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FBXO11: a novel germinal center B-cell regulator?

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In this issue of *Blood*, Schneider et al report that F-box protein 11 (FBXO11) inactivation leads to abnormal germinal center (GC) formation and lymphoproliferative disease.[1]

Ubiquitin-mediated proteasomal degradation is a conserved cellular mechanism for regulation of protein turnover that is used for the regulation of critical biological processes. F-box proteins are important substrate-specific adaptors in the SKP1–cullin-1–F-box protein (SCF) family of cullin-ring ligases, which represent the largest family of multicomponent E3 ubiquitin ligases involved in proteasomal degradation of proteins. Significantly, multiple F-box proteins are dysregulated or targeted by structural alterations, including point mutations and deletions in multiple human cancers.

FBXO11 is evolutionarily conserved from worms to mammals.[2] Deletion of the worm ortholog of FBXO11, DRE-1, results in larval lethality, and DRE-1 mutation results in improper patterning of gonadal outgrowth, suggesting a critical role in the determination of cell fate. Homozygous mutation of *FBXO11* in murine models is associated with facial clefting, cleft palate, and perinatal lethality. As a result, tissue-specific deletion of FBXO11 is required to delineate its function in specific tissues or organs. With regard to cancer, FBXO11 has been reported to be recurrently mutated or deleted in a subset of diffuse large B-cell lymphomas (DLBCLs), suggesting a putative tumor suppressor role.[3] FBXO11 has also been identified as the E3 ubiquitin ligase that targets the BCL6 oncoprotein for ubiquitin-mediated proteasomal degradation.[3]

BCL6 is a transcriptional repressor and oncoprotein whose expression is largely restricted to GCs of the B-cell follicle. BCL6 is functionally critical for initiating and terminating the GC reaction. BCL6-deficient mice fail to generate GCs or produce high-affinity antibodies. The *BCL6* locus is targeted by genetic aberrations in up to 30% of DLBCLs, and the oncogenicity of BCL6 deregulation has been demonstrated in mouse models harboring a BCL6-immunoglobulin heavy chain translocation analogous to that observed in humans.[4]

Prior work has demonstrated that overexpression of FBXO11 results in enhanced BCL6 degradation, inhibition of cell growth, and cell death in human DLBCL-derived cells.[3] An inverse correlation between the expression of FBXO11 and BCL6 protein expression and copy number losses as well as mutations targeting *FBXO11* were identified in approximately 6% of patients with DLBCL, provoking the consideration that FBXO11 could be a tumor suppressor that plays a role in the pathogenesis of B-cell lymphomas. In an effort to formally explore a tumor suppressor role for FBXO11 in B-cell lymphoma, Schneider et al generated a GC-specific knockout mouse model for FBXO11. By using this in vivo model, Schneider et al confirmed that targeted FBXO11 deletion results in prolonged half-life and kinetic delays of BCL6 degradation. In addition, B-cell receptor signaling-induced physiologic downregulation of BCL6 at the end of the GC reaction is impaired.

The GC-specific deletion of FBXO11 was associated with significantly higher numbers of GC B cells compared with the wild-type and heterozygously deleted mice. In addition, in vivo FBXO11 haploinsufficiency or loss was associated with increased numbers of GC B cells and an altered ratio of GC dark zone to light zone cells. Taken together, these findings suggest a role for FBXO11 in B-cell maturation through the GC.

The GC-specific FBXO11 knockout phenotype is complex and intriguing. In this regard, detailed characterization by Schneider et al describe a phenotype of the GC-specific FBXO11-deficient mice that is less penetrant than that observed in mice engineered to constitutively express BCL6. Furthermore, the heterozygous mice showed a somewhat higher predilection for development of lymphoproliferative disease than the homozygous mice. Only 1 mouse with a heterozygous deletion of FBXO11 developed a DLBCL-like lymphoid proliferation. Intriguingly, no DLBCLs were observed in homozygously deleted mice.

The complexity of the GC-specific FBXO11 knockout can be partly explained by the fact that F-box–containing proteins may have many different context-specific substrates. In this regard, DRE-1, the *Caenorhabditis elegans* analog of FBXO11, has been demonstrated to target the transcription factor BLIMP1 for proteasomal degradation.[5] B lymphocyte–induced maturation protein (BLIMP-1) is a transcriptional repressor that controls terminal differentiation of mature B cells to plasma cells. In previous work, the Dalla-Favera[6] and Tam laboratories[7] demonstrated that the genomic locus (6q21) harboring the *BLIMP-1* gene is recurrently deleted or mutationally inactivated in a subset of DLBCLs (activated B-cell type), lending support for a possible tumor suppressor function in B cells.

Accordingly, the interpretation of the phenotype of the FBXO11 null in GC B cells needs to take in account the accumulation of BLIMP-1, which conceptually could counteract the effects of BCL6 accumulation. Furthermore, FBXO11 has been demonstrated to target CDT2 for degradation. Cul4-Cdt2 (CRL4Cdt2) is an E3 ubiquitin ligase that regulates cell cycle progression and genome stability via its central role in the degradation of Cdt1, p21, and Pr-Set7/Set8.[8,9] The functional interaction between FBXO11 and CDT2 is evolutionarily conserved from nematodes to humans in regulating the timing of cell cycle exit.

CDT2 is amplified and/or overexpressed in Ewing sarcoma[10] and epithelial cancers, suggesting an oncogenic function. The phenotypic consequences of deregulated expression of CDT2 have not been explored in the context of B-cell development or lymphoma generation in vivo. Thus, the extent to which simultaneous degradation of BCL6 and CDT2 contributes to the FBXO11 null phenotype in GC cells can only be speculated on. Furthermore, the impact of the yet undiscovered FBXO11 substrates that contribute to the phenotype of GC-specific FBXO11 null phenotype is unknown.

The study by Schneider et al confirms that FBXO11 regulates the stability of BCL6 but also raises some important questions. In this regard, the previously described role of phosphorylation in BCR signaling linked degradation of BCL6, but data presented in this article contradict the original findings by Duan et al,[3] which suggest that BCL6 degradation by FBXO11 does not require phosphorylation and may occur in the absence of BCR signaling.[3] In addition, the BCL6 degron has not been identified and thus there is not yet a full understanding of the mechanistic aspects of the role of FBXO11 in BCL6 regulation. It is anticipated that future studies identifying additional substrates for FBXO11 and delineating their degrons as well as elucidating the structure of FBXO11 will further enhance our understanding of the mechanisms by which FBXO11 plays a role in B-cell biology and DLBCL pathogenesis.

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Versican vs versikine: tolerance vs attack

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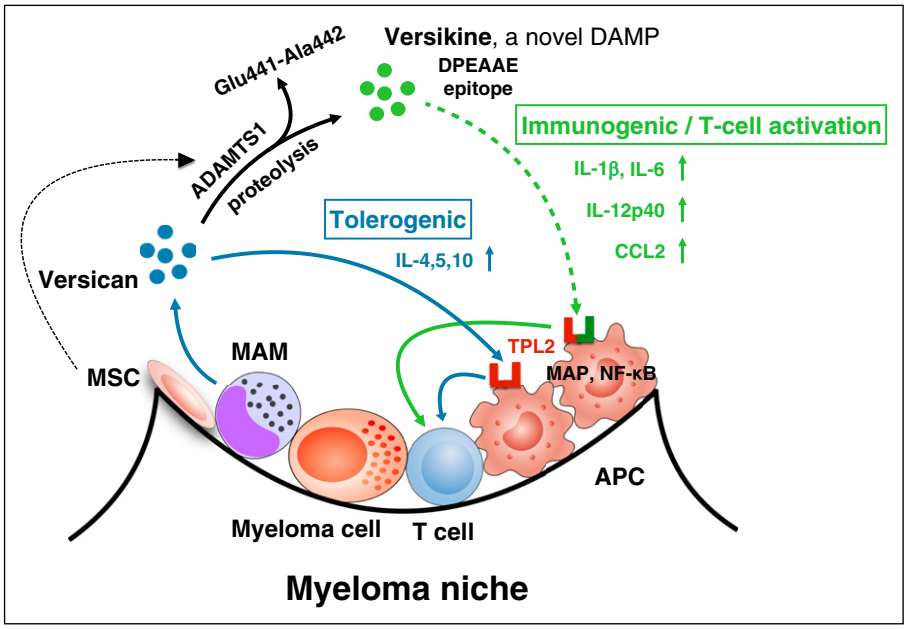
In this issue of *Blood*, Hope et al demonstrate the differential influence of versican and its proteolytic derivate, versikine, on the immune system. This discovery opens a new avenue for immunotherapies in multiple myeloma patients.[1]

Multiple myeloma is an (oligo)clonal plasma cell disease which can develop in the bone marrow and also, in some cases, as extramedullary disease in soft tissue. Tumor escape mechanisms in multiple myeloma still need to be identified. The graft-versus-myeloma effect observed after allogeneic stem cell transplantation clearly demonstrates that T lymphocyte can play a role in the antitumor defense of the immune system. In the last 2 decades, several tumor-associated antigens (TAAs) with therapeutic relevance have been defined.[2] Such TAA-specific T cells can be elicited and augmented, for example, by vaccination with tumor antigen-derived peptides.[3] These antimyeloma T-cell responses may be hampered by standard drugs used in myeloma therapy like steroids (prednisone, dexamethasone) which have a potent antiproliferative action. In modern myeloma therapy, thalidomide and its derivatives play a crucial role. These immunomodulatory drugs are not only antiproliferative agents, but they also exert effects on antigen-presenting cells (APCs) and T cells, thus modulating and enhancing or suppressing TAA-directed T-cell responses.[4]

Immunotherapy, with breakthrough potential, has also reached myeloma therapy: treatment of myeloma with monoclonal antibodies against signaling lymphocytic



MAMs secrete the matrix proteoglycan versican which can be cut into versikine and a small peptide dimer (glutamine position 441–alanine position 442; Glu^{441}-Ala^{442}) by ADAMTS1. This ADAMTS1 is produced by mesenchymal stroma cells (MSCs). Both versican (blue lines) and versikine (green lines) exert an influence on T cells: while versican binds to TLR2 on APCs which block T cells through type II cytokines (interleukin-4 [IL-4], IL-5, and IL-10). On the other hand, versikine, a novel damage-associated molecular pattern (DAMP), binds to TLR2 but perhaps also other receptors, subsequently using intracellular mitogen-activated protein (MAP) and nuclear factor κB (NF-κB) thus mediating T-cell activation through type I cytokines like IL-1β, IL-6, IL-12p40, and CCL2.