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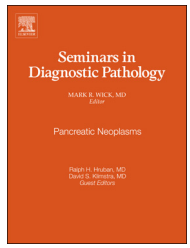

Immunohistochemical markers in lymphoid malignancies: Protein correlates of molecular alterations

*Caleb Ho, MD, Scott J. Rodig, MD, PhD**

Department of Pathology, Brigham and Women 's Hospital, Boston, Massachusetts

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ABSTRACT

Histomorphology, immunohistochemistry (IHC), and genetics are essential tools for the evaluation and classification of lymphoid malignancies. Advances in diagnostic techniques include the development of immunohistochemical assays that can serve as surrogates for genetic tests. We review the performance of a select subset of assays that detect the aberrant expression of onco-proteins secondary to chromosomal translocations (*MYC; BCL2*), somatic mutations (*BRAF* V600E; *NOTCH1*), and gene copy number gains (*CD274* (encoding PD-L1); *PDCD1LG2* (encoding PD-L2)) in fixed tissue biopsy sections. We discuss the limitations of IHC, but also its primary advantage over genetics; specifically, its ability to assess the final, common phenotypic consequences of a multitude of genetic and non-genetic events that influence protein expression. The information provided by IHC and genetic testing are thus intimately related; surgical pathologists will increasingly need to interpret and integrate the results of both to provide a comprehensive assessment of tumor biology and guide therapy.

Introduction

Our understanding of the classification and underlying pathogenesis of lymphomas has improved tremendously in the past few decades, as recurrent genetic alterations have been described in a large number of lymphoproliferative disorders. In some cases, these alterations are known to be oncogenic drivers, while others have unclear functions. The cumulative effect is to promote tumorigenesis through the aberrant expression and function of proteins that affect critical intracellular signaling pathways, growth factors or their receptors, and the cellular microenvironment. Genetic testing is increasingly being used in conjunction with immunohistochemistry (IHC) to facilitate tumor classification in routine diagnostics. Common tests include tumor cell karyotype to establish the identity, number, and structural rearrangements of chromosomes by morphology, fluorescence *in situ* hybridization (FISH) of labeled DNA probes to tumor cell nuclei to screen for specific chromosomal translocations or genetic copy gains or loss, and next-generation sequencing of nucleic acids from unfractionated tumors to survey for hundreds or thousands of somatic mutations. Genetic testing is appealing because the results are easily quantifiable and come from an analyte (DNA) that is extremely stable.

IHC uses antibodies to detect the expression of specific proteins in tissue sections and, in contrast to genetic testing, the analytes are labile. The results of IHC can be seriously affected by pre-analytical variables, such as the chemicals

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* *Correspondence to*: Department of Pathology, Brigham and Women 's Hospital, 75 Francis St, Boston, Massachusetts 02115.
E-mail address: srodig@partners.org (S.J. Rodig).

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used for tissue fixation, the duration of tissue fixation, and the protocols used for tissue processing. In addition, the results of IHC are reliant upon the performance of specific antibodies on specific tissue types. Given these factors, optimal staining protocols must be determined empirically for every individual test. In addition, substantial experience in recognizing the staining patterns resulting from any of the hundreds of tests available to diagnostic pathologists is critical for accurate interpretations.

Despite these limitations, IHC remains an essential tool in diagnostic laboratories for several reasons. First, pathology laboratories have had decades of experience with IHC, which can be performed routinely, economically, and quickly. Second, IHC captures essential phenotypic information that cannot be determined by genetic analysis alone, such as a tumor 's cell of origin. Third, IHC preserves the histomorphological features of a tumor, which facilitates the interpretation of the results. Fourth, IHC can detect disrupted protein expression due to a variety of genetic, epigenetic, translational, post-translational, and microenvironmental aberrations rather than focusing on specific genetic changes.

This article will focus on a few examples of how recent scientific discoveries have improved our understanding of IHC findings in correlation with the underlying genetic lesions in various lymphoid neoplasms, and hence our abilities to utilize IHC results in detecting or screening for these lesions.

MYC

The *MYC* (*c-MYC*) gene on chromosome 8 at band q24 codes for a multifunctional nuclear protein that acts as a transcriptional activator or repressor, which plays a critical role in cell growth, cell cycle progression, apoptosis, angiogenesis, and cellular transformation.[1] A balanced translocation involving the *MYC* gene is the hallmark feature of Burkitt lymphoma (BL), the first lymphoma in which a recurrent chromosomal abnormality was described.[2,3] Most commonly, the translocation juxtaposes *MYC* with the immunoglobulin heavy chain gene (*IGH*) enhancer on chromosome 14q32 (approximately 80% of cases) or, less frequently, the immunoglobulin kappa gene (*IGK*) on chromosome 2p12 or the lambda light chain gene (*IGL*) on chromosome 22q11.[4] *MYC* translocations involving non-immunoglobulin genes, such as *PAX5* (chromosome 9p13) and *BCL6* (chromosome 3q27), have also been described.[5–7] Each of these translocations leads to constitutive expression of the MYC transcript and protein in the malignant cells.

Traditionally, BL is diagnosed through recognition of stereotypical histomorphological features, coexpression of antigens associated with mature, germinal center B-cells, and genetic studies demonstrating a chromosomal rearrangement involving 8q24. The detection of the MYC oncoprotein in tumor cells by IHC would seem an obvious way to confirm a diagnosis of BL in the absence of genetic testing. Yet, early studies on MYC IHC yielded conflicting results, possibly due to the lack of antibodies amenable to detecting the protein in fixed tissues.[8,9] More recently, however, it was found that a novel monoclonal rabbit anti-human MYC antibody (Y69 clone, Epitomics Inc.) was useful for detecting MYC in formalin-fixed, paraffin-embedded (FFPE) tissue sections.[10] In an initial study of MYC IHC using BL cases, most (15/17 cases) with confirmed *MYC* translocations, it was found that the tumor cells comprising the majority of the cases (88%) demonstrated intense nuclear staining for the oncoprotein, while the remaining cases showed equal intensity of nuclear and cytoplasmic staining. In contrast, almost all cases (18/19 cases; 95%) of diffuse large B-cell lymphoma (DLBCL) without *MYC* rearrangement (*MYC*–DLBCL) lacked nuclear staining of the tumor cells for MYC.[10] This initial study also included a small number of DLBCL with *MYC* rearrangement (*MYC*+DLBCL) and B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (BCL-U). MYC IHC showed intense nuclear staining for the oncoprotein in these cases as well. Overall, nuclear staining for MYC was found to be 96% sensitive and 90% specific for *MYC* rearrangement, with high positive and negative predictive values (0.92 and 0.95, respectively) and a high inter-observer concordance in classifying the MYC staining pattern between two pathologists (kappa statistic = 0.90). The results suggested that IHC effectively captures the dysregulated expression of the MYC oncoprotein in BL, the prototypical tumor harboring a *MYC* translocation.

MYC translocations occur in additional lymphoid malignancies, including plasmablastic lymphoma, transformed follicular lymphoma, blastoid mantle cell lymphoma, and *de novo* DLBCL, albeit at much lower frequencies than observed for BL.[11–13] Approximately 10–15% of patients with *de novo* DLBCL are *MYC*+, and a number of studies have demonstrated that these patients have inferior 5-year progression-free survival and overall survival rates, higher rate of central nervous system relapse, as well as poor responses to CHOP or R-CHOP chemotherapy compared to patients with *de novo* *MYC*– DLBCL when treated with standard immunochemotherapy.[14–17]

MYC IHC has been tested on genetically annotated DLBCLs in several studies. It was found that high nuclear MYC staining by IHC (>50% of tumor cells) was detected not only in all *MYC*+ DLBCLs, but also in a subset of *MYC*– DLBCLs.[18] Both *MYC*+ and *MYC*– DLBCLs with high MYC protein were found to exhibit high *MYC* transcript and MYC target gene expression by gene set enrichment analysis (GSEA). Moreover, the transcriptional signature of DLBCLs with high MYC protein expression resembled that of the so-called molecular Burkitt lymphoma (mBL) reported previously.[14,19] Finally, DLBCL cases with high MYC protein expression were shown to have poorer overall survival after R-CHOP chemotherapy compared to DLBCLs with low MYC protein expression.[18] The mechanisms responsible for MYC dysregulation in *MYC*–DLBCL with high MYC protein are under active investigation but are likely multi-factorial and include *MYC* copy number gain, dysregulated micro-RNAs that govern MYC transcript abundance, and mutations in *MYC* itself.[1,18,20] Fig. 1 shows examples of two cases of DLBCL with high nuclear MYC staining by IHC. One showed *MYC* gene rearrangement as demonstrated by FISH study, while the other one did not show *MYC* gene rearrangement.

Several subsequent studies have validated the negative prognostic significance of MYC overexpression in patients with DLBCL treated with R-CHOP, especially in the context of