Molecular pathogenesis of mantle cell lymphoma

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Mantle cell lymphoma is a B cell malignancy in which constitutive dysregulation of cyclin D1 and the cell cycle, disruption of DNA damage response pathways, and activation of cell survival mechanisms contribute to oncogenesis. A small number of tumors lack cyclin D1 overexpression, suggesting that its dysregulation is always not required for tumor initiation. Some cases have hypermutated *IGHV* and stable karyotypes, a predominant nonnodal disease, and an indolent clinical evolution, which suggests that they may correspond to distinct subtypes of the disease. In this review, we discuss the molecular pathways that contribute to pathogenesis, and how improved understanding of these molecular mechanisms offers new perspectives for the treatment of patients.

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Introduction
Mantle cell lymphoma (MCL) is a B cell malignancy with a broad spectrum of clinical, pathological, and biological features. The identification of the translocation event t(11;14)(q13;q32) and the resulting cyclin D1 overexpression were of paramount importance in recognizing the clinical and biological diversity of this tumor. In addition to this constitutive dysregulation of the cell cycle, other mechanisms such as DNA damage response alterations and activation of cell survival pathways are integrated to drive MCL pathogenesis (1, 2). New observations are expanding our views on the ontogeny and pathogenesis of this lymphoma. Furthermore, these new insights into MCL oncogenesis are promoting the development of new therapeutic strategies, intended to target the molecular mechanism of the disease, and opening up new clinical perspectives for optimal diagnosis and management of the patients.

Initial oncogenic steps
The t(11;14)(q13;q32) translocation that juxtaposes the proto-oncogene CCND1 at 11q13 to the immunoglobulin heavy chain complex (IGH) at chromosome 14q32 is considered the primary oncogenic mechanism in the development of MCL. This translocation forces the constitutive overexpression of cyclin D1, which is not detected in normal B lymphocytes, and deregulates the cell cycle at the G1/S phase transition (1). In addition to the primary translocation event, additional mechanisms that further increase cyclin D1 expression are frequently observed in MCL, underscoring its central importance in MCL lymphomagenesis. These mechanisms include secondary chromosomal rearrangement at 3′ of the CCND1 locus or mutations in the 3′ untranslated region (3′UTR) that lead to the expression of truncated cyclin D1 transcripts missing part of the 3′UTR (3, 4). These shorter transcripts, depleted of the destabilizing AU-rich elements and the binding sites for different microRNAs, have an extended half-life resulting in higher cyclin D1 protein levels and increased tumor aggressiveness (4, 5). Alternatively, increased overexpression of cyclin D1 can occur in MCL following the amplification of the translocated t(11;14) allele (6).

Cell(s) of origin and ontogeny
The initial translocation event t(11;14)(q13;q32) occurs at the pre-B stage of differentiation during the recombination of the V(D)J segments of the IGH variable region (IGHV) in the bone marrow. However, the tumor is composed of a specific population of mature B lymphocytes, indicating that the full neoplastic phenotype is acquired at later stages of the B cell differentiation process (7). Historically, naive B cells have been considered the normal counterpart to MCL tumor cells based on IgM/IgD and CD5 expression by the tumor cells, their topographic distribution in the mantle zones, and early descriptions of the predominant use of unmutated IGHV. More recently, comprehensive analysis of B cell receptor (BCR) diversity in MCL has shifted this view to a more complex ontogenetic model, in which antigen selection plays an important role in pathogenesis, at least for a subset of tumors. Recent studies have shown that 15%–40% of MCLs carry IGHV hypermutations, with a strong bias in the IGHV gene repertoire (8–10). As is the case for chronic lymphocytic leukemia (CLL), stereotyped heavy complementarity-determining region 3 (VH CDR3) sequences have been recognized in 10% of MCL. Although the stereotyped subsets are clearly distinct from those described in CLL, their existence suggests a strong role of antigen-driven selection in the clonogenic expansion of MCL tumor cells. These findings open a complex scenario with more than one possible cell subtype dominating in different subtypes of MCL (Figure 1). In the absence of IGHV mutations, MCL may still derive from naive B cells, but cases with stereotyped BCR are likely antigen selected. Furthermore, MCL carrying a high mutational load may originate from cells strongly influenced by the germinal center microenvironment. Finally, the progenitor cells of cases with a low number of somatic mutations may derive from cells of the marginal zone, intermediate cells between naive and germinal center cells already expressing AID or transitional B cells resembling murine B-1 B cells (11, 12).

Genetically deregulated oncogenic pathways
The role of cyclin D1 in promoting MCL lymphomagenesis is related to its function in the cell cycle regulating the cyclin-dependent kinases CDK4 and CDK6. Cyclin D1 binding to CDK4/6 activates the transcription factor EZF2 by phosphorylating its inhibitor, retinoblastoma 1 (RB1), and further promotes cyclin E/CDK2 activation to trigger entry into the S phase of the cell cycle (1). Cyclin D1 may have additional oncogenic effects beyond its role in the cell cycle. Studies in solid tumor models have shown cyclin D1 in transcription regulation as interacting with transcription factors, chromatin-remodeling, and histone-modifying enzymes (13–15). Cyclin D1 may also promote chromosome instability...
by binding to genes that regulate chromosome segregation and chromatin reorganization (16). Intriguingly, cyclin D1 has been implicated in promoting DNA repair by binding to RAG1 and homologous DNA recombination (17). How this DNA-repairing function reconciles with the cyclin D1 oncogenic role is not clear, but it may help the cells to survive under DNA-damaging oncogenic stress or the action of genotoxic drugs (18). These non–cell cycle–related functions of cyclin D1 have not been properly investigated in MCL. However, the complete inactivation of the RB1 by mutations and deletions in some MCLs (19), making cyclin D1 dispensable for cell cycle functions, would support the idea that cyclin D1 may play additional oncogenic roles in these tumors. Interestingly, the recent description of cyclin D1 as promoting cell survival in MCL by sequestering the proapoptotic BAX protein reinforces this concept (20).

Several observations suggest that cyclin D1 dysregulation is not sufficient for cell transformation, nor does it explain the aggressive behavior of MCL. Secondary chromosome alterations that target genes involved in molecular pathways, such as cell cycle control, DNA damage response, and cell survival pathways, are frequently found in aggressive MCL. The INK4a/CDK4/RB1 and ARF/MDM2/p53 cell cycle pathways are very frequently targeted by secondary genetic alterations in MCL. Both pathways are connected through the CDKN2A locus (9p21), which encodes for both the CDK inhibitor INK4a and the positive p53 regulator ARF, and this locus is frequently deleted in MCL. Other key elements of these pathways, such as TP53 and RB1, are also frequently inactivated by point mutations or gene deletions (19, 21). In addition, gene amplification events have been found to deregulate additional genes, including CDK4, polycomb ring finger gene BMI1, and MDM2 (19, 22). Accordingly, the proliferation gene expression signature is the best predictor of patient survival, underscoring the importance of cell cycle dysregulation in dictating the behavior of MCL (23).

Figure 1
Hypothetical models of two different molecular subtypes of MCL. The naïve B cell carrying the t(11;14) colonizes the mantle zone of the lymphoid follicle and generates an in situ MCL lesion. Most MCLs evolve from these cells or cells in the marginal zone with no or limited IGHV somatic mutations. These tumors express SOX11, are genetically unstable, and tend to accumulate alterations in genes dysregulating cell cycle, DNA damage response pathways, and cell survival mechanisms. Alternatively, some cells with the t(11;14) may enter the germinal center and undergo IGHV somatic hypermutations. These cells are genetically stable and do not express SOX11. The tumors derived from these cells tend to spread to the peripheral blood and spleen more than to lymph nodes. The disease seems to be stable for long periods of time, but some of these tumors may acquire mutations in genes such as TP53 that lead to disease progression. Intriguingly, some tumors with the pathological and genetic features of MCL do not carry translocations of the CCND1, CCND2, and CCND3.
Chromosome aberrations observed in MCL are consistent with an important role for dysregulation of the DNA damage response in this lymphoma (24). The ataxia telangiectasia mutated (ATM) gene, located at 11q22-23, is frequently deleted and mutated in MCL cases with increased genomic instability (25). Additional downstream elements of this pathway, including CHK1 and CHK2, are occasionally deregulated in MCL, suggesting that mutation of DNA damage response contributes to oncogenesis (1). Recent studies have also shown that genes involved in cell survival are targets of recurrent genetic alterations in MCL. Amplifications and overexpression of antiapoptotic genes such as BCL2 (18q21) (6) and homozygous deletions of proapoptotic genes such as BCL2L11 (2q13) have been described in primary tumors (26).

A recent study found NOTCH1 mutations in 12% of MCL cases to be associated with poor survival (27). This gene encodes for a transmembrane protein that functions as a ligand-activated transcription factor. Similarly to NOTCH1 mutations described recently in CLL (28, 29), mutations in MCL occur in the PEST domain and generate a truncated, more stable and transcriptionally active protein. In the study mentioned above, inhibition of this pathway reduced proliferation and induced apoptosis of MCL cells (27).

Cyclin D1 may be dispensable for MCL pathogenesis
The existence of uncommon lymphomas with pathological features of MCL in the absence of cyclin D1 dysregulation suggests that cyclin D1 overexpression is not the only mutation that can initiate MCL development (Figure 1). These tumors have a similar gene expression profile and share the same type of secondary chromosomal alterations, supporting the idea that they correspond to the same molecular disease. Chromosomal translocations fusing the CCND2 gene, but not the CCND3 gene, to IG loci are found in 50% of the cases, suggesting that this may be an alternative mechanism to CCND1 translocation (30, 31). However, the absence of any cyclin D gene alterations in a subset of otherwise conventional MCL raises the intriguing question about the initial driver for pathogenesis in these cases.
In a search for new genes relevant in MCL pathogenesis, SOX11, a neuronal transcription factor, was identified as a very specific marker of MCL (32). SOX11 is highly expressed in virtually all MCL tumors but is not expressed in other mature lymphoid neoplasms and normal lymphocytes at any stage of differentiation. Interestingly, SOX11 is highly expressed in both cyclin D1–negative and –positive MCL, suggesting that in addition to its value as a diagnostic biomarker, it may be an important factor in the pathogenesis of MCL (33). SOX11 expression in normal and neoplastic lymphoid cells is regulated by epigenetic changes, but its function in tumor development is not well understood yet (34). Further studies of potential oncogenic mechanisms common to cyclin D1–positive and –negative MCL may provide new insights into the primary genetic events that drive the development of one of the most aggressive lymphomas.

**Signaling pathways deregulated in MCL**

In addition to the multiple genetic alterations observed in MCL, these tumors carry dysregulation of different signaling pathways involved in cell proliferation, survival, and interactions with the microenvironment. Some of them are beginning to be targeted with new therapies and open a new perspective for the treatment of patients (Figure 2).

The PI3K/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway plays a critical regulatory role in cell growth and death processes. The causes for the activation of this pathway are not well understood but may include BCR, tumor necrosis factor (ligand) superfamily, member 13b (BAFF), and CD40 signaling. High levels of activated AKT, mTOR, and their respective downstream targets have been identified in MCL (35–37). Loss of expression or inactivating phosphorylation of PTEN, a negative PI3K regulator, have been found in some MCLs associated with the constitutive activation of AKT (37). No genetic or epigenetic alterations of PTEN have been identified in MCL, but the microRNA (miRNA) miR17–92 cluster, which is frequently amplified and overexpressed in MCL (38), was recently shown to target the PTEN transcript (39). Overexpression of these miRNAs causes downregulation of PTEN and activation of the AKT pathway and promotes resistance to chemotherapy-induced apoptosis in these tumors (39).

The influence of the microenvironment in the pathogenesis of different lymphoid neoplasms is receiving increased interest, but its potential role in MCL has been less investigated. The recent studies of the BCR supporting an antigen selection in the pathogenesis of at least a subset of MCL suggest a role for the stimulation of this pathway in the clonal expansion of tumor cells. However, the specific mechanisms and downstream effectors potentially involved are not well known. Amplification and overexpression of the spleen tyrosine kinase (SYK) and the PI3K catalytic α polypeptide have been found in a subset of MCL (40, 41). Furthermore, different kinases of the BCR signaling, including SYK, LYN, and BTK, have been found to be phosphorylated in primary MCL, suggesting activation of the pathway (42).

Activation of the JAK/STAT pathway may be a relevant mechanism connecting external stimuli with cell survival and proliferation. Phosphorylated STAT3 has been observed in primary MCL (43, 44). This activation may occur through a cytokine-dependent autocrine IL-6 and/or IL-10 secretion loop and/or induced upon BCR engagement (43). The IL-10 receptor is overexpressed in some MCLs (45), and IL-10 has been shown to sustain cell proliferation in MCL primary cells (46). Inactivating mutations of SOCS1, an inhibitor of the JAK/STAT pathway, have been described in some MCLs (47). Interestingly, STAT3 activation seems to be more common in MCLs with hypermutated (compared with unmutated) IGHV, suggesting that these two subtypes of MCL may use different cell survival mechanisms (43).

Expression of CD40 and responsiveness to the CD40 ligand (CD154) (48) suggest that MCL cells can interact with CD154-positive T cells in tissues, where such cellular interactions could promote MCL cell survival and expansion (49). High levels of functional CXCR4, CXCR5, and VLA-4 (CD49d) have been reported, suggesting a role in the migratory properties of the tumor cells (50). Further, the IL-22 receptor is aberrantly expressed in MCL, and stimulation by IL-22 promotes tumor cell growth and survival (51).

Constitutive activation of NF-κB has been detected in MCL cell lines and primary tumors with overexpression of several target genes, including the antiapoptotic proteins cFLIP, BCL2, BCLXL, and XIAP (52, 53). BCR signaling, microenvironmental stimuli, and TNF signaling may mediate NF-κB activation. Inactivation of the NF-κB pathway inhibitors TNFAIP3/A20 (6p23) and EAFI...
Are all MCLs created equal? An alternative pathogenetic pathway with clinical implications

MCL is classically considered an aggressive lymphoma. However, some studies have described a subset of patients with an indolent evolution of MCL even in the long-term absence of chemotherapy. The identification of these patients is puzzling given the current understanding of MCL as an aggressive tumor, which leads to the frequent recommendation of immediate treatment in almost all patients. These patients may benefit from a “watch and wait” management approach, at least for some period of time, but the challenge is in distinguishing them from patients who need aggressive therapy as soon as the disease is identified (56, 57).

MCLs with a very low proliferation index, a histological growth pattern restricted to the mantle zone of the lymphoid follicles (mantle zone pattern), and a limited clinical stage may have a more indolent behavior than MCLs without these features (58–61). These cases may correspond to early stages in the development of conventional MCL.

In addition, clinical studies have recognized a subgroup of MCL patients with an indolent behavior who, contrary to most cases of conventional MCL, present with a predominantly leukemic non-nodal disease. The genetic and molecular mechanisms underlying this distinct biological behavior are not fully understood, but recent studies comparing the groups of indolent nonnodal and conventional MCLs have revealed marked differences between the two (Figure 1). Most MCLs have complex karyotypes, but the nonnodal tumors have very few, if any, chromosomal alterations in addition to the t(11;14) translocation. The nonnodal MCLs show differences in certain gene expression have been occasionally found in the mantle zones of otherwise reactive lymphoid tissues in healthy individuals. These clones can persist for long periods, but their potential to evolve into an overt lymphoma is not clear (70). Their high frequency in healthy individuals and low prevalence of MCL suggests that most clones bearing only the t(11;14) translocation will never transform into a malignant tumor. On the other hand, the observation of a simultaneous MCL with the same clonal origin in a recipient and donor 12 years after an allogeneic bone marrow transplant underscores the long latency required by initial clones to develop an overt lymphoma (71).

Cells carrying the t(11;14) translocation and cyclin D1 overexpression have been occasionally found in the mantle zones of otherwise reactive lymphoid tissues in healthy individuals. These lesions have been referred to as “in situ MCLs,” but their malignant potential seems very limited (Figures 1 and 3). In a recent study, only 1 of 12 of these lesions developed an overt lymphoma four years after its detection. To avoid overtreatment, the alternative term “in situ MCL-like B cells” has been proposed (72). Retrospective analysis in seven patients identified in situ lesions in tissues obtained 2 to 15 years prior to diagnosis, suggesting that all MCLs proceed through a stage of in situ lesions (73). Some in situ lesions express SOX11, whereas others are SOX11 negative, suggesting that the in situ lesion stage may be a common step in both SOX11-negative and -positive subtypes of MCL (ref. 71 and Figure 1). Incidents of in situ lesions have also been found in patients who are apparently in complete remission following treatment, suggesting that this microenvironment may sustain residual tumor cells resistant to chemotherapy (ref. 71 and Figure 3).

Multistep development and progression in MCL

The contribution of multiple mutations to tumor development is not as well characterized in lymphoid neoplasms as in solid tumors. Recent observations have identified what may correspond to early molecular and pathological phases of MCL oncogenesis (Figure 3). Cells carrying the t(11;14) translocation have been detected at very low levels in the peripheral blood of a number of healthy individuals (8%). These clones can persist for long periods, but their potential to evolve into an overt lymphoma is not clear (70). The high frequency in healthy individuals and low prevalence of MCL suggests that most clones bearing only the t(11;14) translocation will never transform into a malignant tumor. On the other hand, the observation of a simultaneous MCL with the same clonal origin in a recipient and donor 12 years after an allogeneic bone marrow transplant underscores the long latency required by initial clones to develop an overt lymphoma (71).

Target therapy in MCL: a promise of more successful treatments

The heterogeneous biology and clinical presentation of MCLs are major challenges to define standard therapies (74). Recent protocols adjusted for the patients’ conditions are improving the
clinical outcome, but frequent relapses and progressive resistance to treatment emphasize the need for new approaches (75). The increasing understanding of the pathogenetic mechanisms provides attractive and promising targets. In the last six years, two new drugs for relapsed or refractory MCL have been approved: the proteasome inhibitor bortezomib (approved by the US FDA; ref. 76) and the mTOR inhibitor temsirolimus (approved by the European Medicines Agency; ref. 77).

Proteasome inhibitors disrupt the ubiquitin-proteasome system (78). Bortezomib induces apoptosis in MCL through upregulation of the BH3-only protein NOXA (79). NOXA acts as a downstream effector of an integrated cellular stress response triggered by the accumulation of undegraded, polyubiquitinated proteins that induce endoplasmic reticulum stress and the generation of reactive oxygen species (80). Single-agent bortezomib therapy was shown to induce responses in 30%-50% of cases, even in patients who failed to respond to prior dose-intensive therapy or who had bulky disease (81). A second generation of proteasome inhibitors, including NPI-0052 (salinosporamide A), PR-171 (carfilzomib), and MLN9708, are now entering the clinic. Efficacy has also been reported for proteasome inhibitors in combination with other cytotoxic agents and targeted therapies such as histone deacetylase inhibitors and HS990 inhibitors (78, 82). Several proteins central to MCL, including cyclin D1, CDK4, AKT, and p53, bind to the molecular chaperone HSP90, suggesting that HSP90 inhibitors may be a suitable therapeutic target (83). However, HSP90 inhibitors as well as histone deacetylase inhibitors have demonstrated only moderate clinical antitumor activity when used alone (78, 82, 84).

mTOR is another attractive target for new therapies (85). Temsirolimus is a rapamycin analog that allosterically inhibits mTORC1. As a single agent, temsirolimus was shown to induce responses in up to 40% of relapsed or refractory patients, and its efficacy was superior to that of traditional monotherapies (86). Phase I/II studies are currently being performed to explore the best chemotherapy that may be added to temsirolimus and its potential role as maintenance therapy (87–92). Other rapalogs, such as everolimus and deforolimus, and several PI3K inhibitors offer additional means to target the PI3K/AKT/mTOR pathway, although deforolimus has not showed clinical activity in relapsed MCL (93, 94). The PI3Kδ inhibitor CAL101 has shown an overall response rate of 62% in relapsed and refractory MCL cases (95). In addition, several ongoing clinical trials are using MK2206, an allosteric inhibitor of AKT, and OSI-027, a dual mTORC1 and mTORC2 inhibitor (82, 96).

Some therapies target cell cycle regulators and other key patho-genetic pathways in MCL (22, 97, 98). The pan-CDK inhibitor flavopiridol has shown minimal antitumoral activity (99), but in contrast PD-0332991, an inhibitor of CDK4 and CDK6, has shown biological effects (reduced retinoblastoma phosphorylation and Ki67 index) and some clinical benefit in a subset of patients (100). Promising results are being obtained with drugs targeting the DNA repair pathway (25, 101), such as the poly(ADP-ribose) polymerase 1 (PARP) inhibitor AG014699 (102–104). The use of proapoptotic molecules that mimic the activity of BH3-only proteins (105) has shown promising Results in preclinical models (106–108) but no responses or only modest activity in clinical studies (82, 109). However, these compounds may sensitize cells to the effect of other therapeutic agents.

Novel approaches include targeting the BCR pathway. Unfortunately, PKCB inhibitors showed no objective responses in MCL patients (110), and an objective response to SYK inhibitors was reported in only 11% of patients (111). In contrast, the BTK inhibitor PCI-32765 (ibrutinib) induced a high rate of objective responses in patients with relapsed or refractory MCL (111, 112). In addition, a Phase I clinical trial using the oral JAK-2 inhibitor SB1518 showed activity in two of three MCL cases studied (113). The identification of recurrent NOTCH1 mutations provided a preclinical rationale for therapeutic inhibition of the NOTCH pathway using γ-secretase inhibitors or for immunotherapy using antibodies against NOTCH1 (114, 115).

Conclusions and future directions
The study of MCL, integrating multidisciplinary research from molecular biology and pathology to the clinic, has improved the understanding of its pathogenesis and guides the search to design new therapeutic strategies. Although advances in the last decade have been remarkable, recent clinical and biological observations have shown that our understanding of this tumor is far from complete. The increasing evidence for a role for cyclin D1 beyond cell cycle in some tumor models raises the question of similar functions in MCL. On the other hand, the absence of cyclin D1/D2 dysregulation in some cases argues for additional mechanisms in the initiation of the tumor. The intricate networks of genetic alterations, deregulated signaling pathways, and their triggering elements are only starting to be elucidated. The identification of two subtypes of the disease sharing the cyclin D1 dysregulation but with different molecular, genetic, and clinical characteristics and evolution is intriguing and requires further study. The role of antigen selection and different cells of origin for these subtypes of MCL may highlight the influence of the microenvironment in the development of the disease. The investigation of these new observations should render a better understanding of the tumor and facilitate a more precise diagnosis and tailored management of patients, particularly in light of an increasing number of new potential therapeutic drugs.

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