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MOLECULAR DIAGNOSIS OF MALIGNANT LYMPHOMA: MANTLE CELL LYMPHOMA, ANAPLASTIC LARGE CELL LYMPHOMA, AND MARGINAL ZONE B-CELL LYMPHOMA OF MALT

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ABSTRACT

Malignant lymphoma is a heterogeneous category embracing three major types of lymphoid neoplasms: B cell neoplasms, T and NK cell neoplasms, and Hodgkin lymphoma. Within each type, distinct disease entities are defined based on a combination of morphology, immunophenotype, genetic features and clinical syndromes, the emphasis on which represents a new paradigm in the lymphoma classification of the World Health Organization (WHO). These lymphoma entities often have distinctive cytogenetic abnormalities, usually involving translocations that place a potential cellular oncogene under the influence of the immunoglobulin in some low-grade B-cell lymphomas. Both pathologists and oncologists are now concerned with better understanding each disease entity and its spectrum of morphology, genetic events, and clinical behaviors.

Over the last decade, significant progress has been made in the molecular characterizations of mantle cell lymphoma, anaplastic large cell lymphoma, and marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT), which have not only provided insights into the pathogenesis of lymphomas, but also valuable data that could lead to therapies based on their clinical behavior.

Key Words: Malignant lymphoma, Genetic alteration, Translocation, Prognosis, Therapy

INTRODUCTION

Malignant lymphoma classification is still evolving, including a diverse spectrum of malignancies containing many different histologic categories.[1-5] Earlier classification schemes relied strictly on morphologic or phenotypic features, which remain the most important criteria for current lymphoma categorization. However, a recurrent problem with classification by morphology alone has been the lack of adequate reproducibility. Therefore, it is thought that the recognition of precise disease entities as defined by a combination of clinical, morphologic, phenotypic and genotypic features would benefit pathologists with enhanced diagnostic accuracy and reduced subjectivity. This consensus has been embodied in both the Revised European-American Classification of Lymphoid Neoplasms (REAL)[2] and the World Health Organization (WHO) schemes.[5] They emphasize that biological approaches, such as immunohistochemistry and molecular biology,

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play a critical role in the definition of disease entities. This new emphasis is best exemplified by testing for cyclin D1 overexpression in mantle cell lymphoma (MCL), anaplastic lymphoma kinase (ALK) in anaplastic large cell lymphoma (ALCL), and API2/MALT1 chimeric transcript in extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma).

1. MANTLE CELL LYMPHOMA (MCL)

MCL is a relatively recent nosological term proposed by Banks *et al.*[6] Since its original recognition in the mid-1970s was based on morphology rather than on immunophenotype, this distinct entity has been described by various diagnostic terms, e.g., lymphocytic lymphoma of intermediate differentiation by Berard, centrocytic lymphoma by Lennert, and mantle zone lymphoma by Weisenburger.[5,6] This history reflects the process of identifying the relatively divergent histologic patterns (diffuse, nodular, and mantle zone patterns) of this entity, which may sometimes create diagnostic pitfalls even for expert pathologists. In 1992, Banks *et al.*[6] showed that these differently named lymphomas fell within the same entity which they named *mantle cell lymphoma*.

MCL is mostly characterized by a monotonous proliferation of small to medium-to-large lymphocytes with scant cytoplasm and slightly irregularly contoured nuclei (Fig. 1A). Immunophenotypically, coexpressions of CD5 and pan B-cell antigens (CD19, CD20, CD22, and CD24) are characteristic of MCL, though this feature is also observed in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). CD23, which is positive for CLL and generally negative to MCL, is a distinguishing feature, though some contradictory findings have been reported. Clinically, patients with MCL are characterized by advanced stages (III and IV) and frequent involvement of bone marrow (BM), peripheral blood, and other extranodal sites. Despite the use of combination chemotherapy for aggressive lymphoma, the median survival of patients has been only 3 to 4 years in most large-scale series, indicating MCL as one of the most incurable lymphomas.

Typical MCL contains a cytogenetic abnormality with translocation of t(11;14)(q13;q32), and, less commonly, t(11;22)(q13;q11), which involves a rearrangement of the BCL-1 locus. The putative oncogene derregulated by this alteration has subsequently been identified as cyclin D1, which belongs to the G1 cyclin family and plays a key role in cell cycle regulation during the G1/S transition by cooperating with cyclin-dependent kinases (CDKs). Further evidence suggests that cyclin D1 can function as an oncogene, the overexpression of which may promote the growth of tumor cells by way of cell cycle progression. Indeed, its overexpression has also been reported in various other human cancers, e.g., esophageal, breast, and bladder carcinomas. Among hematolymphoid malignancies, cyclin D1 overexpression resulting from translocational activation has also been recognized in a subset of B-CLL, multiple myeloma, splenic marginal zone lymphoma, and hairy cell leukemia, though the relationship between them remains to be clarified. Since the breakpoint on chromosome 11q13 covers a wide range between 15 kb and more than 400 kb distant from the cyclin D1 gene, Southern blotting or polymerase chain reaction (PCR) analysis can not detect all of t(11;14).

The expression of cyclin D1 protein can now be readily detected using the 5D4, DCS-6, or P2D11F11 monoclonal antibody.[7] Normal lymphocytes do not stain for cyclin D1, and positive staining in the nuclei of lymphoid cells correlates well with the presence of t(11;14) translocation and the overexpression of cyclin D1 mRNA (Fig. 1B). Cyclin D1 immunohistochemistry is now essential for the routine diagnosis of MCL, since patients with cyclin D1-positive MCL have had

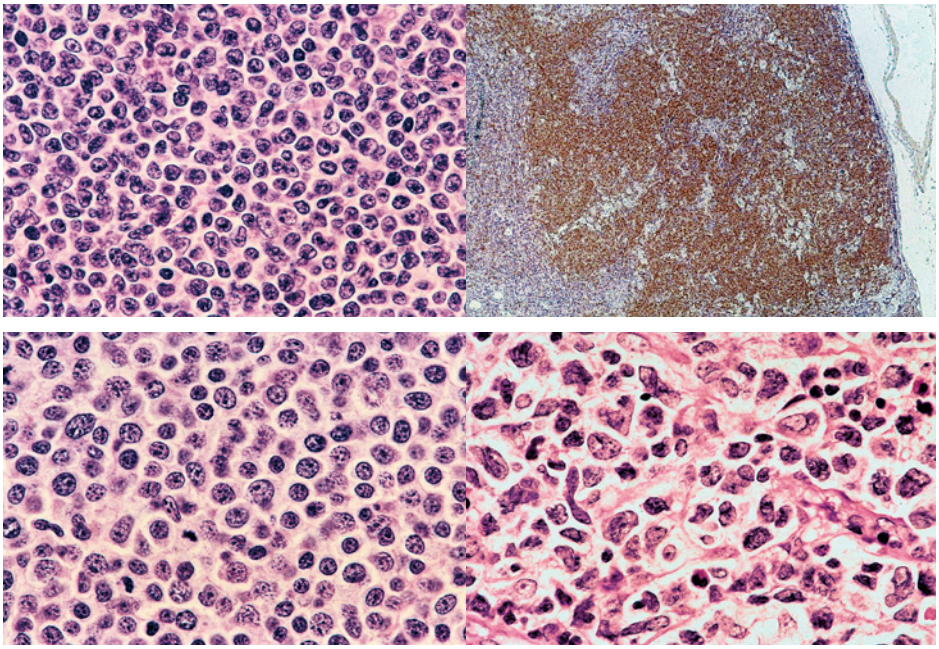

Fig. 1 A (upper left); B (upper right); C (lower left), and D (lower right).

Fig. 1 Mantle cell lymphoma. **A.** In this prototypical case, cells closely resemble centrocytes with irregular nuclei (HE ×200). **B.** Cyclin D1 immunostain shows nuclear positivity (×40). **C.** Blastoid mantle cell lymphoma with tumor cells somewhat resembling lymphoblasts (HE ×200). **D.** Pleomorphic mantle cell lymphoma clearly shows evidently pleomorphic nuclei (HE ×200).

a much poorer survival rate than those with cyclin D1-negative lymphomas of a similar morphology. Our previous studies have highlighted the importance of cyclin D1 immunohistochemistry for predicting prognoses and providing a guide to clinical management by clearly defining a homogeneous group of patients, and illuminating the clinical aggressiveness of their disease as contrasted with the indolent course of the negative group (5-year survival: 30% versus 86%, P = .0002).[8] This suggested that cyclin D1-positive and -negative groups represent different entities, and that the former closely fits the characteristics of typical classic MCL. The pathologic features of MCL have been refined in parallel, and the histologic spectrum of the disease has been expanded in recent years through the detection of cyclin D1. Immunostaining for cyclin D1 is recommended to establish a reliable diagnosis of MCL beyond the broad morphologic spectrum of this aggressive lymphoma (Fig. 1C and 1D).[9]

2. ANAPLASTIC LARGE CELL LYMPHOMA (ALCL)

ALCL was originally defined by expression of CD30 by Stein *et al.*,[10] and further originally defined by expression of CD30 (Ki-1 antigen) and a peculiar anaplastic morphology that often infiltrates the nodal sinuses and paracortical areas as sheets of lymphoma cells (Fig. 2A). CD30 is an activation-associated marker, belonging to the tumor necrosis factor (TNF)/nerve growth factor

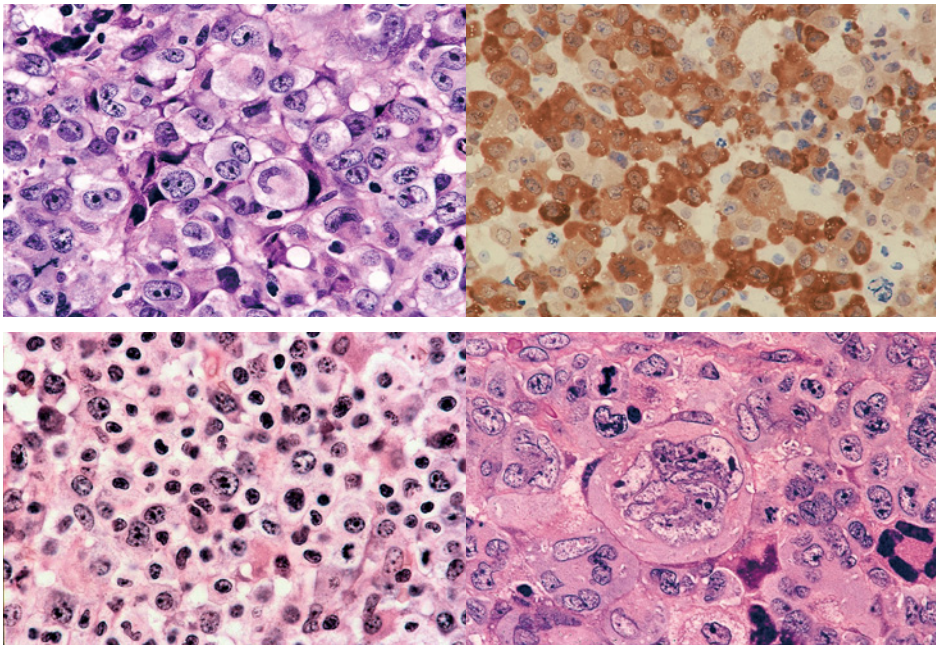

Fig. 2 A (upper left); B (upper right); C (lower left), and D (lower right).

Fig. 2 Anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma (ALCL). **A.** Common type of ALK+ ALCL showing predominant population of large cells with irregular nuclei (HE ×200). Note large cells ("hallmark" cells) with eccentric kidney-shaped nuclei (×125). **B.** Tumor cells positive for ALK in their cytoplasm and nuclei. **C.** Small cell variant of ALK+ ALCL showing predominant population of small cells with irregular nuclei and admixture of "hallmark" cells (HE ×200). **D.** Giant cell-rich variant of ALK+ ALCL consisting of pleomorphic giant cells (HE ×200).

(NGF) receptor family, which is also expressed by Reed-Sternberg cells of Hodgkin's disease and subsets of common types of B- and T-cell lymphomas. Both the B- and T-/null cell type ALCLs were initially recognized in the updated Kiel classification,[1] but only the latter has been included in the REAL[2] and WHO[5] classifications. Although some morphological variants were subsequently proposed, ALCL has been recognized as a distinct disease entity. The nonrandom chromosomal translocation, t(2;5)(p23;q35), is highly associated with ALCL, especially that of nodal origin, reported in various studies to be present in from 15 to 65% of cases. This translocation has recently been cloned and shown to result in the fusion of the *NPM* gene located on chromosome 5 with a newly described kinase gene, *ALK*, located on chromosome 2, which leads to the expression of a novel fusion protein, $p80^{NPM/ALK}$. Although the precise function of p80 fusion protein is not yet understood, it is believed to play an important role in the pathobiology of ALCLs that express it. The identification of this protein in lymphoma cells by immunohistochemistry is now available on paraffin sections, and is believed to be a reliable marker for the p80 fusion protein that results from t(2;5) translocation (Fig. 2B), since ALK expression has not been detected in cells of the hematopoietic system. The polyclonal antibody against $p80^{NPM/ALK}$, which recognizes ALK, and the subsequently established monoclonal antibodies ALK1 and ALKc, have made it possible to further categorize ALCL as an entity distinct from Hodgkin's disease, lymphomatoid papulosis, and primary cutaneous ALCL.[10] Using $p80^{NPM/ALK}$

antibody, Shiota *et al.*[11] clearly demonstrated that p80-positive ALCL is a distinct entity both clinically and pathogenetically and should be differentiated from p80-negative ALCL. Since then, accumulated evidence such as immunohistochemical, cytogenetic, and reverse genetic detection, also supports the recognition of ALK-positive ALCL as a distinct subtype with a much younger age distribution, nodal predilection, and better prognosis.[12,13] Our improved understanding of ALK-positive ALCL as a distinct molecular pathologic entity has simultaneously enabled us to document the divergent morphologic spectrum ranging from small cell (Fig. 2C) to ginat cell-rich variants (Fig. 2D). On the other hand, ALK-negative ALCL is currently less characterized, and is suggested to represent different entities, including at least the anaplastic variants of peripheral T-cell lymphoma and aggressive Hodgkin's lymphoma.

3. EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF MALT

MALT lymphoma was originally described by Isaacson,[14] and is listed as a distinct low-grade lymphoma in the WHO classification.[5] The stomach is the most common site of MALT lymphoma, constituting nearly half of all cases. Notably, most gastric MALT lymphomas are strongly associated with *Helicobacter pylori* (*H. pylori*) infection in terms of their histopathologic, epidemiological, and clinical characteristics.[15] On the other hand, recent studies including ours have shed light on the genetic mechanism of the pathogenesis of MALT lymphomas. Genetically, t(11;18)(q21;q21), which represents the most frequent structural chromosomal abnormality, is restricted to MALT lymphoma, ccurring in approximately 30% of cases.[16] This translocation was first described by Levine *et al.*,[17] and was subsequently identified in some MALT lymphoma cases. Recently, we[18] and Dierlamm *et al.*[19] demonstrated that the apoptosis inhibitor-2 (*API2*) gene at 11q21, which is involved in the anti-apoptotic signal transduction pathway, is fused in frame with a novel gene, the MALT lymphoma-associated translocation gene 1 (*MALT1*) at 18q21 in t(11;18)(q21;q21) of MALT lymphomas, resulting in API2-MALT1 chimeric products. Furthermore, we reported that gastric MALT lymphomas without regression in response to *H. pylori* eradication expressed the API2-MALT1 chimeric transcript mediated by t(11;18)(q21;q21)translocation, and that this gene aberration was thus a reliable predictive marker for responsiveness to anti-*H. pylori* treatment.[20] We subsequently documented that MALT

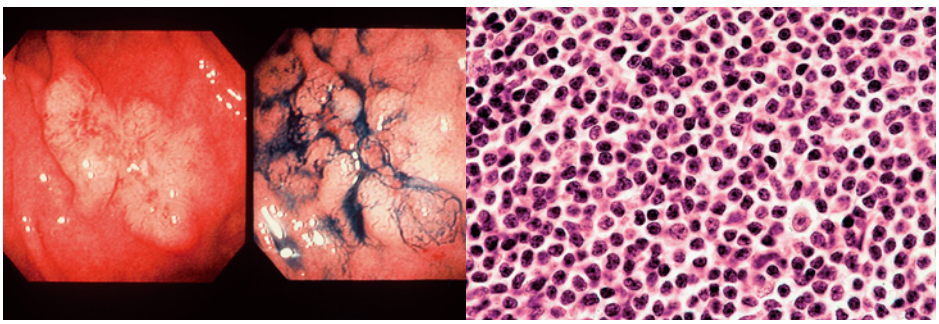

Fig. 3 A (left) and B (right).

Fig. 3 Extranodal marginal zone B-cell lymphoma of MALT with API2-MALT1 chimeric transcript. **A.** Submucosal tumor-like gross appearance on endoscopic examination. **B.** Tumor cells resembling centrocytes are designated as "centrocyte-like" cells (HE ×200).

lymphoma possessing API2-MALT1 chimeric products constitutes a distinct group with peculiar gross and histopathologic characteristics. This tumor often endoscopically features cobblestone mucosa and submucosal tumor-like gross appearances (Fig. 3A) derived from a dense lymphocytic infiltration predominantly in the submucosa, and reveals a relatively monotonous proliferation of centrocyte-like cells with infrequent lymphoepithelial lesions and often a few reactive components such as plasma cells and eosinophils compared with those of many genetically negative tumors (Fig. 3B).[21]

Recently, Streubel *et al.* reported on a novel recurrent translocation, t(14;18)(q32;q21), in MALT lymphoma involving *IGH* at 14q32 and *MALT1* at 18q21.[22] The documentation of these 2 structural aberrations, t(14;18)(q32;q21) and t(11;18)(q21;q21), indicated that *MALT1* is the common genetic denominator, possibly constituting a unifying feature of MALT lymphoma (Fig. 1). The clinicopathologic significance of t(14;18)(q32;q21) still remains to be clarified, though it was recently reported to have gone undetected in 31 cases of gastric MALT lymphoma.[23] These data suggest that the genetic alteration of *MALT1* is unrelated to *H. pylori* infection in its pathogenesis, and that MALT lymphomas characterized by the chromosomal translocation affecting MALT1 gene may constitute a distinct group.

CONCLUSION

Malignant lymphoma contains a large number of distinct disease entities associated with distinctive epidemiology, aetiology, clinical features and, often, distinctive responses to therapy. They are now defined by various principles, including their postulated normal counterpart in the immune system as well as their morphologic or clinical features. The knowledge provided by these abundant data is now essential in establishing a reliable diagnosis that will facilitate therapeutic decisions for both pathologists and oncologists.

Currently, we have both a wide range of monoclonal antibodies reactive in paraffin sections as well as molecular techniques, enabling the detection of genetic alterations from formalin-fixed paraffin-embedded materials. These developments have transformed our understanding of lymphoid neoplasia and permitted an impartial resolution of questions that remained unresolved when addressed using only H & E sections. Such new biological insights will lead to the recognition of new subtypes of malignant lymphomas, further facilitating progress in their understanding and treatment.

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