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# II. New perspectives and challenges in the understanding of mantle cell lymphoma

A. Navarro & E. Campo

*Hematopathology Section, Department of Anatomic Pathology, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain*

## introduction

Mantle cell lymphoma (MCL) is a B-cell neoplasia that has been well characterized in recent years. Recognition of the t(11;14)(q13; q32) and cyclin D1 overexpression as the genetic and molecular hallmarks of the tumor have been key elements in recognizing the broad spectrum of clinical, pathological and biological manifestations of the disease [1]. Cyclin D1 expression is now routinely used as one of the most important elements in the diagnosis of this lymphoma. The term mantle cell lymphoma reflects the idea that the normal cell counterpart of this tumor is a lymphocyte whose physiological microenvironment is the mantle zone of the secondary lymphoid follicle. This view is supported by the tendency of the tumor cells to grow and expand this area and to express a phenotype also found in a subset of mantle cells. MCL is considered clinically one of the most aggressive lymphomas with short responses to current therapies, frequent relapses and a relatively short median survival. This behavior has led to the recommendation of intense therapeutic regimens and exploration of new strategies with more recently developed drugs targeting specific pathogenetic mechanisms. In spite of the good characterization of MCL, recent molecular studies and clinical observations are opening new perspectives on the ontogeny and pathogenesis of this lymphoma that challenge some of our previous ideas about the disease. Here we consider new developing concepts in MCL that may have an impact on our understanding of its pathogenesis and may influence our clinical practice.

## MCL does not always carry the t(11;14) translocation and express cyclin D1

The t(11;14)(q13;q32) translocation and upregulation of cyclin D1, which is not expressed in normal B lymphocytes, are considered the primary pathogenetic mechanisms in the development of MCL. Cyclin D1 is a key regulatory element of the cell cycle at the $G_1$–S phase transition [1]. The relevance of cyclin D1 has been highlighted by the identification of several mechanisms used by the tumor cells to increase further the expression of cyclin D1 beyond the constitutive activation provided by the translocation. The cyclin D1 gene (*CCND1*) is transcribed in two main isoforms of 4.5 and 1.5 kb. Both mRNA transcripts contain the whole coding region of the gene and differ only in the length of their 3′ untranslated region (UTR). Some tumors have secondary chromosomal rearrangement or point mutations in the 3′ UTR of the longer isoform that truncate the sequence and generate more stable transcripts by eliminating the destabilizing AUUUA sequences and binding sites for different microRNAs [1–3]. These alterations generate higher expression of cyclin D1 and are associated with more proliferative and aggressive tumors. An additional mechanism targeting cyclin D1 is the amplification of the translocated t(11;14) allele, which results in very high levels of cyclin D1 expression [4].

Given the relevance of the t(11;14) translocation and cyclin D1 deregulation in MCL, it was very puzzling for pathologists to recognize some lymphomas with the morphological and phenotypic features of MCL that were negative for cyclin D1 expression and also lack the t(11;14) translocation. The marked difference in the clinical outcome of patients with true MCL compared with the more indolent behavior of other small B-cell lymphomas mimicking MCL made this differential diagnosis of paramount importance in the management of the patients [5]. For some time, initial difficulties in the immunohistochemical detection of cyclin D1 raised the controversy of whether those cyclin D1-negative tumors resembling MCL were real, were technical artifacts or corresponded in fact to other small B-cell lymphomas not related to this entity. This controversy was over when a gene expression profile study of a number of these putative cyclin D1-negative MCLs showed that they had a global expression signature similar to that of conventional cyclin D1-positive tumors confirming that they corresponded to the same entity [6]. This idea was further supported by the identification of a similar profile of secondary genomic alterations [7].

Intriguingly, the cyclin D1-negative MCLs had high expression of cyclin D2 or D3 suggesting that the upregulation of these $G_1$ cyclins could take over the oncogenic role of cyclin D1 in these tumors. In fact, some cyclin D1-negative MCLs carry chromosomal translocations, sometimes cryptic, fusing *CCND2* or *CCND3* to *IG* loci, particularly light chain genes. These cases have extremely high levels of expression of these cyclins suggesting that they may be useful in recognizing the tumors. However, the use of cyclin D2 and D3 in routine diagnosis is hampered by the fact that both are also expressed in other B-cell lymphomas. Their mRNA levels in the cyclin D1-negative MCLs with translocations of the genes are

significantly higher than in other tumors but the differences are difficult to discriminate using routine immunohistochemistry [8]. On the other hand, the number of cyclin D1-negative MCLs carrying *CCND2* or *CCND3* translocation is low, raising the question of the potential oncogenic mechanism involved in most MCLs lacking the translocation of all these cyclins [8].

Searching for new genes differentially expressed in MCL, Ek et al. [9] identified SOX11, a neuronal transcriptor factor of the high mobility group, as a very specific marker of MCL since it was highly expressed in virtually all these tumors whereas it was negative in normal lymphocytes and most other lymphoid neoplasms [9–11]. Interestingly, a study of the intial cyclin D1-negative MCLs identifed by gene expression profiling and an additional subset of cases showed that SOX11 was positive in all of them, indicating that it may be an excellent marker by which to recognize these tumors [10]. SOX11 is also expressed in 30%–50% of Burkitt's lymphoma at lower levels and virtually all B- and T-lymphoblastic lymphomas but not in other types of lymphoma [10, 11]. The different morphological and phenotypic features of these tumors compared with MCL should not represent a problem in the use of SOX11 to identify cyclin D1-negative MCLs in clinical practice. The availability of a reliable marker may open the possibility of studying the cyclin D1-negative MCLs and define whether these patients may also benefit from the new therapeutic strategies.

## is MCL derived from a naïve B-cell?

The t(11;14)(q13;q32) translocation occurs in the bone marrow in the early B cell at the pre-B stage of differentiation when the cell is initiating *IG* gene rearrangement with the recombination of the V(D)J segments [1]. Although the translocation occurs at a very early stage, the MCL cells are mature B lymphocytes that express genes normally detected in naïve B cells, like IgD and the T-cell-associated CD5 antigen. The phenotype and topographic distribution of these cells are similar to a small subpopulation of naïve B CD5+ cells, producing low-affinity polyreactive antibodies that colonize the normal mantle zone of the lymphoid follicles and tend to recirculate [12]. These observations and the predominance of unmutated *IG* genes observed in most MCL lead to the common understanding that the normal counterpart of this tumor is a mature naïve B-cell.

Several studies have shown that 15%–40% of MCLs carry *IGHV* somatic hypermutations and have a strong bias in the *IGHV* gene repertoire with *IGHV3-21*, *IGHV4-34*, *IGHV1-8* and *IGHV3-23* used by 46% of the cases [13]. The mutational load and the associated light chain gene also differ between the *IGHV* families. Thus *IGHV3-21*, in contrast to *IGHV3-23* and *IGHV4-59*, is almost exclusively found in germline configuration and is commonly paired with the light chain *IGLV3-19*. The fact that the mutation frequency is not random and is related to the utilization of specific *IGHV* genes suggests that at least a subgroup of MCL is not derived from naïve B cells but from cells expanding under the stimulation of certain antigens. This idea is further supported by the recognition of a bias association of certain *IGHV*, *IGHD* and *IGHJ* genes with restricted VH CDR3 motifs in 10% of tumors. This scenario is similar to the stereotyped *IG* rearrangements observed in chronic lymphocytic leukemia (CLL) [14]. However, the family usage and clusters of the rearranged *IG* genes are different in the two diseases suggesting that the potential mechanisms involved in the clonal selection may be different. On the other hand, in contrast to CLL, there is no clear evidence of a relationship with *IGHV* mutational status using a 2% cut-off [15]. All this information suggests that most MCLs may derive from antigen-experienced cells. However, the different levels of somatic mutations in the *IGHV* genes observed in MCL may indicate that different subpopulations of B cells could be considered normal counterparts of the tumor. Thus, the fact that ~15% of the tumors have a large number of somatic mutations would suggest that these tumors originate in cells that have had strong experience through the germinal center. Conversely ~30%–50% of the cases have total homology with the germline sequence of the *IGVH* genes and these cases may derive from cells without any exposure to the mutational machinery. Finally, a number of cases have a low number of somatic mutations. These cases may be related to marginal zone or early germinal center cells similar to the cells described by Kolar et al. [16] that express $IgD^{+}CD38^{-}CD23^{-}CD71^{+}$ cells, have few somatic *IGHV* hypermutations and express AID.

## indolent MCLs: do they exist?

Most patients with MCL present with generalized lymphadenopathy and disseminated disease and follow an aggressive clinical evolution. Some observations indicate, however, that some patients may have a disease with a more indolent clinical course. The recognition of these patients is important because they may benefit from more conservative management for some time without apparently harming their global outcome [17].

Studies of prognostic factors in MCL have indicated that tumors with very low proliferation fraction, limited stage or a mantle zone pattern may have a significantly better prognosis with longer survival than the global series of patients [1, 5]. In addition to these parameters, clinical observations recognized a subgroup of patients with MCL of indolent behavior that presented with non-nodal disease, splenomegaly and a leukemic phase [15, 18]. The clinical similarities between this subset of MCL and splenic marginal zone lymphomas raised some questions about the identity of these categories. In a recent study we addressed this question by comparing the gene expression profile of a group of indolent leukemic MCLs that did not require chemotherapy for >2 years with a group of conventional MCLs and other leukemic lymphoid neoplasias including CLL, splenic marginal zone lymphoma, hairy cell leukemia and leukemic follicular lymphoma. Interestingly, the indolent MCLs were molecularly more similar to conventional MCL than to any other type of lymphoid neoplasia supporting the idea that they correspond to the same molecular disease [19]. However, they also had differential expression of a small signature of genes that included among others the lack or low levels of SOX11. Other biological differences were the predominance of highly mutated IGHV and very simple karyotypes in the indolent tumors [19]. These findings suggest that MCL with a predominant non-nodal and leukemic disease, frequently associated with splenomegaly and an indolent

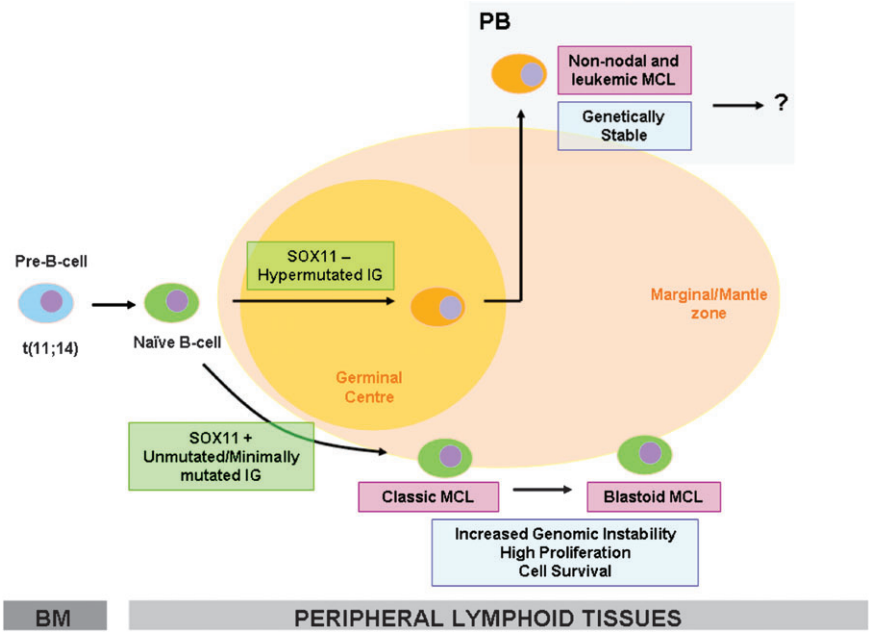


**Figure 1.** Hypothetical models of two different molecular subtypes of MCL. The naïve B-cell carrying the t(11;14) may evolve into a classic MCL in the mantle or marginal zone with no or limited somatic mutations. These tumors express SOX11 and are genetically unstable. Alternatively, some cells with the t(11;14) translocation may enter the germinal center and undergo somatic hypermutation. These cells are genetically stable and do not express SOX11.

clinical course may correspond to a different molecular subtype of MCL (Figure 1).

The possibility of demonstrating SOX11 by immunohistochemistry in routinely processed biopsies suggests that this detection may assist in recognizing these patients at diagnosis. However, one major limitation is that most patients with an indolent clinical course usually present with a non-nodal and leukemic disease and therefore with limited tissue availability to perform the studies. On the other hand, a recent immunohistochemical study showed that SOX11-negative MCLs had a worse prognosis than SOX11-positive tumors [20]. One possible explanation for this apparent discordance is that the SOX11-negative cases observed in the later study could correspond to a progressed or transformed stage with generalized lymphadenopathy of SOX11-negative tumors. Although this idea needs to be confirmed by further studies it would suggest that SOX11-negative MCLs may have an indolent phase and eventually some of the tumors could progress with a generalized lymphadenopathy and aggressive behavior.

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## references

1. Jares P, Colomer D, Campo E. Genetic and molecular pathogenesis of mantle cell lymphoma: perspectives for new targeted therapeutics. Nat Rev Cancer 2007; 7: 750–762.
2. Wiestner A, Tehrani M, Chiorazzi M et al. Point mutations and genomic deletions in CCND1 create stable truncated cyclin D1 mRNAs that are associated with increased proliferation rate and shorter survival. Blood 2007; 109: 4599–4606.
3. Jiang Q, Feng MG, Mo YY. Systematic validation of predicted microRNAs for cyclin D1. BMC Cancer 2009; 9: 194.
4. Bea S, Salaverria I, Armengol L et al. Uniparental disomies, homozygous deletions, amplifications, and target genes in mantle cell lymphoma revealed by integrative high-resolution whole-genome profiling. Blood 2009; 113: 3059–3069.
5. Tiemann M, Schrader C, Klapper W et al. Histopathology, cell proliferation indices and clinical outcome in 304 patients with mantle cell lymphoma (MCL): a clinicopathological study from the European MCL Network. Br J Haematol 2005; 131: 29–38.
6. Fu K, Weisenburger DD, Greiner TC et al. Cyclin D1-negative mantle cell lymphoma: a clinicopathologic study based on gene expression profiling. Blood 2005; 106: 4315–4321.
7. Salaverria I, Zettl A, Bea S et al. Specific secondary genetic alterations in mantle cell lymphoma provide prognostic information independent of the gene expression-based proliferation signature. J Clin Oncol 2007; 25: 1216–1222.
8. Quintanilla-Martinez L, Slotta-Huspenina J, Koch I et al. Differential diagnosis of cyclin D2+ mantle cell lymphoma based on fluorescence in situ hybridization and quantitative real-time-PCR. Haematologica 2009; 94: 1595–1598.
9. Ek S, Dictor M, Jerkeman M et al. Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma. Blood 2008; 111: 800–805.
10. Mozos A, Royo C, Hartmann E et al. SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1-negative subtype. Haematologica 2009; 94: 1555–1562.
11. Dictor M, Ek S, Sundberg M et al. Strong lymphoid nuclear expression of SOX11 transcription factor defines lymphoblastic neoplasms, mantle cell lymphoma and Burkitt's lymphoma. Haematologica 2009; 94: 1563–1568.
12. Dono M, Cerruti G, Zupo S. The CD5+ B-cell. Int J Biochem Cell Biol 2004; 36: 2105–2111.
13. Hadzidimitriou A, Agathagelidis A, Murray F et al. Sequence-based evidence for antigen selection in mantle cell lymphoma: remarkable immunoglobulin gene repertoire biases, stereotyped antigen-binding sites and recurrent hypermutations in certain subsets. 2009 ASH Annual Meeting Abstracts. Blood 2009; 114 (22): Abstract 1933.

14. Murray F, Darzentas N, Hadzidimitriou A et al. Stereotyped patterns of somatic hypermutation in subsets of patients with chronic lymphocytic leukemia: implications for the role of antigen selection in leukemogenesis. Blood 2008; 111: 1524–1533.
15. Orchard J, Garand R, Davis Z et al. A subset of t(11; 14) lymphoma with mantle cell features displays mutated IgVH genes and includes patients with good prognosis, nonnodal disease. Blood 2003; 101: 4975–4981.
16. Kolar GR, Mehta D, Pelayo R et al. A novel human B cell subpopulation representing the initial germinal center population to express AID. Blood 2007; 109: 2545–2552.
17. Martin P, Chadburn A, Christos P et al. Outcome of deferred initial therapy in mantle-cell lymphoma. J Clin Oncol 2009; 27: 1209–1213.
18. Angelopoulou MK, Siakantariz MP, Vassilakopoulos TP et al. The splenic form of mantle cell lymphoma. Eur J Haematol 2002; 68: 12–21.
19. Fernandez V, Salamero O, Espinet B et al. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res 2010; 70: 1408–1418.
20. Wang X, Asplund AC, Porwit A et al. The subcellular Sox11 distribution pattern identifies subsets of mantle cell lymphoma: correlation to overall survival. Br J Haematol 2008; 143: 248–252.