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INSIGHTS

Takes one to B1a: Dismantling the origin of mantle cell lymphoma

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Therapeutic discovery for mantle cell lymphoma (MCL) has been hindered by a lack of preclinical mouse models that recapitulate human disease. In this issue, Pieters and colleagues (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20202280) establish a novel mouse model of MCL driven by overexpression of cyclin D2 and identify fetal-derived B1a cells as putative cell of origin for MCL.

Mantle cell lymphoma (MCL) is a rare and aggressive subtype of non-Hodgkin's B cell lymphoma that remains incurable. Despite considerable advances in defining pathology and expanding nonchemotherapy treatment options within the last decade, limited understanding of the mechanisms underlying disease pathogenesis continues to hamper discovery of new therapeutic targets. In particular, the inability to replicate disease in mouse models represents a real constraint within the field. In this issue, Pieters and colleagues (Pieters et al., 2021) have now circumvented this major limitation by creating a novel preclinical mouse model that recapitulates classic features of MCL disease.

Human MCL is characterized genetically by overexpression of D-type cyclins, including cyclin D1, D2, and D3, with the most common chromosomal translocation t(11;14)(q13;32) causing overexpression of cyclin D1. Cyclin D1 (*Ccnd1*) is a widely studied oncogene (Musgrove et al., 2011), and cyclins broadly regulate cell cycle progression, proliferation, and differentiation through their interactions with cyclin-dependent kinase 4 (cdk4) and cdk6. Previous attempts to drive overexpression of *Ccnd1* alone in mice, however, did not recapitulate disease or result in spontaneous MCL in the absence of immune stimulation or an additional driver mutation (Montalto and De Amicis, 2020). Pieters et al. generated mice overexpressing cyclin D2 (*Ccnd2*) that spontaneously develop MCL. Although human MCL is most commonly associated with *Ccnd1* overexpression, translocations in *Ccnd2* have also been frequently identified (Salaverria et al., 2013). Notably, *Ccnd2* overexpression in this new model was driven off of the ubiquitous Rosa promoter and induced broadly across the hematopoietic system during development by Vavi-Cre. Whereas previous attempts to overexpress cyclins have used the heavy chain locus to drive overexpression in order to mimic the most common human translocation, success of the *Vav-Ccnd2* model suggests either that (1) broad overexpression within the hematopoietic hierarchy or (2) overexpression earlier during ontogeny aptly targeted cells that were previously excluded by regulation at the IgH locus. The success of modeling MCL by driving *Ccnd2* overexpression with the Vav promoter suggests that a similar approach to driving overexpression of *Ccnd1* might be advantageous in future studies.

30% of $Ccnd2^{Vav}$ mice developed spontaneous tumors that shared many overlapping phenotypic and genotypic features of classic human MCL, including expression of CD5 and Sox11. Still, the latency to spontaneous tumor progression remained long, over a year, as with previous models, and transplantation of primary tumors driven by *Ccnd2* overexpression alone into immunodeficient mice failed to propagate disease. To increase disease penetrance and severity, the authors crossed mice overexpressing *Ccnd2* to conditional p53 KO mice. Deletion of TP53 is a genetic lesion in ∼13% of human MCL (Meissner et al., 2013); p53 was specifically deleted in B cells using Mb1-Cre. Deletion of p53 alone did not cause any

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incidence of MCL-like lymphoma. In contrast, combined deletion of p53 and Ccnd2 overexpression in *Ccnd2/p53^{mbl}* mice resulted in a spontaneous, aggressive, transplantable lymphoma, with a significant bias toward an MCL-like immunophenotype, comparable to phenotype observed in *Ccnd2* overexpression alone and classic human MCL alike.

The generation of a novel mouse model that recapitulates human disease phenotype provided a unique opportunity to dissect molecular profiles and identify novel druggable targets. Extensive transcriptional profiling of transplantable MCL-like and non-MCL-like tumors revealed a striking similarity and transcriptional overlap of MCL-like tumors to B1 B cells. B1 B cells are distinct lymphocytes derived from fetal hematopoiesis that persist into adult life. B1 B cells can be further classified into additional subsets based on expression of CD5, with CD5+ "B1a" cells considered as the first B cells to arise during ontogeny. B1a cells are primarily IgM-producing cells with limited B cell receptor (BCR) repertoires that primarily recognize self-antigens. Beyond a common immunophenotype, MCL-like lymphomas in *Ccnd2/p53^{mbl}* mice displayed many key features of B1a cells, including secretion of IgM, evidence of restricted BCR repertoire, and reactivity to phosphatidyl choline ligands, suggesting a B1a cell of origin. It is notable that despite a homogeneous immunophenotype, many of these features were heterogenous among phenotypically comparable MCL-like tumors, possibly reflecting heterogeneity both in function (Baumgarth, 2016) and in ontogeny (Hadland and Yoshimoto, 2018) described among B1a cells in mice.

Notably, whereas B1a cells had the highest endogenous expression levels of *Ccnd2*, overexpression of *Ccnd2* had no effect on normal B cell development or B1a cell expansion in vivo. Transformation of B1a cells to MCL therefore requires an additional "hit"—in this case, deletion of p53—that makes B1a cells specifically sensitive to cyclin overexpression. B1a cells are not maintained by adult bone marrow hematopoiesis, and they therefore have a unique capability to self-maintain or self-renew within the pleural cavities in which they reside. Self-renewal is thought to be driven by continuous and sustained BCR signaling trigged by self-antigen exposure (Huizar et al., 2017), which gradually restricts BCR repertoire with aging (Yang et al., 2015). MCL-like tumors expressing restricted BCRs in *Ccnd2/p53^{mbl}* mice were particularly enriched for increased BCR activity. Similarly, a previous publication has demonstrated that overexpression of *Ccnd1* in combination with enforced expression of a restricted B1 B cell–specific BCR also resulted in the formation of MCL-like disease (Hayakawa et al., 2018). Together, these observations indicate that the longevity of fetal-derived B1a cells and the specific features that drive their long-term self-renewal may make them particularly susceptible to transformation.

Identification of B1a cells as the cell of origin in this model provided the necessary insight to identify MALT1 as a novel therapeutic target for MCL. MALT1 protease is an essential member of the CARD11-BCL10-MALT1 (CBM) signaling complex regulating NF-κB signaling. Although canonical NF-κB signaling is the central target of current therapies for MCL, MALT1 has only been specifically investigated in the context of MCL in one previous publication, where MALT1 inhibition was broadly linked to MYC regulation (Dai et al., 2017). Importantly, MALT1 is specifically required for the generation and maintenance of innate-like B cells, including B1 B cells and marginal zone cells (Ruland et al., 2003). MALT1 protease activity was detected in 3/4 classic MCL-like tumors induced by *Ccnd2/p53mb1*. Importantly, targeting MCL-like tumors with two different MALT1 inhibitors showed striking efficacy in reducing tumor growth, not only in in vivo tumor models, but also in an MCL patient-derived xenograph model. Both endogenous B1 B cells and MCL tumors were sensitive to MALT1 inhibition, indicating that both lymphomas and cell of origin can be targeted directly by MALT1 inhibition. The specific requirement of MALT1 for innate-like B cells and the efficacy of MALT1 inhibitors for reducing tumor growth in this novel mouse model makes a particularly strong argument for pursuing it as a novel therapeutic target in MCL.

The last decade has witnessed major advances in treatment of lymphomas including MCL with the use of Bruton's tyrosine kinase inhibitors such as ibrutinib that constrain chronic BCR signaling. Still, major limitations associated with relapse and toxicity persist (Stephens and Spurgeon, 2015), and the absence of appropriate mouse models that replicate disease phenotype, penetrance, and severity has prevented further discovery of druggable targets. Pieters and colleagues have provided strong evidence for identifying a distinct cell of origin for this rare and particularly devastating lymphoma. Because B1a cells are dispensable in the adult, this discovery represents a very distinct path forward for identifying new treatment options. Adult mice can survive without B1a cells, suggesting that the cell of origin for MCL can be entirely eradicated without direct effects on survival. Although targeting MALT1 may have considerably broader effects beyond B1a cells, the identification of fetal-derived B1a cells as the cell of origin for MCL may serve as a wellspring for identifying additional, highly specific therapeutic targets that can be directed at these cells while sparing adult lymphopoiesis.

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