MYD88 L265P Mutation in Lymphoid Malignancies
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Abstract
Next-generation sequencing has revealed cancer genomic landscapes, in which over 100 driver genes that, when altered by intragenic mutations, can promote oncogenesis. MYD88 is a driver gene found in hematologic B-cell malignancies. A missense mutation (L265P) changing leucine at position 265 to proline in MYD88 is found in ∼90% of Waldenström macroglobulinemia (WM) cases and in significant portions of activated B-cell diffuse large B-cell lymphomas and IgM monoclonal gammopathy of undetermined significance. Few cancers such as WM have a single amino acid substitution in one gene like MYD88 L265P that occurs in ∼90% of cases, making WM paradigmatic for study of a single causative mutation in oncogenesis. In this review, we summarize the frequency and cancer spectrum of MYD88 L265P and its downstream effects in lymphoid cancers. Malignant B cells with MYD88 L265P are likely transformed from IgM-producing B cells either in response to T-cell–independent antigens or in response to protein antigens before class switching. We also discuss therapeutic strategies that include targeting Bruton tyrosine kinase and other kinases, interfering with the assembly of MYD88 and its interacting partners, and MYD88 L265P–specific peptide-based immunotherapy. Cancer Res. 78(10): 2457–62. ©2018 AACR.

Introduction
Myeloid differentiation factor 88 (MYD88) was discovered in the 1990s as a primary differentiation response gene in myeloid precursors that was activated following terminal differentiation and growth arrest induced by IL6 (1, 2). As a general adaptor protein, MYD88 contains three main structures: a death domain (DD) at the N terminus, an intermediate linker domain (ID), and a Toll/interleukin-1 receptor domain (TIR) at the C terminus. Following ligand binding, the cytoplasmic TIR domain of Toll-like receptors (TLR) or IL1R associates with the TIR of MYD88 (3–5). Then, the serine–threonine kinase, IL1-receptor associated kinase 4 (IRAK4), is recruited to MYD88 through the interaction of the death domains of both molecules. IRAK4 interacts and phosphorylates IRAK2 and IRAK1 to form a complex known as the “Myddosome” (6). Phosphorylated IRAKs 1 and 2 interact with the E3 ubiquitin ligase TNF receptor–associated factor 6 (TRAF6), via their TRAF binding domains. TRAF6 ultimately recruits TAK1-binding protein 2 (TAB2) and activates TAB2–associated TGF-

Frequency of MYD88 L265P Mutation in B-cell Neoplasms
Recurrent MYD88 L265P mutation was first found in diffuse large B-cell lymphoma (DLBCL), which can be classified into two major molecular subtypes based on cell of origin (11). The germinal center B-cell–like (GCB) subtype possesses gene expression patterns that resemble those of nonmalignant B cells that have entered the germinal center, whereas the activated B-cell–like (ABC) subtype expresses genes induced by NF-kB during in vitro activation of peripheral blood cells. Patients with ABC-DLBCL have poor survival compared with GCB-DLBCL. Staudt and colleagues first found MYD88 mutations in ABC-DLBCL biopsies with L265P as the most frequent, seen in about 29% of cases (12). In contrast, this mutation was rare or absent in patients with GCB-DLBCL, primary mediastinal B-cell lymphoma, and Burkitt lymphoma. shRNA knockdown and reintroducing wild-type (WT) or mutant MYD88 revealed that the survival of ABC-DLBCL cells bearing the L265P mutation is sustained by the mutant but not WT MYD88, supporting the idea that L265P is a gain-of-function driver mutation in ABC-DLBCL (12). Lymphoplasmacytic lymphoma (LPL) is rare, representing ∼1% of all of non-Hodgkin lymphomas. LPL is characterized by predominantly small B lymphocytes in varying degrees of plasmacytic differentiation ranging from plasmacytic lymphocytes to plasma cells. Waldenström macroglobulinemia (WM) is defined as a subset of LPL in which patients have bone marrow...
involvement and produce large amounts of monoclonal IgM macroglobulin. WM represents ~95% of LPL cases, whereas other LPLs are associated with IgA or IgG paraprotein (13). In 2012, MYD88 L265P was found in tumor samples from 49 of 54 (91%) patients with WM (14). Ensuing studies indicate that the frequency of MYD88 L265P mutation in non-IgM LPL is ~25%. In contrast, MYD88 L265P was found in 87% (1,324 of a total of 1,520) of patients with WM as reported from 25 publications (Supplementary Table S1). Across all cancers, few other than WM have a missense mutation like MYD88 L265P that occurs in >85% of cases, making WM paradigmatic for study of a single causative mutation in oncogenesis (15).

Patients with monoclonal gammapathy of undetermined significance (IgM class [IgM MGS]) are at increased risk for WM, DLBCL, and mucosa-associated lymphoid tissue (MALT) lymphoma, as well as chronic lymphocytic leukemia (16, 17). Staudt and Langdren determined the MYD88 gene status in IgM MGS and found MYD88 L265P expression in 5 of 9 patients (56%; ref. 18). We summarize the MYD88 L265P mutation frequency rates in different B-cell neoplasms: MYD88 L265P mutation occurs frequently in WM (87%), primary DLBCL of the central nervous system (CNS; 70%), cutaneous DLBCL, leg-type (54%), IgM MGS (52%), testicular DLBCL (74%), and ABC-DLBCL (24%; Fig. 1A; Supplementary Table S1). Though they are classified as independent entities of mature B-cell neoplasms, the vast majority of CNS DLBCL (19), cutaneous DLBCL, leg-type (20), and testicular DLBCL (21) are of ABC origin. Mutation of MYD88 has not been found in IgG or IgA MGS, nor in multiple myeloma (22, 23). Therefore, IgM MGS is more closely related to LPL and other B-cell lymphomas and is segregated from IgG or IgA MGS, which are more closely related to multiple myeloma (23).

Origin of MYD88 L265P Mutation

Malignant B-cell neoplasms develop during various stages of normal B-cell differentiation and development (Fig. 1A; ref. 24). In humoral immune responses, B cells are activated by both protein and nonprotein antigens and secrete immunoglobulins to eliminate the antigen. In response to protein antigens, with the contribution of CD4+ helper T cells, B cells in the germinal center reaction are subjected to two distinct modifications: somatic hypermutation (SHM) and heavy-chain isotype (class) switching. Class switching alters the immunoglobulin heavy-chain class from IgM to IgG, IgA, or IgE and requires the induction of activation-induced cytokine deaminase (AID), which is also required by SHM. Many nonprotein antigens, such as polysaccharides, membrane glycolipids, and nucleic acids, are multivalent and can activate B cells without T cell help; these T-cell independent (TI) antigens stimulate immunoglobulin responses with limited class switching. MAR-15 activated mouse primary B cells of acquiring MYD88 L265P, L265P induces NF-kB activation and shifts B-cell proliferation, which are nevertheless rapidly countered by the induction of TNFAIP3 (i.e., A20) and extinguished by Bim-dependent apoptosis (25). In the presence of BCL2 overexpression or A20 inactivation, self-reactive B cells with MYD88 L265P continue to proliferate. A20 inactivation accompanies MYD88 L265P in ~24% of ABC-DLBCL (12) and A20 gene loss or inactivating mutation occurs in ~40% of WM (32). These results support that MYD88 L265P triggers the anti-apoptotic NF-kB signaling that may enable cell survival during B-cell development and yet needs a second somatic mutation to provide an advantage for continuous B-cell clonal selection (25). In WM, these two hits include TNFAIP3, CCR4, ARID1A/B, and CD79A/B (33); among them, CCR4 mutation is detected in 9% of IgM MGUS (34).

The second question is why does L265P heterozygous mutation predominate, particularly in WM? A possible explanation lies in the MYD88 protein structure and the disrupting power of the Proline residue. Proline is the strongest structural disruptor in the middle of α-helices and β-sheets and is commonly found in β-turns and polyproline II helices. The change of Leucine at position 265 to Proline in one of the β-sheets at the hydrophobic core (Fig. 1B) would radically disrupt the secondary structure of the TIR domain, resulting in stabilization of the core of the dimer interface of the MYD88-TIR domain (31) and an intrinsic propensity for augmented oligomerization of MYD88 to facilitate spontaneous formation of cytosolic Mydsosom aggregates in lymphoma cell lines. The L265P TIR domain, but presumably less so for other MYD88 mutants, cooperates with WT MYD88 for NF-kB hyperactivation via constitutive TIR-TIR oligomer formation (35). Based on these, one allelic copy of MYD88 L265P would be sufficient to initiate potent NF-kB signaling in lymphoma cells through TIR-TIR oligomerization.
MYD88 L265P mutation in B-cell malignancies and targeted therapy. A, Top, MYD88 L265P mutation frequency. Bottom, B-cell malignancy arises from different stages of B-cell development and differentiation. Red lines, pathogenic transformation; green lines, normal physiological B-cell development. MCL, mantle cell lymphoma; CLL, chronic lymphocytic leukemia; BL, Burkitt lymphoma; GCB-DLBCL, follicular lymphoma; MZL, marginal zone lymphoma; PTL, primary testicular DLBCL; LT, primary cutaneous DLBCL, leg type; CNS, primary CNS DLBCL; MM, multiple myeloma. B, Structure of a human MYD88 TIR monomer (Protein Data Bank accession number 2JS7). The α-helices are shown in bright plum, β-sheets in light yellow, and BB loop in blue; the lymphoma-associated mutations are marked by red triangles. C, MYD88 L265P gain-of-function mutation drives oncogenesis through regulation of kinases and transcription factors. D, MYD88 L265P can be targeted directly by small-molecule inhibitors and engineered TCRs or indirectly by attenuation of MYD88 L265P-activated kinases.
**Functional Consequences of MYD88 L265P Mutation**

The primary consequence of MYD88 L265P mutation is NF-κB activation, most likely through IRAK4 and IRAK1 (Fig. 1C; ref. 12). Phosphorylation of endogenous IRAK1 is observed in ABC- but not GCB-DLBCL cells, and phosphorylated IRAK1 (pIRAK1) interacts with MYD88 L265P but not with WT MYD88 or other MYD88 mutants (12). Thus, MYD88 L265P forms a signaling complex that includes pIRAK1 in ABC-DLBCL. A selective small-molecule inhibitor of IRAK1 and IRAK4 induces cell death in ABC-DLBCL cell lines but not in GCB-DLBCL and myeloma cell lines (12). Therefore, ABC-DLBCL relies upon IRAK4 and pIRAK1 to transduce signals from MYD88 L265P that promote cancer cell survival. JAK–STAT3 signaling gene signature overlapped significantly with the MYD88- and IRAK1-regulated gene signatures in ABC-DLBCL (12). Knockdown of MYD88 significantly diminished the secretion of IL6, IL10, and IFNβ and the phosphorylation of STAT3 in ABC-DLBCL cells. In addition, IL6, IL10, and IFNβ secretion was blocked by IRAK1/4 kinase inhibitor or MYD88 knockdown, indicating that MYD88 mutations, along with IRAK1/4, contribute to JAK–STAT3 and type I interferon signaling in ABC-DLBCL. IFNβ-mediated type I interferon signaling is proapoptotic. Rui and colleagues demonstrated that STAT3 is a critical transcriptional regulator of ABC-DLBCL, and STAT3 upregulates genes related to NF-κB, PI3K–AKT–mTORC1, and ERK/C–JNK cell-cycle checkpoint and downregulates the expression of IRF7, IRF9, STAT1, and STAT2 in ABC-DLBCL cell lines with MYD88 L265P (36). Thus, STAT3 accentuates survival signaling pathways while dampening the lethal type I interferon pathway in ABC-DLBCL cells with MYD88 L265P.

Bruton tyrosine kinase (BTK) is a critical node in B-cell receptor (BCR) signaling cascades, mediating the signal from BCR to downstream pathways such as NF-κB, PI3K/AKT, and NFAT (37). MYD88 is preferentially complexed to phosphorylated BTK (pBTK) in WM cells with MYD88 L265P, yet little complexing is observed in lymphoma cells with WT MYD88 (38). The level of pBTK is higher in WM cells with MYD88 L265P than lymphoma cells with WT MYD88. Importantly, inhibition of either BTK or IRAK1–4 induced WM cell apoptosis. These data not only establish BTK as a downstream target of MYD88 L265P, but also provide a novel strategy to treat WM and other MYD88 L265P-expressing tumors (38). In an ensuing study, MYD88 L265P is found to be associated with hematopoietic cell kinase (HCK), a member of the SRC family of protein tyrosine kinases, and one of the most aberrantly upregulated genes in WM cells (39). MYD88 L265P overexpression triggered HCK and IL6 transcription via NF-κB activation, whereas HCK knockdown significantly reduced cell survival and attenuated BTK, PI3K/AKT, and MAPK/ERK signaling in WM and ABC-DLBCL cells (40). Heterozygous MYD88 L265P expression in HEK293 cells led to higher MAPK/ERK phosphorylation and higher NF-κB activation than that of WT MYD88 (41). Reinhardt and colleagues generated a B-cell-specific conditiona\

**MYD88 L265P in Therapeutic Implications**

Based on the functional consequence of MYD88 L265P activity, several strategies have been devised to halt its oncogenic activation by targeting IRAK1/4, JAK, and, the most successful target, BTK, or by disrupting the myddosome assembly. Moreover, MYD88 L265P-containing peptides can elicit human leukocyte antigen (HLA) class I–restricted cytotoxic T-cell responses (46, 47), supporting the potential for T-cell receptor (TCR)-based immunotherapy (Fig. 1D).

The BTK inhibitor ibrutinib (also known as PCI-32765) reduces the binding strength of BTK to MYD88 L265P. Inhibition of either BTK or IRAK1/4 induced apoptosis of WM cells, and their combination resulted in more robust inhibition of NF-κB signaling and synergistic WM cell killing (38, 48, 49). In a phase I/II clinical trial that involved 80 patients with relapsed or refractory DLBCL, ibrutinib produced complete or partial responses in 37% of those with ABC-DLBCL (50). ABC tumors with BCR mutations responded to the drug (56%), especially those with concomitant MYD88 mutation (80%), in agreement with in vitro data on BCR and MYD88 pathways (50). In a phase II trial of patients (n = 63) with relapsed or refractory WM, patients received ibrutinib until disease progression or unacceptable toxicity occurred (51); the objective response rate was 91%, and the major response rate was 73% (51). It was therefore concluded that ibrutinib was highly active, associated with durable responses, and safe in patients with WM who had previously received therapy (51). Based on these data, ibrutinib (Imbruvica) was approved for the treatment of WM (52). Ibrutinib is particularly effective in CNS DLBCL patients: 94% showed tumor reductions with ibrutinib alone and 86% of evaluable patients achieved complete remission with ibrutinib plus chemotherapy (53).

Compared with WT MYD88, mutated MYD88 protein has enhanced IRAK4 and pIRAK1 binding and can form the myddosome without external stimuli, thus heightening canonical NF-κB signaling in MYD88 L265P WM cell lines (12, 38). Using minipeptides designed to compete with MYD88 TIR and DD domain interactions to disrupt the myddosome self-assembly, Liu and colleagues found that expression of two MYD88 minipeptides (MYD8881–202 and MYD8840–85) blocked the growth of MYD88 L265P BCWM.1 and MWC1–1 cells, but not that of WT MYD88 Ramos cells (54). Furthermore, these minipeptides induced apoptosis, reduced IRAK1 phosphorylation, and attenuated NF-κB activation in MYD88 L265P WM cells (54). Inhibition of MYD88 homodimerization...
using another peptide IMG-2005-5 (IMGENEX) reduced NF-κB activation in WM cell lines (BCWM.1 and MWCL-1) expressing MYD88 L265P (14). Olson and coworkers performed high-throughput computational screening to identify drug-like inhibitors of MYD88 homodimer formation; biochemical and cellular assays revealed that a promising compound, T6167923, disrupts MYD88 homodimer formation by targeting its TIR domain, though its efficacy for MYD88 L265P was not tested (55). These data indicate that interference of mydosome assembly is an attractive option for developing target therapies for WM and other lymphoproliferative disorders driven by MYD88 L265P.

Tumor-specific neoepitopes, derived from protein-altering mutational events like missense mutations, may be perceived as foreign by the human immune system and induce tumor-specific T-cell immunity (56, 57). Growing evidence demonstrates that therapeutic peptide-vaccines that target tumor neoepitopes elicit specific immune responses and improve outcome (58, 59). MYD88 L265P is one such tumor-specific mutation. Based on in silico predictions, Nelde and colleagues identified potential MYD88 L265P-containing ligands of HLA for several HLA class I restrictions (46). A few MYD88 L265P-derived ligands elicited specific cytotoxic T-cell responses for HLA-B07 and -B15. The HLA-B15-restricted peptide is HQKRPIPIKY (named P4B15; the L265P is underlined). P4B15-specific CD8+ T cells from healthy blood donors showed IFNγ and TNFα secretion after stimulation with the peptide P4B15, but not with the WT peptide. Furthermore, P4B15-specific CD8+ T cells elicited mutant peptide-restricted cytotoxicity (46). In a separate study, Nielsen and colleagues assessed T cells from healthy donors for recognition of MYD88 L265P-containing peptides and identified CD8+ T cells against RPIPIKYKA when presented by HLA-B07:02 (47). In both cases, healthy individuals harbor T cells with specific TCRs that recognize L265P-containing neoantigens restricted by MHC, highlighting the potential of TCR recognition of MYD88 L265P-derived peptides as a novel personalized therapeutic strategy.

Summary and Future Directions

To summarize, MYD88 L265P is a prevalent somatic mutation in patients with WM, IgM MGUS, ABC-DLBCL, and other non-Hodgkin lymphomas. The discovery of this somatic mutant affords us numerous opportunities to improve lymphoma diagnosis and treatment. Further dissection of MYD88 L265P at the molecular and cellular levels and using mouse models will produce insights into the underlying mechanisms of its oncogenic action and devise novel avenues for drug development against lymphoid malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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