



***In vivo* modeling of diffuse large B cell lymphoma (DLBCL) with the myeloid differentiation primary response gene 88 (MYD88) L265P mutation**

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Diffuse large B cell lymphoma (DLBCL) is the most common lymphoid malignancy, accounting for 30–40% of adult lymphomas. It is a difficult-to-treat disease; only 60% of patients with DLBCL can achieve long-term remission with contemporary immune chemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; or R-CHOP). Gene expression profiling has identified at least two biologically distinct DLBCL subtypes based on their cell of origin: the germinal center B cell-like (GCB) and the activated B cell-like (ABC) DLBCL (1). Patients with the ABC subtype have a poorer outcome with only 40% of 3-year progression-free survival after R-CHOP therapy compared to 75% for the GCB subtype (2). Therefore, there is an urgent need for better understanding of disease mechanisms and development of more effective therapies for ABC DLBCL. Recent genomic profiling has uncovered novel mutations that may provide clues to the development of DLBCL. Notable mutations are found in the key components of the NF- κ B signaling, including CARD11, CD79B, myeloid differentiation primary response gene 88 (MYD88) and TNFAIP3 (also known as A20). These studies also uncovered complexity in the DLBCL genome, which has an average of 50 to 100 variable genetic lesions in each tumor biopsy (3,4). Identification of which mutations are causative and which ones are secondary is challenging due to confounding effects of concomitant genetic alterations. Therefore, an experimentally tractable system such as genetically engineered mouse (GEM)

modeling becomes necessary to study the consequence and contribution of individual mutations. To date, GEM models of human DLBCL-derived CARD11 mutation and A20 deficiency have been generated (5,6). Knittel and colleagues has recently expanded this list with a new mouse model of the *MYD88 L265P* mutation, which is frequently found in ABC DLBCL (7). These GEM models have provided critical insights into DLBCL pathogenesis and a useful platform for new drug development for this aggressive disease.

The MYD88 encodes a central adaptor protein downstream of the pattern recognition toll-like receptors (TLRs). There are currently ten human TLRs, which recognize a wide variety of non-self and self-antigens ranging from lipopolysaccharides to double and single-strand RNA and DNA. Except for TLR3, all other TLRs transmit signals through MYD88, leading to NF- κ B activation. While the critical role of MYD88 in TLR activation and innate immune response is well established, its role in cancer is less understood. Discovery of MYD88 in B-cell malignancy began with an early study by the Staudt laboratory, which found that ABC DLBCL was dependent on constitutive NF- κ B activity for proliferation and survival (8). NF- κ B is a family of transcription factors that play a central role for activation, proliferation and survival of immune cells in response to infection. Although inhibition of the I κ B kinase β (IKK β), a central kinase of NF- κ B signaling, can suppress the growth

of ABC DLBCL, this approach causes severe side effects, preventing many active IKK β inhibitors from regulatory approval. A more rational approach is to identify abnormal signaling that activates NF- κ B only in tumor cells, but not in normal cells. Realizing the complex and diverse signaling networks upstream of NF- κ B, Staudt and colleagues have integrated unbiased, high-throughput techniques such as RNA interference (RNAi) screen and next-generation sequencing to unravel molecular targets that are highly specific in the ABC DLBCL subtype. Remarkably, among targets identified by the RNAi screen that are essential for the survival of ABC DLBCL, many harbor somatic mutation that hyper-activate NF- κ B, including *CD79A*, *CD79B*, *CARD11* and *MYD88* (9). Mutations in *MYD88* account for 37% of ABC DLBCL cases, making it the most mutated gene in this subtype. *MYD88* mutations are located within the evolutionarily conserved Toll/IL-1 receptor (TIR) domain of MYD88. The most common mutation is *L265P*, which occurs at the conserved residue in the hydrophobic core of the TIR domain. MYD88 mutants associate with hyper-phosphorylated IRAK1, leading to constitutively active NF- κ B and JAK-STAT3 signaling that provide survival signals for the lymphoma cells. ABC DLBCL is not the only lymphoma that harbors *MYD88* mutations. Subsequent studies uncovered the *L265P* mutation in 100% of Waldenström's macroglobulinemia (10), 13% of splenic marginal zone lymphoma (11), 10% of mucosa-associated lymphoid tissue (MALT) lymphoma (9), and 3% of chronic lymphocytic leukemia (CLL) (12), underscoring the important role of this mutation in B-cell malignancies.

To determine whether the human *MYD88 L265P* mutation promotes lymphomagenesis *in vivo*, Knittel *et al.* knocked in a mouse orthologous *Myd88 L252P* allele to the endogenous locus, an elegant approach to utilize the natural regulatory machinery (7). The mutant allele was expressed by Cre-mediated recombination using promoter-driven Cre expression at three different B-cell differentiation stages: pre-B and pan-B (Cd19 promoter), mature B cells (Cd21 promoter) and germinal center B cells (Aid promoter). The indistinguishable development of lymphoproliferative disease (LPD) and ABC DLBCL-like lymphoma in these mouse models suggest that *Myd88 L252P*-mediated neoplastic transformation of B cells is likely a late event, occurring after germinal center reaction. The *Myd88 L252P* mouse model by Knittel *et al.* has provided several important implications for the oncogenic potential of aberrant TLR signaling. First, the long latency

of tumor onset (about 60 weeks) in the *Myd88 L252P* mice, which is substantially shortened by a *BCL2* transgene (under 20 weeks), indicate that lymphoma development through Myd88-mediated NF- κ B activation requires additional anti-apoptotic signals. The cooperation between MYD88 and BCL2 is further supported by enrichment of *MYD88* mutations and high BCL2 expression in human ABC DLBCL (1,7). Furthermore, such cooperation was also observed in a previous study in which *Bcl2* overexpression rescued apoptotic cell death of adoptively transferred self-reactive B cells that expressed the *Myd88 L252P* mutant (13). Interestingly, overexpression of *Myd88* mutations in this study promoted B-cell proliferation for only a few cell divisions before NF- κ B activity was shut down. This self-limiting activity of Myd88 mutants appeared to be mediated by induction of the NF- κ B negative regulator TNFAIP3/A20, which is frequently inactivated in *MYD88 L265P*-bearing lymphoma (9). Thus, *Myd88 L252P*-mediated lymphomagenesis may also require inactivation of the negative feedback loop mediated by TNFAIP3. With the availability of the B cell-specific *TNFAIP3/A20* deficient mice (6), testing this possibility would be quite feasible. In this regard, cooperation between *MYD88 L265P* mutation and other highly overlapping *CD79A* and *CD79B* mutations (9) may also represent a viable alternative for lymphoma development, pending *in vivo* validation using appropriate mouse strains. Finally, the predominant LPD with occasional transformation into aggressive B-cell lymphoma in the *Myd88 L252P* mice provides a useful model to tract the natural history of lymphoma development. Sequencing of early LPD clones to detect potential cooperating mutations before and after malignant transformation will provide a powerful insight into the pathogenesis of DLBCL.

In summary, the new mouse model by Knittel *et al.* has provided an excellent *in vivo* system to study the natural development and outcome of DLBCL mediated by the *MYD88 L265P* mutation. Despite the strong oncogenic potential of *MYD88 L265P*-mediated signaling, cooperation with secondary mutations is necessary to overcome pro-apoptotic and NF- κ B inhibitory signals inherently induced by the *MYD88* mutation. The mouse model will be useful for testing the cooperation with other mutations that are frequently found in *MYD88 L265P*-bearing DLBCL, including *TNFAIP3/A20* and *CD79A/B*. This model also provides an excellent preclinical platform to test drug combinations and predict clinical outcome of DLBCL.

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Footnote

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Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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