Best Practice & Research Clinical Haematology 23 (2010) 417–431

Contents lists available at ScienceDirect

Best Practice & Research Clinical Haematology

journal homepage: www.elsevier.com/locate/beha

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# Immunohistochemical Surrogates for Genetic Alterations of *CCDN1*, *PML*, *ALK*, and *NPM1* Genes in Lymphomas and Acute Myeloid Leukemia

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*Keywords:*
Monoclonal antibodies
CCND1
PML
ALK
Nucleophosmin
Lymphoma
Leukemia
Immunohistochemistry

The World Health Organization (WHO) classification of lympho-hemopoietic neoplasms is increasingly based on genetic criteria. Detection of tumor-associated primary genetic lesions is usually performed using the polymerase chain reaction (PCR) and/or fluorescence in-situ hybridization (FISH). This review focuses on alternative techniques for detecting genetic lesions in biopsy samples. Immunohistochemical surrogates for the detection of genetic alterations involving the *CCND1*, *PML*, anaplastic lymphoma kinase (*ALK*) and nucleophosmin (*NPM1*) genes are presented as examples for this approach. Because of their high specificity, rapidity, low costs and ease of performance, these assays have the potential for being extensively applied in developing countries. In some instances (e.g. detection of ALK protein) immunohistochemistry has fully replaced molecular studies for routine diagnosis in paraffin-embedded specimens. Genome wide based discovery of new tumor-associated genetic lesions that are suitable for antibody targeting promises to further expand the application of immunohistochemistry for the molecular classification of hematological neoplasms.



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doi:10.1016/j.beha.2010.09.001

## Introduction

The World Health Organization (WHO) classification of lympho-hemopoietic neoplasms is oriented, whenever possible, towards categorization of disease entities according to underlying genetic alterations [1]. Primary genetic lesions not only help to define distinct entities but may also serve as specific diagnostic and prognostic markers. Several highly sensitive and specific techniques have been proposed for the detection of tumor-associated molecular lesions. Among these, the polymerase chain reaction (PCR) and fluorescence in-situ hybridization (FISH) are the ones most commonly applied in the Western World. However, because of their high costs and the need of qualified personnel, they cannot be easily handled in all centers, especially in resource constrained countries, and this hinders the worldwide use of the WHO classification. Such problems could potentially be solved by developing simple, low-cost surrogate assays for molecular studies. This approach has the potential to introduce molecular diagnoses, indirectly, even in developing countries.

Here, we briefly review immunohistochemical methods that allow for the detection of primary genetic lesions in a variety of lymphomas and in acute myeloid leukemias (AML). As a paradigm for the potential of this approach, we will focus on immunohistochemical surrogates for genetic alterations involving the *CCND1*, *PML*, anaplastic lymphoma kinase (*ALK*) and nucleophosmin (*NPM1*) genes.

## Immunohistochemical detection of protein products of genes activated by chromosomal changes

The most illustrative example of this type of gene activation is the recurrent t(11;14) chromosomal translocation that affects the *CCND1* and immunoglobulin (Ig) heavy chain genes in mantle cell lymphoma (MCL) [2]. As a consequence of this translocation, the product of the *CCDN1* gene (the cyclin D1 protein), which is not detectable in normal lympho-hemopoietic tissues, is expressed at high levels at the nuclear level and therefore identifiable by immunohistochemistry in MCL [3].

### *Mantle cell lymphoma (MCL) with t(11;14)*

MCL is a relatively rare form of peripheral B-cell lymphoma that mostly affects middle-aged to older males (male:female ratio = 2–7:1) and is generally regarded as an aggressive, incurable disease with a median survival of 3–4 years [4]. It is believed that MCL derives from the transformation of peripheral B-cells of the inner mantle zone of secondary follicles, mostly of naïve pre-germinal center (GC) type, as a result of the (11;14) translocation [2].

### *CCND1 (*BCL-1*) gene*

The (11;14)(q13;q32) translocation is recognized as the primary genetic event in MCL [2]. It juxtaposes the *CCDN1* (*BCL1*) locus encoding cyclin D1 on chromosome 11 to the Ig heavy chain gene at the 14q32 locus. This translocation causes the over-expression of the cyclin D1 protein [3] that is thought to play a pivotal role in the pathogenesis of MCL by promoting neoplastic proliferation through perpetuation of G1 to S cell cycle phase transition.

Occasionally, cases of MCL that exhibited the same clinical, morphological and gene expression profile features as classical MCL but did not carry the t(11;14)(q12;q32) nor expressed cyclin D1 have been reported [5,6]. Interestingly, these cases were found to express either cyclin D2 or D3 [6], strongly suggesting that the upregulation of these cyclins may substitute for cyclin D1 in the pathogenesis of MCL.

### *Antibodies to cyclin D1 and their reactivity in normal tissues*

Difficulties in reliably detecting nuclear cyclin D1 positivity in paraffin tissues sections have troubled hematopathologists for a long time. The situation has markedly improved following the introduction of a rabbit monoclonal antibody with enhanced affinity for the cyclin D1 molecule [7–9]. The intensity of nuclear staining for cyclin D1 is quite variable in MCL and, not infrequently, only a fraction