicular lymphoma [5], gene expression profiling was used to better understand the biological underpinnings of long-term survival. In that study, tumor biopsy samples were obtained at the time of diagnosis from 191 patients, and their profile for gene expression was analyzed. Hierarchical clustering was applied separately to genes associated with longer survival (good prognosis) and shorter survival (poor prognosis). Clusters of genes with highly correlated expression were identified as gene expression signatures associated with survival (Figure 1). Within each survival-associated gene expression signature, the expression levels of the component genes were averaged to create a signature average. These signature averages were combined into multivariate models and tested for their association with overall survival. By using this technique, an optimal model of survival in follicular lymphoma was created by using 2 gene expression signatures. The signatures were called im-
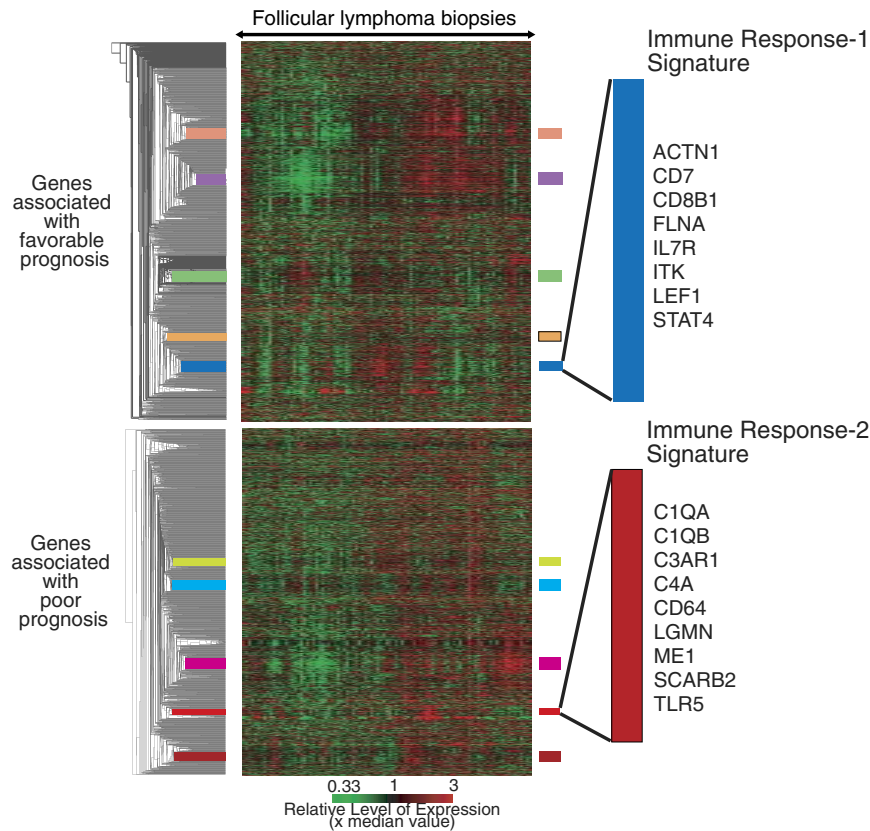
**Figure 1.** Hierarchical clustering identifies survival-associated gene expression signatures in biopsy samples from patients with follicular lymphoma. Two gene expression signatures associated with the host immune response (shown) were combined into an optimal model that predicts survival in follicular lymphoma.

mune response 1 and immune response 2 because their component genes included those known to be differentially expressed in normal immune cells. Although the immune response 1 and immune response 2 gene expression signatures were relatively weak predictors of survival as univariate variables, there was a strong statistical synergy between the 2 variables. Thus, the model comprising these 2 signatures was the most significant such survival model, and no other gene expression signatures contributed significantly to the predictive ability of this 2-variable model. The association of the immune response signatures with survival is summarized in Table 1. The model generated a survival predictor score for each patient, and a higher survival predictor score was associated with poorer survival. When the patients were ranked according to their survival predictor score and divided into 4 equal quartiles, they were found to have dramati-

cally different median survival times, ranging from 3.9 to 13.6 years (Figure 2).

Notably, both the immune response 1 and immune response 2 signatures were highly differentially expressed in the CD19-negative, nonmalignant fraction of tumor biopsy samples. Both these gene expression signatures were also found to be highly expressed in T cells and monocytes compared with germinal center B cells, which represent the cell of origin in follicular lymphoma.

The immune response 1 signature is composed of several genes that are believed to be restricted to T cells, including genes such as *CD7, CD8B, ITK,* and *LEF1*. However, it is noteworthy that the signature is not simply a reflection of the cells present in the tumor biopsy specimen. Several other genes known to be restricted to T cells were not associated with survival; this suggests that the genes in the immune response 1 signature might reflect a particular T-cell subpopulation

**Table 1.** *Predictive Power of Immune Response Signatures in Follicular Lymphoma*

| Gene Expression Signature | Contribution of Signature to Model (*P* Value) | Relative Risk of Death (95% Confidence Interval) | Effect on Survival of Increased Expression |
|---|---|---|---|
| Immune response 1 | <.001 | 0.15 (0.050-0.46) | Favorable |
| Immune response 2 | <.001 | 9.35 (3.02-28.9) | Poor |

A

Follicular Lymphoma Biopsy Samples

Immune Response 1
Signature Average

Immune Response 2
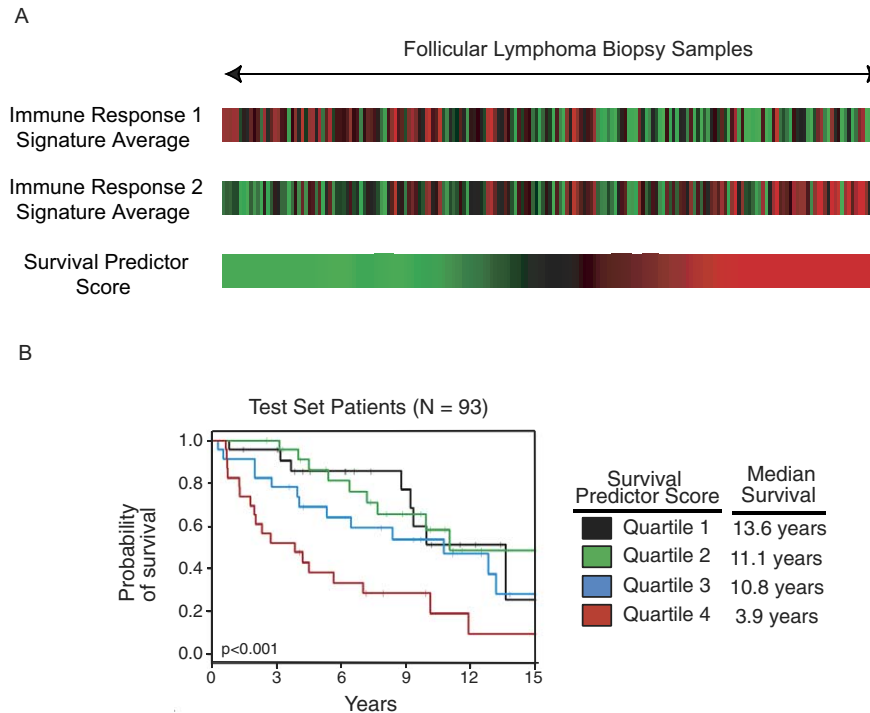Signature Average

Survival Predictor
Score

B



**Figure 2.** A, Association of immune response survival signature averages with the survival predictor score. A higher survival predictor score is associated with poorer survival. B, Results of applying the gene expression predictor to an independent set of patients with follicular lymphoma. The survival predictor score was divided into 4 equal quartiles and plotted as a Kaplan-Meier curve with distinct median survival.

or activation state. Conversely, the immune response 2 signature consists of genes known to be overexpressed in macrophages and dendritic cells, such as *CD64*, *TLR5*, and several components of complement.

The strong statistical synergy between the 2 immune response signatures likely reflects the relative abundance of tumor-infiltrating nonmalignant immune cell populations that interact strongly with the malignant cells in the tumor. These findings provide new insight into the mechanisms that underlie disease progression.

There are 2 major implications of these findings. First, the molecular predictor of survival can be used to risk-stratify patients at the time of diagnosis. Patients who are predicted to have low-risk disease may benefit the most from a watchful waiting strategy. However, patients identified as having a poor prognosis could be enrolled in clinical trials to modify the expected aggressive disease course. Second, it seems that the tumor is strongly dependent on signals from nonmalignant immune cells in its microenvironment. It is not currently clear whether this relationship is predominantly a property of the tumor cells, the microenvironment, or both. However, it is likely that altering the microenvironment will shift the equilibrium of tumor-host interactions. Thus, the microenvironment can be viewed a therapeutic target in follicular lymphoma, and this suggests promise for immunologic interventions.

Allogeneic transplantation has been used with some success in patients with relapsed follicular lymphoma [6]. Findings from gene expression profiling suggest a role for allogeneic transplantation in follicular lymphoma, especially in the multiply relapsed patient. Thus, gene expression profiling provides new opportunities for understanding the molecular underpinnings of non-Hodgkin lymphoma and for identifying new therapeutic options to improve patient outcomes.

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