THE DIRECT AND STRUCTURAL UNIQUE INTERACTION OF LMP1 AND TRAF6 MEDIATES LMP1 SIGNALING AND VIRUS-DRIVEN B CELL LYMPHOMA

Fabian Giehler[1,2,3], Michael S. Ostertag[4], Thomas Sommermann[5], Daniel Weidl[6], Kai R. Sterz[2], Helmut Kutz[1,2], Brigitte Biesinger[6], Grzegorz M. Popowicz[4], Johannes Kirchmair[7], Arnd Kieser[1,2,3]

[1]Institute for Molecular Toxicology and Pharmacology, Helmholtz Center Munich - German Research Center for Environmental Health (HMGU), 81377 Munich, Germany; [2]Research Unit Gene Vectors, HMGU, Munich, Germany; [3] German Center for Infection Research (DZIF), Partner Site Munich, Germany; [4] Institute of Structural Biology, HMGU, 85764 Neuherberg, Germany; [5] Immune Regulation and Cancer, Max Delbrück Center for Molecular Medicine, 13125 Berlin, Germany; [6]Institute of Clinical and Molecular Virology, University Erlangen-Nuremberg, 91054 Erlangen, Germany; [7] Division of Pharmaceutical Chemistry, Department of Pharmaceutical Sciences, University of Vienna, 1090 Vienna, Austria

E-mail a.kieser@helmholtz-muenchen.de

Introduction: Epstein-Barr virus (EBV) latent membrane protein 1 (LMP1) drives viral B cell transformation and oncogenesis. LMP1's transforming activity depends on its cytoplasmic C-terminal activation region 2 (CTAR2), which induces NF-kappaB and JNK by engaging TNF receptor-associated factor 6 (TRAF6). The molecular mechanism of TRAF6 interaction with LMP1 and its critical role in LMP1 signaling remain unknown.

Objectives: We aimed at identifying the molecular basis of LMP1 interaction with TRAF6 to clarify the role of TRAF6 in LMP1 signaling and EBV-driven lymphoma development.

Methods: We established AlphaScreen-based protein-protein interaction (PPI) assay technology with recombinant proteins to characterize and quantify the interaction of LMP1 WT and mutants with TRAF6 WT and mutants. An *in silico* model of the LMP1-TRAF6 structure was generated and further validated by mutant analysis as well as NMR spectroscopy studies of the LMP1-TRAF6 complex. Confocal immunofluorescence and rescue experiments in TRAF6-KO cells were applied to confirm the mechanism of LMP1-TRAF6 interaction and its role in LMP1 signaling in vivo. The CRISPR/CAS9-mediated knockout of TRAF6 in LMP1-driven B cell lymphomas derived from a transgenic mouse model provided insight into the role of TRAF6 in lymphoma development. Cell-penetrating peptides were used to block the LMP1-TRAF6 interaction in lymphoblastoid cells (LCLs) to demonstrate the relevance of this complex for LCL survival.

Results: We demonstrate that TRAF6, but no other TRAF protein, interacts directly with the novel TRAF6 binding motif P379VQxxY384 within CTAR2 of LMP1. TRAF6 is, thus, the first identified cellular factor whose binding site exactly matches the signaling-active site of CTAR2. Structural modeling supported by in vitro and in vivo mutant analysis of LMP1 and TRAF6 provides insight into the architecture of the LMP1-TRAF6 complex and reveals substantial differences to CD40-TRAF6 interaction. NMR spectroscopy further confirms the structural model and reveals shifting of TRAF6 residues upon LMP1 binding. The direct recruitment of TRAF6 to LMP1 is essential for NF-kappaB activation and the survival of LMP1-driven B cell lymphoma cells. Disruption of the LMP1-TRAF6 complex by inhibitory peptides interferes with the survival of EBV-transformed B cells.

Conclusion: We identify and characterize the LMP1-TRAF6 complex as critical virus-host interface and validate this interaction as novel therapeutic target against EBV.