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Loss of *ETV6* enhances lymphomagenesis in a murine model of diffuse large B cell lymphoma in a cell-of-origin dependent manner

S. Höfmann[1], A. Tabatabai[1], R. Flümann[2, 3], S. Klein[1], I. Kisis[1], R. Öllinger[4], M. Möllmann[1], M. Hartnack[1], S. Kesper[1], A. Arora[1], B. v. Tresckow[1], R. D. Jachimowicz[2, 5], R. Rad[4, 6], G. Knittel[1], H.C. Reinhardt[1]

[1] *University Hospital Essen, Department of Hematology and Stem Cell Transplantation, Essen, North Rhine-Westphalia, Germany*
[2] *University of Cologne, Faculty of Medicine and University Hospital Cologne, Department I of Internal Medicine, Center for Integrated Oncology, Aachen Bonn Cologne Duesseldorf, North Rhine-Westphalia, Germany*
[3] *University of Cologne, Center for Molecular Medicine, Cologne, North Rhine-Westphalia, Germany*
[4] *Technische Universität München, Institute of Molecular Oncology and Functional Genomics, School of Medicine, Munich, Bavaria, Germany*
[5] *Max Planck Institute for Biology of Ageing, Cologne, North Rhine-Westphalia, Germany*
[6] *Technische Universität München, Center for Translational Cancer Research (TranslaTUM), School of Medicine, Munich, Bavaria, Germany*

DLBCL is a highly heterogeneous disease and the most common non-Hodgkin lymphoma. The MCD genetic subtype harbors recurrent *CD79B* and *MYD88* mutations affecting B cell receptor- (BCR) and Toll-like receptor signaling. B cell-specific expression of the murine *MYD88* p.L252P orthologue in $Myd88^{cond\_p.L252P}$;$Cd19^{Cre}$ (MC) mice develop a mild lymphoproliferative phenotype and occasional lymphoma, dramatically enhanced by *BCL2* overexpression (MBC) and *Prdm1* deletion (PPMBC).

To identify modifiers of *Myd88*-driven lymphomagenesis, we performed an *in vivo piggyBac (PB)* insertional mutagenesis screen. Mice carrying a conditional transposase and mobilizable transposons, which can activate or disrupt genes upon reintegration, were crossed to MC animals. *Myd88*/*PB* mice showed reduced overall survival and increased lymphoma development. Clonal $B220^{+}$/$CD138^{-}$ lymphomas displayed signs of somatic hypermutation, indicating germinal center (GC) origin. QiSeq and bioinformatic analysis of tumor DNA identified ~350 genes with significantly enriched integrations. A KEGG gene set enrichment analysis highlighted BCR signaling, NFκB activation and apoptosis, reflecting MCD features. We also detected recurrent MCD mutations, including *Pim1*, *Tbl1xr1* and *Etv6*.

To investigate the role of ETV6 in lymphomagenesis, we introduced an *Etv6*-flox allele into our lymphoma mouse models, representing distinct cell of origins. $Myd88^{cond\_p.L252P/wt}$;$Rosa26^{LSL.BCL2\text{-}IRES\text{-}GFP/wt}$;$Cd19^{Cre/wt}$ (MBC) mice develop $B220^{-}$/$CD138^{+}$ plasmablastic/plasmacytic lymphomas, whereas additional *Prdm1* loss ($Prdm1^{flox/flox}$; PPMBC mice) results in $B220^{+}$/$CD138^{-}$ clonal lymphomas with memory/light zone features. Additional *Etv6* loss drastically reduced survival in PPMBC mice (median 20 vs. 37 wks) and also accelerated lymphomagenesis in MC animals compared to controls. In contrast, *Etv6* loss did not affect plasmablastic MBC disease dynamics, suggesting a differentiation stage-dependent role of *Etv6* in lymphomagenesis. Developing tumors were characterized transcriptionally, genetically and histologically.

To assess the role of *ETV6* in GC response, an NP-OVA immunization was performed in mice carrying a conditional *Etv6*-flox allele in combination with $Ighg1^{Cre}$. $Etv6^{flox/wt}$ and $Etv6^{flox/flox}$ animals showed reduced NP-specific GC B cells compared to control animals, decreased apoptosis in NP-specific LZ GC B cells, increased proliferation, and less NP-specific plasmablasts and plasma cells, indicating a less

efficient GC output.