Transcription Factors Come of Age in Lymphoma Immunophenotyping

A (T-)bet That Has Paid Off

Adam Goldfarb, MD

DOI: 10.1309/6UYK3P1JBAN5FLCA

In this issue of the *Journal*, Dorfman and colleagues describe the usefulness of T-bet as a marker for specific subtypes of B-cell lymphoma. T-bet originally was identified as a T-box family transcription factor that dominantly programs CD4 and CD8 T-cell development along Th1 and Tc1 pathways, respectively. It accomplished this impressive task in part through the coordinated transcriptional activation of the IFN-γ (interferon-γ) gene and repression of the IL-4 (interleukin-4) gene. The clinical importance of T-bet emerged in studies of T-bet knockout mice, which showed airway abnormalities remarkably similar to those in human asthma; furthermore, airway samples from human patients with chronic asthma showed marked reduction of T-bet expression within CD4+ T cells. By contrast, overexpression of T-bet occurred in lamina propria T cells of colonic biopsy specimens from patients with Crohn disease, a causal role for which was supported by a murine model system of enforced T-bet expression in CD4+ T cells. Thus either overexpression or underexpression of T-bet in T cells might contribute to the development of certain types of human inflammatory or autoimmune disease, presumably through the dysregulation of the balance of Th1 and Th2 cells.

Initial studies of human tumors logically focused on T-cell lymphomas, in which T-bet expression occurred in a high percentage of angioimmunoblastic, lymphoepithelioid, and Th1 T cell–like lymphomas. Cases with no or low-incidence expression included T-cell precursor (0%), anaplastic large cell (25%), and Th2 T cell–like (18%) lymphomas. Strikingly little T-bet expression manifested in reactive tonsil or normal adult thymus. These results corroborated recent subtyping of peripheral T-cell lymphomas (PTCLs) by a panel of surface markers that assigned angioimmunoblastic lymphoma to Th1-like and anaplastic large cell lymphoma to Th2-like categories. Important questions for the future include how well T-bet expression can predict a Th1-like phenotype in PTCL and the prognostic relevance of T-bet expression as well as Th category. More basic questions include whether T-bet expression simply reflects the preexisting phenotype of the T cell destined for malignancy or whether T-bet expression itself might contribute in some way to the pathogenesis of cases of PTCL.

In normal B cells, previous studies had shown minimal baseline expression but strong up-regulation in response to certain activating stimuli, eg, those that promote IFN-γ production and CpG-containing DNA. Again, the T-bet knockout mice shed light on function, showing a role in initiating immunoglobulin heavy chain (IgH) gene class switching, preferentially to IgG2A. This function is fascinating in the light of the absence of detectable T-bet expression in normal germinal centers, the usual site of class switching. Another consequence of loss of T-bet function in these studies was reduction in autoantibody production in the murine lupus model. This intriguing finding suggests that T-bet might promote development or activation of B-1 B cells and anticipates the findings in the present study in which T-bet was expressed frequently in cases of chronic lymphocytic leukemia (CLL), a malignancy of the human counterpart of the B-1 B cell (the CD5 B cell) and characterized by a propensity for autoantibody production.

The present analysis of T-bet expression in a variety of human B-cell malignant neoplasms yielded novel and potentially useful results. T-bet expression occurred in all B-cell precursor malignant neoplasms (BCP-ALL), in stark contrast with the absence of T-bet expression in all Burkitt
lymphomas and in all T-cell precursor malignant neoplasms. T-bet expression manifested in a high percentage of CLL cases (72%) and in no mantle cell lymphomas. A high percentage of marginal zone lymphomas (MZL) (83%) but no follicular lymphomas showed T-bet expression. Thus, in practical terms, T-bet might offer an additional tool in the separation of certain cases with morphologic and/or immunophenotypic overlap: BCP-ALL vs Burkitt lymphoma and T-cell precursor malignant neoplasm; CLL vs mantle cell lymphoma; and MZL vs follicular lymphoma.

These results with human B-cell malignant neoplasms raise many clinical and biologic questions. Can T-bet expression be used as yet another marker to stratify risk in CLL patients? Does any correlation exist with mutational status of IgH variable regions, CD38 expression, etc? Can T-bet expression be applied to split MZL cases into clinically meaningful subclasses? On a more basic level, does T-bet expression tell us anything about the specific cell of origin in B-cell malignancy, for example, do normal T-bet+ B cells represent a distinct lineage of B-1 B cells that mature outside the germinal center? Alternatively, does T-bet have any role in lymphomagenesis, for example, in the BCP-ALL developmental blockade?

T-bet can be added to a growing list of transcription factors that have proven valuable as markers in diagnostic subtyping and biologic characterization of human lymphomas. Traditional immunophenotyping of lymphomas depended largely on profiling of cell surface antigens; one important exception involved the use of the nuclear enzyme terminal deoxynucleotidyl transferase as a marker of lymphoid immaturity. However, basic discoveries in mechanisms of B-cell development have turned the spotlight on transcription factors as programmers and markers of specific lineages and stages. Transcription factors critical in the earliest stages of B-lineage commitment include BSAP/PAX-5, a member of the paired box family, and PU.1, a member of the Ets family. Factors involved in later, germinal center–dependent stages of B-cell maturation include bcl-6, a zinc finger factor, and Oct-2, a POU homeodomain factor. The last phases of B-cell maturation, from post–germinal center to plasma cell, are marked by the expression of MUM1/IRF-4, a member of the IRF family of transcription factors. Interestingly, the genes encoding 3 of these factors (BSAP/PAX-5, bcl-6, and MUM1/IRF-4) also undergo chromosomal translocations in specific subsets of B-lineage malignant neoplasms.

One established application of transcription factor profiling is in the analysis of Hodgkin lymphomas. Evidence for the B-cell derivation of classic Hodgkin lymphoma (cHL) originally came from the analysis of IgH configurations in microdissected Reed-Sternberg cells, with clonal rearrangements found in a majority of cases. Despite compelling genomic evidence for a B-cell origin, Reed-Sternberg cells from cHL rarely express B-cell surface antigens such as CD20 and surface immunoglobulin. By contrast, the lymphocyte predominance type of Hodgkin disease frequently displays clonal IgH rearrangements and B-cell antigen expression within the neoplastic cells. Transcription factor profiling has provided a satisfying explanation for this discrepancy: the neoplastic cells of cHL express BSAP/PAX-5, permitting B-lineage commitment, but lack PU.1 and Oct-2, thus precluding normal B-cell maturation and preventing expression of B-cell surface antigens. By contrast, the neoplastic cells of lymphocyte predominance Hodgkin disease have the full complement of transcription factors (BSAP/PAX-5, PU.1, and Oct-2), permitting expression of B-cell surface antigens. By translating these findings into clinical practice, one can incorporate transcription factors as part of a standard marker panel for the immunohistochemical separation of cHL, nodular lymphocyte predominance Hodgkin disease, and anaplastic large cell lymphoma, the last of which lacks all B-cell transcription factors.

Transcription factor profiling also might assist in the subclassification of diffuse large B-cell lymphoma (DLBCL). Original expression profiling of DLBCL cases using complementary DNA (cDNA) microarrays led to the creation of 3 subclasses: germinal center B-cell (GCB), activated B-cell, and miscellaneous others. Of prognostic importance, the GCB subclass showed significantly superior survival compared with the other 2 non-GCB subclasses. Owing to the current impracticality of cDNA microarray analysis for routine diagnostic use, a subsequent approach used immunohistochemical analysis on a refined set of markers, CD10, bcl-6, and MUM1/IRF-4. If cases expressed the CD10 surface antigen, they immediately were subclassified as GCB. If cases were CD10–, as in the majority of DLBCL cases, the expression of the transcription factors bcl-6 and MUM1/IRF-4 determined subclassification: bcl-6+ MUM1/IRF-4– cases were GCB and all other combinations, non-GCB. This simple algorithm permitted separation of GCB and non-GCB cases with prognostic accuracy equivalent to that obtained with cDNA microarray analysis.

Because transcription factors often preside at the top of developmental regulatory hierarchies, ie, a single transcription factor can regulate the expression of numerous target genes, one might be able to capture comprehensive phenotypic information about a given cell through analysis of a limited number of transcription factors. Thus, immunohistochemical profiling for 3 or 4 well-chosen transcription factors conceivably could provide information equivalent to that obtained in comprehensive microarrays. Furthermore, transcription factors provide certain types of lineage information, about developmental potential or thwarted maturation, that
might not be accessible solely through surface and cytoplasmic marker analysis. With improvements in antibodies and detection systems, the perennial problems of low antigen abundance and poor stability no longer limit detection of many transcription factors in appropriately handled specimens. Further exciting technical advances include development of antibodies that might discriminate phosphorylation status of some transcription factors, providing information not only on expression but also on activation states. Thus, much work remains to be done in characterizing lymphomas by their transcription factor profiles. The data presented herein on T-bet expression in B-cell lymphomas foreshadow the tremendous rewards to be gained from taking such an approach.

From the Department of Pathology, University of Virginia School of Medicine, Charlottesville.

References


