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MYD88 L265P mutation promoted malignant B cell resistance against T cell-mediated cytotoxicity via upregulating the IL-10/STAT3 cascade

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ABSTRACT

The myeloid differentiation factor 88 (MYD88) signaling plays critical roles in the developments of B cells. Recent studies demonstrated that in the activated B cell subtype of diffuse large B cell lymphoma (DLBCL), approximately one-third of the patients harbored somatically acquired MyD88 L265P mutation in their lymphomas. It remains unclear whether B cell lymphomas with MYD88 L265P mutation respond differently toward $CD8^+$ T cell-mediated cytotoxicity. Here, we demonstrated that, when incubated with autologous $CD8^+$ T cells, the MYD88 L265P mutant lymphomas were more resistant to granzyme B- and perforin-mediated killing than MYD88 wild-type (WT) lymphomas. Interestingly, in the absence of autologous lymphomas, the granzyme B and perforin expression levels in $CD8^+$ T cells from patients with MYD88 WT lymphomas and from patients with MYD88 L265P mutant lymphomas were comparable; however, in the presence of autologous lymphomas, the $CD8^+$ T cells from patients with MYD88 L265P mutant lymphomas presented significantly lower granzyme B and perforin expression than $CD8^+$ T cells from patients with MYD88 WT lymphomas. We further found that the IL-10 expression level and the STAT3 activation level were significantly higher in MYD88 L265P mutant lymphomas than in MYD88 WT lymphomas. Suppressing IL-10 significantly reduced STAT3 activation in both MYD88 WT and MYD88 L265P mutant lymphomas. Blocking either STAT3 or IL-10 could significantly increase the susceptibility of MYD88 L265P mutant lymphomas toward $CD8^+$ T cell-mediated cytotoxicity. Together, these data revealed a mechanism of immune evasion in MYD88 L265P mutant lymphomas.

1. Introduction

The myeloid differentiation factor 88 (MYD88) gene encodes an adaptor protein used by the IL-1 receptor (IL-1R) and by all toll-like receptors (TLRs) except TLR3 [1]. The protein MYD88 has three major structures, including an N-terminal death domain, a C-terminal Toll/IL-1R domain, and a linker domain [2]. Upon ligand binding, the cytoplasmic region of TLRs or IL-1R engages with MYD88, which then recruits IRAK4, a serine-threonine kinase. IRAK4 then interacts and phosphorylates IRAK1 and IRAK2 [3], which transmit inflammatory signals further downstream and ultimately result in the activation of NF-κB and NF-κB controlled genes [1]. MYD88 can also interact with IRF-7 directly without IRAKs [4]. Via these pathways, MYD88 activation promotes the production of pro-inflammatory and anti-inflammatory cytokines, as well as type I interferon (IFN) [5,6]. In addition, B cell-intrinsic MYD88 signaling is required for the migration, proliferation, and class switch recombination in activated B cells [7,8]. B cells with MYD88 deficiency fail to form protective antibody responses following vaccination [9,10].

Diffuse large B cell lymphoma (DLBCL) is an aggressive B cell lymphoma commonly categorized into two major subtypes, including the germinal center B cell (GCB) subtype and the activated B cell (ABC) subtype, which are biologically and molecularly distinctive [11]. Most DLBCL cases are treated with the R-CHOP regimen, a combination of the monoclonal anti-CD20 antibody rituximab and four chemotherapeutic drugs (cyclophosphamide, doxorubicin, vincristine, and prednisone). Under the current treatment strategy, approximately 40% of patients exhibit a refractory or relapsed disease, which has limited treatment options. The ABC subtype is especially difficult to treat with < 40% success rate [12]. Compared to the GCB-DLBCL, the ABC-DLBCL presents higher activation of NF-κB and NF-κB controlled genes. Mutations in the NF-κB modifiers and B cell receptor (BCR) proximal components are identified in a subset of ABC-DLBCL [13,14]. In addition, Ngo et al. found that 29% of ABC-DLBCL biopsies contained a L265P somatic mutation in the MYD88 gene [15]. This mutation is rare or absent in GCB-DLBCL and Burkitt's lymphoma. Unlike the wild-type

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Table 1
Characteristics of study subjects.

Characteristics	WT	L265P	P
N	30	20	
Female (N, %)	14, 47	7, 35	> 0.05
Age in years (mean, SD)	60.4, 7.2	62.3, 6.6	> 0.05
Stages (N, %)			> 0.05
II	5, 17	4, 20	
III	15, 50	8, 40	
IV	10, 33	8, 40	

(WT) MYD88 isoform, the MYD88 L265P mutant could spontaneously form a protein complex with IRAK1 and IRAK4, and initiates a series of downstream activation events ultimately leading to the phosphorylation of STAT3. Phosphorylated STAT3 then synergizes with NF-κB to promote cell survival [16,17]. Meta-analysis of 40 studies in over 2000 DLBCL patients found that this MYD88 L265P mutation was significantly associated with older age and poorer survival [18]. Together, these results illustrated the MyD88 L265P mutation could contribute to a more aggressive disease in B cell lymphomas.

In recent years, cytotoxic T cells expressing anti-CD19 chimeric antigen receptor (CAR) are being tested for treating refractory/relapsing DLBCL and are showing great therapeutic potential [19]. However, the patient response to CAR-T is highly variable, possibly related to differences in the individual lymphoma genome. It is yet unclear how B cell lymphomas with MYD88 L265P mutation would respond in the presence of cytotoxic T cells. In this study, we investigated the interactions between cytotoxic T cells and MyD88 WT and MyD88 L265P mutant lymphomas.

2. Materials and methods

2.1. Identification of MYD88 WT and MYD88 L265P mutant in ABC-DLBCL patients

This study was approved by the Ethical Review Committee of Changhai Hospital. ABC-DLBCL was diagnosed by immunohistochemistry staining and the Hans algorithm at Changhai Hospital [20]. Peripheral blood samples were gathered from untreated ABC-DLBCL patients and processed with standard Ficoll-Paque PLUS (GE Healthcare) gradient centrifugation to isolate mononuclear cells. The B cell lymphomas were then extracted using the Pan B Cell Isolation kit, human (Myltenyi Biotec). The MYD88 WT and L265P mutant identification on the MYD88 transcripts was then performed using previously established sequencing methods with minor modifications [15]. Total RNA was then extracted using the Trizol reagent (Invitrogen), and the mRNA was reverse transcribed into cDNA using the High-Capacity cDNA Reverse Transcription kit (Invitrogen). The MYD88 cDNA was then amplified using PCR and sequenced using Sanger sequencing with previously published primers [15]. A total of 20 ABC-DLBCL patients carrying the MYD88 L265P mutation and 30 ABC-DLBCL patients carrying the MYD88 WT were recruited. The age, gender, and staging of the patients are listed in Table 1.

2.2. Cytotoxicity assay

Isolated B cell lymphomas were loaded with 50 μCi of radioactive chromium-51 (PerkinElmer), washed, and loaded into a 96-well U-bottom plate (Corning) at 5×10^3 cells per well. For STAT3 inhibition, lymphoma cells were first incubated with S31–201 (Santa Cruz Biotechnology) at various concentrations for 6 h and washed twice

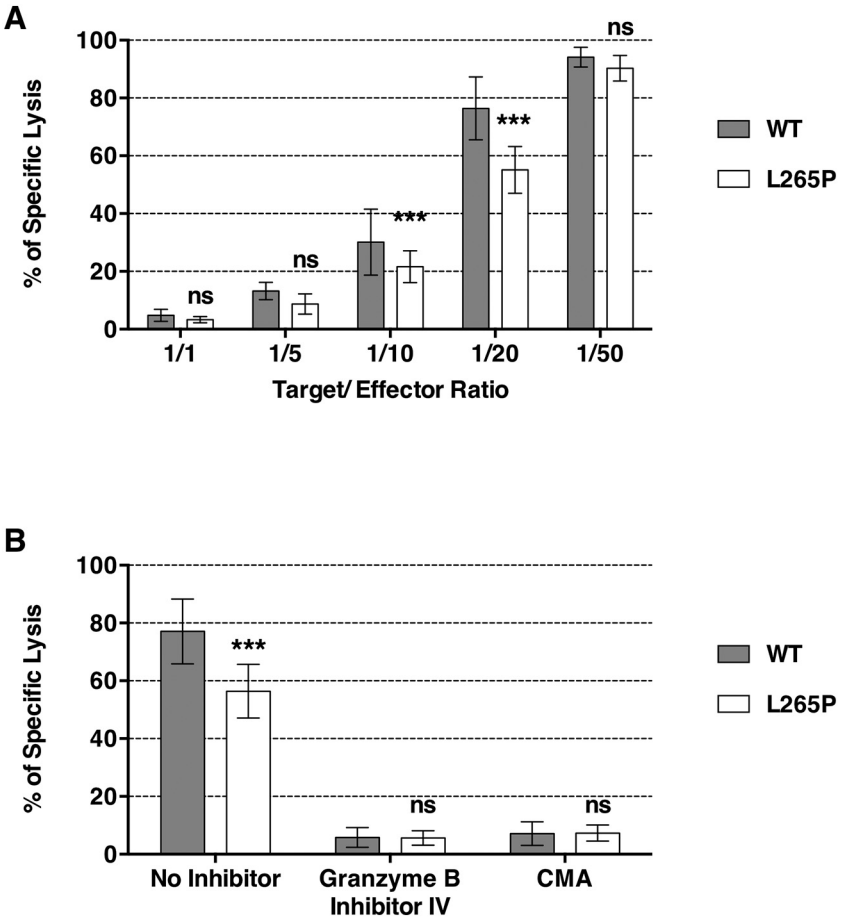

Fig. 1. Cytotoxicity of $CD8^+$ T cells toward autologous MYD88 WT or L265P mutant lymphomas. (A) Circulating $CD8^+$ T cells from each subject carrying MYD88 WT or MYD88 L265P mutant lymphomas were isolated, activated by anti-CD3/CD28 stimulation, and co-incubated with autologous WT or L265P lymphomas for 6 h at various target/effector ratios. The specific lysis was then measured using a chromium-51 release assay. (B) The specific lysis of activated circulating $CD8^+$ T cells toward autologous WT or L265P lymphomas at 1/20 target/effector ratios, without inhibitors, or with granzyme B inhibitor IV or CMA at 2 μg/mL each after 6 h co-incubation. 2-way ANOVA followed by Sidak's test. ns, not significant. ***P < 0.001. Data were represented as mean ± standard deviation.