ABSTRACT

The interferon (IFN) system is the first line of defense against viral infections. The recognition of viral infection by pattern recognition receptors (PRRs) results in the transcriptional induction of IFN. IFN, secreted from the virus-infected cells, acts on infected and yet uninfected cells to trigger the transcription of IFN-stimulated genes (ISGs). All biological functions of IFN are executed by the ISG-encoded protein products that inhibit virus replication. Due to the virus-specific nature, and replication stage-specific action, of the ISGs, there is a need to identify new antiviral ISGs and understand the mechanisms of their action. We carried out a high-throughput screen, using an shRNA library of human ISGs, to identify novel antiviral ISGs against Sendai virus (SeV), a model paramyxovirus. The screen isolated a novel antiviral ISG, Tudor domain containing protein 7 (TDRD7), which we studied in-depth for its antiviral function, using knockout, knockdown and ectopic expression systems. TDRD7 deficiency led to increased virus replication, and reciprocally, ectopic expression of TDRD7 caused inhibition of SeV replication. TDRD7 inhibited cellular autophagy pathway, which was activated by SeV infection and required for its replication, to exhibit its antiviral activity. Further studies revealed that TDRD7 inhibited autophagy by blocking the activation of AMP-dependent kinase (AMPK), which is required for the initiation of autophagy pathwa. y. The antiviral activity of TDRD7 was further expanded to human parainfluenza virus (HPIV3) and respiratory syncytial virus (RSV), two closely related RNA viruses, which also require AMPK-mediated autophagy pathway. We further explored the antiviral activity of TDRD7 against HSV-1, which also activates the autophagy pathway. Our results, supported by the current literature, indicate that HSV-1 induced, but did not require, cellular autophagy for its replication. We validated this using chemical inhibitors as well as genetic manipulation of the autophagy pathway. Surprisingly, HSV-1 replication was dependent on AMPK activity but not its autophagy pathway. Genetic and chemical inhibition of AMPK, in the absence or the presence of autophagy, inhibited HSV-1 replication *in vitro*. TDRD7 inhibited HSV-1-induced AMPK activation to block virus replication in human and mouse cell types, and the antiviral activity of TDRD7 was dependent on AMPK activity. Finally, we examined whether inhibition of AMPK in vivo restricts virus replication. The AMPK inhibitor *in vivo* resulted in suppression of SeV and HSV-1 replication in a mouse infection model. Therefore, our studies revealed that the host IFN system uses a previously unrecognized antiviral strategy by inhibiting the virus-activated AMPK, to inhibit both autophagy-dependent and independent viruses

COLLEGE OF MEDICINE AND LIFE SCIENCES
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THE UNIVERSITY OF TOLEDO
1872

DISSERTATION PRESENTATION

by

Gayatri Subramanian

April 30th, 2020

TDRD7, a novel viral restriction factor, inhibits cellular AMP-dependent kinase to inhibit virus replication

Ph.D. in Biomedical Sciences

AWARDS/ LEADERSHIP

2020 Recipient of the COMSLS Class of 2020, Doctor of Philosophy in Biomedical Sciences Program Outstanding Student.

2019 Recipient of the 2019-2020 Graduate Research Award from the University of Toledo Graduate Student Association (GSA) and College of Graduate Studies that provides $2000 towards laboratory expenses based on a grant-style application submitted by a graduate student.

2019 Student Travel Award to attend the Midwest Virology Symposium (MVS) 2019 hosted by Ohio State University, abstract selected for short talk

2019 First place for oral presentation at the Graduate Research Forum (GRF) 2019, University of Toledo, College of Medicine and Life Sciences

2019 Winner of the Three Minute Thesis® (3MT®) 2019 competition, at the University of Toledo and represented University of Toledo at the Midwestern Association for Graduate Studies (MAGS 2019), at St. Louis, Missouri

2019 Health Science Campus Volunteer at the 10th annual WISDOM (Women in STEMM Day of Meetings), May 9, 2019 (held on both UT campuses). The purpose is to increase awareness and enthusiasm for STEMM fields, with the long-term goal of helping to bridge the gender gap in these fields.

2019 Subramanian, G. 2019. Battling the cause, not the symptoms, of viruses. Toledo Blade.

2018 Recipient of the UTRA Scholarship Award (2018-2019) of $1500 from the UT Health Science Campus Retirees Scholarship Fund for scientific productivity and community service.

2018 President for the Council of Biomedical Graduate Students (CBGS) for the year 2017-2018, at the University of Toledo.

2018 Student Travel Award to attend the 37th Annual Meeting of the American Society for Virology (ASV 2018) meeting hosted by the University of Maryland

2018 Finalist in the Three Minute Thesis® (3MT®) 2018 competition (inaugural year) at the University of Toledo

2018 Secretary for the Council of Biomedical Graduate Students (CBGS) for the year 2016-2017, at the University of Toledo.

2016 Travel grant for ZIBI-DAAD Summer School 2016 - Interdisciplinary Centre of Infection Biology and Immunity. (received travel grant but unable to attend)

2015 First Year Representative at the Council of Biomedical Graduate Students (CBGS) for the year 2015-2016, at the University of Toledo.

PUBLICATIONS

Subramanian G, Popli S, Chakravarty S, Taylor R.T, Chakravarti R, Chattopadhyay S. (2020) The interferon-inducible protein TDRD7 inhibits AMP-activated protein kinase and thereby restricts autophagy-independent virus replication. JBC

Glanz A, Chawla K, Fabry S, **Subramanian G**, Garcia J, Jay B, Ciricillo J, Chakravarti R, Taylor R.T, Chattopadhyay S.(2020), High Throughput Screening of FDA-Approved Drug Library Reveals the Compounds that Promote IRF3-Mediated Pro-Apoptotic Pathway Inhibit Virus Replication Viruses

Goswami R, **Subramanian G**, Silayeva L, Newkirk I, Doctor D, Chawla K, Chattopadhyay S, Chandra D, Chilukuri N and Betapudi V (2019) Gene Therapy Leaves a Vicious Cycle. Front. Oncol

Subramanian G, Chakravarti R, Chattopadhyay S. Ifi204/p204, a new piece in the sepsis puzzle. Ann Transl Med (2018);6(Suppl 1):S12. doi: 10.21037/atm.2018.09.22

Subramanian G, Kuzmanovic T, Zhang Y, Peter CB, Veleeparambil M, Chakravarti R, Sen GC and Chattopadhyay S. (2018). A new mechanism of interferon's antiviral action: induction of autophagy, essential for paramyxovirus replication, is inhibited by the interferon stimulated gene, TDRD7. PLOS Pathogens 14(1): e1006877.

Subramanian, G., Pan, K., Chakravarti, R. and Chattopadhyay, S. (2016). Biochemical Analysis of Caspase-8-dependent Proteolysis of IRF3 in Virus-infected Cells. *Bio*

FUTURE PLANS

Gayatri is currently interviewing for postdoctoral fellowships.

PRESENTED ABSTRACTS

Subramanian, G. and Chattopadhyay, S. Interferon-inducible protein TDRD7 inhibits the autophagy-initiating kinase, AMPK to inhibit virus replication. Midwest Virology Symposium (MVS) 2019 (Poster Presentation and selected for short talk)

Subramanian G, Glanz A, Popli S, Chakravarty S and Chattopadhyay S.TDRD7, a new antiviral mechanism of the interferon system. Midwest Microbial Pathogenesis Conference (MMPC) 2019 (Poster Presentation)

Subramanian, G. and Chattopadhyay, S. TDRD7 – a new antiviral effector of the interferon system. Council of Biomedical Graduate Students Graduate Research Forum, University of Toledo Health Science Campus, Toledo, Ohio, March 2019. (Oral Presentation)

Chattopadhyay, S., **Subramanian, G.**, and Chawla K. A genetic screen to identify new antiviral mechanisms of the interferon system. Midwest Microbial Pathogenesis Conference (MMPC) 2018 (Oral Presentation)

Subramanian, G. and Chattopadhyay, S. A new antiviral mechanism of the interferon system by targeting the autophagy pathway. American Society for Virology Meeting (ASV) 2018 (Oral Presentation)

Chattopadhyay S, **Subramanian G**, Zhang Y, Veleeparambil M and Sen G (2017) Transcriptional and non-transcriptional functions of IRF3 in host defense, J Immunol May 1, 2017, 198 (1 Supplement) 203.3; (Oral Presentation)

Subramanian, G. and Chattopadhyay, S. AMPK, a novel checkpoint to control Herpesvirus replication. Council of Biomedical Graduate Students Graduate Research Forum, University of Toledo Health Science Campus, Toledo, Ohio, March 2017. (Oral Presentation)