ABSTRACT

Tick-borne flaviviruses (TBFVs) can cause life-threatening encephalitis and hemorrhagic fever. The means by which TBFVs subvert the immune system and commandeer cellular machinery largely remain unknown. To identify virus-host interactions that may be exploited as new flavivirus therapeutic targets, we analyzed the TBFV polyprotein for known binding motifs of antiviral proteins and found four putative tumor-necrosis factor receptor-associated factor 6 (TRAF6) binding motifs (TBM) within the viral nonstructural 3 protein (NS3). TRAF6 is an E3 ubiquitin ligase that is associated with antiviral innate immune signaling. NS3 plays essential enzymatic roles in cleaving the viral polyprotein and replication of the viral genome, making it an ideal antiviral target. Here we report that NS3 from Langat virus (LGTV), a prototypical TBFV, interacted with TRAF6 during infection, as revealed by co-precipitation assay and confocal microscopy. To determine whether the NS3-TRAF6 interaction inhibits TBFV replication, we infected TRAF6-/- fibroblasts with LGTV. Surprisingly, we found the replication of LGTV in TRAF6-/- fibroblasts was decreased up to 14.2-fold when compared to wild-type cells. Assessment of the highly virulent BSL-4 TBFVs, tick-borne encephalitis virus and Kyasanur Forest disease virus, also found that TRAF6 is needed for optimal replication. The proviral role of TRAF6 appears to specific to TBFVs, as replication of the mosquito-borne flaviviruses, West Nile virus and Kunjin virus, was enhanced, rather than restricted, in the absence of TRAF6. Site-directed mutagenesis revealed that an E117A mutation located in the second TBM of NS3 resulted in disruption of TRAF6 binding and 82.5% reduction of protease activity. Furthermore, replication of LGTV with an E117A mutation in NS3 was attenuated up to 17.5-fold *in vitro* compared to wild-type virus. Taken together, these studies reveal new insights into how flaviviruses exploit innate immune system signaling for the purpose of viral replication and identify a potential target for therapeutic design.

COLLEGE OF MEDICINE AND LIFE SCIENCES
THE UNIVERSITY OF TOLEDO

DISSERTATION COMMITTEE

R. Travis Taylor, Ph.D., (Mentor)

Saurabh Chattopadhyay, Ph.D.

Malathi Krishnamurthy, Ph.D.

Stanislaw Stepkowski, D.V.M., DSc., Ph.D.

R. Mark Wooten, Ph.D.

Graduate School Representative

Kevin Pan, M.D. Ph.D.

Medical Microbiology and Immunology (MMI) Track

Department of Medical Microbiology & Immunology

THE UNIVERSITY OF TOLEDO
1872

DISSERTATION PRESENTATION

by

Brian Youseff

May 24, 2018

The Role of Tumor Necrosis Factor Receptor-Associated Factor 6 (TRAF6) in Tick-Borne Flavivirus Infection

Ph.D. in Biomedical Sciences

AWARDS

2017 - Earl H. Freimer M.D./Ph.D. Award

2017 - Robert R. Buell Memorial Achievement Award

2016 - Council of Biomedical Graduate Students 2016 Graduate Research Forum, Finalist, Poster Presentation, 1st Place

2015 - Professor Daniel A. Koechel Scholarship

2015 - Council of Biomedical Graduate Students 2015 Graduate Research Forum, Finalist, Poster Presentation, 1st Place

2014 - Selected to attend NIAID/IDSA Infectious Disease Research Careers Meeting

2014 - Council of Biomedical Graduate Students 2014 Graduate Research Forum , Finalist, Poster Presentation, 2nd Place

2014 - University of Toledo College of Medicine Honors Scholarship

2013 - University of Toledo College of Medicine Honors Scholarship

2011– present University of Toledo M.D./Ph.D. Program Scholarship

PUBLICATIONS

Youseff BH, Brewer TG, McNally K, Izuogu AO, Lubick KJ, Presloid JB, Alqahtani S, Chattopadhyay S, Best SM, Hu X, RT Taylor. 2018. A proviral role of TRAF6 in tick-borne, but not mosquito-borne, flavivirus infection through interaction with the NS3 protease domain. *Cell Host Microbe (in preparation)*

Chiramel AI, Meyerson N, Montoya V, McNally K, Mendes-Solis O, Robertson S, Bouamr F, Sturdevant G, Lubick K, Nair V, **Youseff BH**, Hirsch V, Taylor RT, Sawyer S, and S Best. 2018. TRIM5a restricts flavivirus replication by targeting the viral protease for proteasomal degradation. *Immunity. (under revision)*

Izuogu AO, McNally K, Harris S, **Youseff BH**, Burlak C, Munshi-South J, Best SM, and RT Taylor. 2017. Interferon signaling in Peromyscus leucopus confers a potent and specific restriction to vector-borne flaviviruses. *Plos One 2017 Jun 26; 12(6):e0179781*

Youseff BH. 2016. UT researchers focus on Zika virus treatment. *Toledo Blade*

PRESENTED ABSTRACTS

2016 Positive-Strand RNA Viruses (N1), Keystone Symposia on Molecular and Cellular Biology

Understanding the role of TRAF6 in tick-borne flavivirus infection.

Brian H. Youseff, Kirk J. Lubick, Sonja M. Best, R. Travis Taylor

2016 Council of Biomedical Graduate Students Graduate Research Forum

Understanding the role of TRAF6 in tick-borne flavivirus infection.

Brian H. Youseff, Kirk J. Lubick, Sonja M. Best, R. Travis Taylor

2015 Council of Biomedical Graduate Students Graduate Research Forum

The proviral role of TRAF6 during flavivirus infection.

Brian H. Youseff, John B. Presloid, R. Travis Taylor

FUTURE PLANS

Brian plans on completing his final year of medical school and applying to residency programs in the specialty of family medicine.