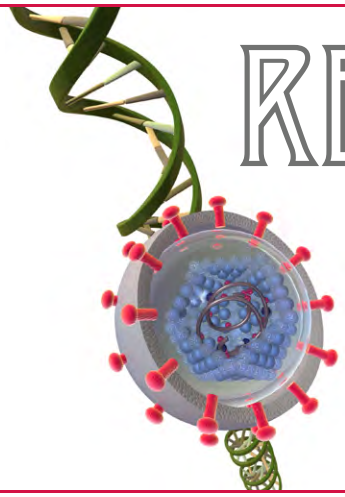
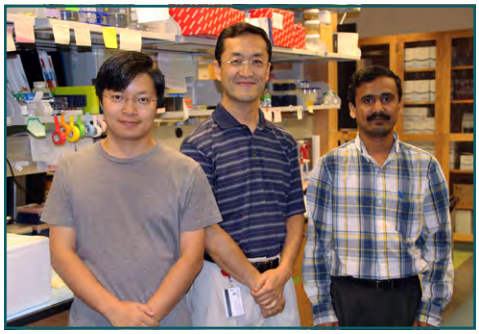


RETRO-ACTIVE NEWS

Newsletter of the Center for Retrovirus Research
at The Ohio State University

2016 Highlights

Wu lab discoveries HIV RNA methylation regulating viral infection



Dr. Li Wu's lab in the Center for Retrovirus Research reported their new findings on HIV RNA modification and its regulatory proteins that modulate viral infection in cells. The paper was published in eLife 2016.

The coauthors include two post-doctoral researchers in Dr. Wu's lab, Nagaraja Tirumuru (right) and Wuxun Lu (left), and their collaborators from University of Chicago.

The team identified where biochemical modifications occur in the HIV RNA, and what happens to the HIV infection when cellular proteins that add, remove or recognize the modification on HIV RNA are altered for their expression levels. The team also found that cellular proteins specifically recognize modified HIV RNA and can inhibit HIV infection in CD4-positive T-lymphocytes — the primary target cells of HIV infection in humans. Dr. Wu and colleagues believe that novel approaches to exploring HIV basic research could provide deeper insights into developing treatments and cures in the future.

This study was supported by the NIH, The Howard Hughes Medical Institute, and a seed grant from the Center for RNA Biology at The Ohio State University. The Wu Lab team was recently awarded a new one-year grant from the NIH for $400,272 toward their continued efforts in this project.

Dr. Shan-Lu Liu, MD, PhD joins the Ohio State faculty and Center

Professor Liu was recruited from the University of Missouri to join the Department of Veterinary Biosciences and the Center for Retrovirus Research, as part of the University's Discovery Themes initiative.

Dr. Liu received his PhD training with Dr. Dusty Miller and his postdoctoral training with Dr. James Mullins, both at the University of Washington and Fred Hutchinson Cancer Research Center. Dr. Liu's laboratory makes use of model viruses of study, including HIV-1, Ebola, influenza A virus, Zika virus, hepatitis C virus, and oncogenic sheep retroviruses.

His major interests are to uncover the mechanisms of host restriction to viral infection and viral counter measures, viral cell-to-cell transmission, innate immunity and sensing to viral infection, viral membrane fusion and entry, and viral dysregulation of cell signaling and cancer. His cutting edge research has been funded by several NIH grants. Welcome Shan-Lu!

Read More: www.dispatch.com/content/stories/local/2016/09/06/ohio-state-lures-zika-researcher-to-campus.html

Dr. Nicholas Funderburg and colleagues receive NIH award to explore cellular mediators of vascular inflammation in treated HIV infection

Dr. Nicholas Funderburg, Assistant Professor in the School of Health and Rehabilitation Sciences, Division of Medical Laboratory Science, and Center member has received a four year grant of $2,990,705 from the NIH, National Heart, Lung, and Blood Institute.

In the antiretroviral therapy (ART) era, HIV-infected persons remain at increased risk for cardiovascular disease linked to persistent inflammation. The precise mechanisms driving persistent inflammation are not known, but may include: low level HIV-1 replication, co-pathogens, microbial translocation, and pro-inflammatory lipids that can each activate innate defenses, inducing inflammatory cytokines, and altering endothelial cell and immune cell function.

This grant will allow Funderburg and his team to define the complex inflammatory interactions among monocytes, effector CD8+ T cells, and the endothelium, cells that likely contribute to the development of vascular disease. This study will ultimately facilitate the development of more effective strategies to prevent or combat HIV associated vascular inflammation and cardiovascular disease.

Dr. Kristine Yoder awarded NIH R01 to visualize HIV-1 integration in real-time.

Dr. Kristine Yoder, Assistant Professor, Department of Cancer Biology and Genetics and member of the Center for Retrovirus Research, has received a five year grant of $1.52M from the National Institutes of Health. Dr. Yoder is utilizing novel single molecule analytical tools to evaluate retroviral integration, including the visualization of intasomes on target DNAs. Important questions to be answered by this study include:

1) What are the protein dynamics of HIV-1 IN during the DNA target search and integration?
2) What are the viral DNA dynamics during the DNA target search and integration process?
3) What constitutes an efficient DNA target site?
4) What factors influence the DNA target search process?
5) What are the dynamics of intasome targeting on chromatin?

Dr. Yoder will utilize several innovative single molecule imaging systems to visualize the HIV-1 integration in real-time. She will also examine DNA lesions and mechanically altered DNA structures that may mimic the preferred target DNA configuration as well as chromatin containing histones with specific PTMs.

These studies are designed to fully interrogate the animated processes associated with HIV-1 integration which will provide critical information for design and development of novel drugs and small molecules for HIV-1 treatment.

The Center for Retrovirus Research

2016 Distinguished Research Career Award

Dr. Genoveffa Franchini, MD, Chief of the Animal Models and Retroviral Vaccines Section, Center for Cancer Research, National Cancer Institute at NIH in Bethesda, Maryland was the 17th recipient of the annual award for her seminal contributions to the field of molecular biology, immunology, and pathogenesis of human and animal retroviruses.

Dr. Franchini's research program has made exceptional contributions to the field of retrovirology from molecular biology to translational vaccine strategies. Her early work characterized the oncogenes of several retroviruses. She has studied the persistence and pathobiology of multiple oncogenic retroviruses and lentiviruses, including HTLV-1, HIV-1, and SIV. She was the first to sequence SIV and made the critical identification of the fusion peptide of SIV and HIV.

Dr. Franchini has made key contributions to our understanding of the first identified human pathogenic retrovirus, HTLV-1. She sequenced multiple HTLV-1 patient isolates and showed that the infected tumor cells were clonal. She has identified and characterized the roles of multiple HTLV-1 proteins, including Tax, p8, p12, and p30. This body of work has led to a deeper understanding of the ability of HTLV-1 to persist and transform T cells leading to leukemia.

Dr. Franchini has been working on HIV vaccine development for over 20 years. A pre-clinical trial of an HIV vaccine regimen developed in her laboratory was the first to demonstrate protection in human subjects. She has extensively characterized the adaptive and innate immune responses to viral infection and vaccination. Her continued development of vaccine strategies in non-human primate models importantly includes evaluation of mucosal transmission and gender differences. Her work has extended to the development of a novel combination DNA and recombinant protein vaccine strategy to prevent monkeypox infection of non-human primates, which could be applied to smallpox.

Dr. Franchini's visit was sponsored by the Center for Retrovirus Research, Departments of Veterinary Biosciences and Molecular Virology, Immunology and Medical Genetics, Public Health Preparedness for Infectious Diseases Program, Center for Microbial Interface Biology, and the Comprehensive Cancer Center.

Dr. Genoveffa Franchini receives Career Award crystal from members of the Center for Retrovirus Research. Shown from left are Drs. Kristine Yoder, Karin Musier-Forsyth, Jesse Kwiek, Genoveffa Franchini, Patrick Green, Li Wu, Sanggu Kim, and Shan-Lu Liu. Dr. Franchini's distinguished award lecture was entitled "Monkeys and Men tales on HIV Vaccine Development".

Dr. Sanggu Kim receives the American Society for Hematology (ASH) Junior Faculty Award.

Recent advances in stem cell gene therapy have generated tremendous hope for the treatment of a wide range of previously incurable diseases, including HIV/AIDS and cancers. The development of gene therapies is however inherently challenging due to difficulties in understanding and controlling both the therapeutic agents themselves and the complex body system that operates on multiple scales, including molecular, cellular, tissue, and environmental.

ASH will fund a three-year research project proposed by Dr. Kim to address hematopoietic stem cells (HSC) and T-cell dynamics in an anti-HIV gene therapy setting. Dr. Kim and his research team will investigate how the vast number of therapeutically engineered HSC and derived T-cells repopulate in various tissues/organs and contribute to maintain homeostasis in the presence of T-cell killing using animal models for HIV gene therapy. Dr. Kim's study will provide systems-level data sets for HSC and T-cell behaviors crucial for understanding the transplant conditions under which genetic engineering of HSC is therapeutically effective and can ultimately cure HIV/AIDS.

Dr. Mamuka Kvaratskhelia and colleagues uncover new approaches for combating HIV

Dr. Mamuka Kvaratskhelia, Kimberly Professor at the Ohio State College of Pharmacy and Center for Retrovirus Research, and his research group have uncovered a new mechanism for halting the spread of infectious viruses that could pave the way for therapies to combat emerging HIV-1 strains resistant to currently available treatments.

These findings were reported in the August 25, 2016 issue of *Cell*. Kvaratskhelia and his team sought to alter the structure of integrase, a key HIV-1 protein, using small molecules called allosteric integrase inhibitors, or ALLINIs. These studies have resulted in discovery of a new and unexpected role for integrase in HIV-1 biology and revealed the mechanism that causes ALLINIs to impair HIV-1 replication.

The HIV-1 life cycle is broadly divided into two phases: the early phase, when the virus invades human cells to establish the infection; and the late phase, when infected cells make new virus particles that infect other cells. Historically, it was thought that integrase acts only during the early phase of HIV-1 infection by integrating the viral copy DNA into the human genome. Yet in this study Kvaratskhelia's team, in collaboration with Sebla B. Kutluay, PhD, Washington University School of Medicine and Paul D. Bieniasz, PhD, The Rockefeller University, have found that integrase is also essential for the late phase where it ensures that viral RNA, which is the genetic code of HIV-1, is correctly positioned within the infectious virus particles.

Furthermore, these investigators have found that ALLINIs altered the structure of integrase and impaired its ability to bind the viral RNA, thus yielding non-infectious particles. The outer shells of the newly formed particles were normal, but the internal components were profoundly altered with the viral RNA genome being misplaced. Kvaratskhelia's team is currently working with medicinal chemists at Ohio State to develop ALLINIs that can be used to treat AIDS patients.

Retrovirology Journal Club Started in 2016

This past year saw the beginning of the Ohio State University Retrovirology Journal Club. The club meets twice per month to discuss the latest retroviral research. This is a formal opportunity for graduate students and postdoctoral fellows to practice their presentation and critical reading skills.

The group has enjoyed discussions of many high profile papers and helpful insights from Center for Retrovirus Research faculty members. Organizer and Faculty advisor Dr. Kristine Yoder maintains the website which posts upcoming papers for discussion as well as other notable advances in the field.

u.osu.edu/retrovirologyjournalclub/

Selected Grant Awards

Kristine Yoder
NIH R21-AI122981 "CRISPR gRNA library screen of the HIV-1 genome" (2016-2018)

Kristine Yoder
NIH R01-GM121284 "Visualization of HIV-1 integration in real time" (2016-2021)

Li Wu
NIH, R56AI127667 "Mechanisms of HIV-1 RNA Methylation in Regulating Viral Replication" (2016-2017)

Sanggu Kim
American Society for Hematology Junior Faculty Award "Hematopoietic stem cells (HSC) and T-cell dynamics in an anti-HIV gene therapy" (2016-2018)

Patrick Green and Amanda Panfil
Cerus Inc "Methods to inhibit HTLV-1 and HTLV-2 infectivity" (2017-2020)

Karin Musier-Forsyth and Michael Ibba, co-PIs
NIH, T32 GM086252 "Cellular, Molecular and Biochemical Sciences Predoctoral Training Grant" (2016-2021)

2016 Graduates

Jacob Al-Saleem, PhD (Green Lab) "Identification of HTLV-1 Tax-1 and HBZ Binding Partners, and Their Role in HTLV-1 Biology and Pathogenesis"

Roopa Comandur, PhD (Musier Forsyth Lab) "Structure of Retroviral 5'-Untranslated Regions and Interactions with Host and Viral Proteins"

Weixin Wu, PhD (Musier Forsyth Lab) "Characterization of Human T-cell Lymphotropic Virus Type 1 Protein-Viral Genomic RNA Interactions and tRNA Primer Annealing"

Lin Chen, PhD (Musier Forsyth Lab) "Characterization of Prolyl-tRNA Synthetase, YbaK, and ProXp-ala Editing Mechanism"

Bradley Howard, MS (Musier Forsyth Lab) "Development of gain-of-function reporters to probe trans-editing of misacylated tRNA in vivo"

Nathan Titkemeier, MS (Musier Forsyth Lab) "Mechanism of Glutamyl-Prolyl-tRNA Synthetase-mediated Repression of HIV-1 Infection"

Chase McVey, MS (Musier Forsyth Lab) "Variants of Human Lysyl-tRNA Synthetase: In vitro Activity and Relevance to Human Disease"

Student, Post-doc, Research Scientist and Visiting Scholar Awards

Jacob Al-Saleem (PhD student; Green)
1st Place poster presentation 2016 College of Veterinary Medicine Research Day.

Jenna Antonnuci (PhD student; Wu Lab)
C. Glenn Barber Fellowship (2016-2018).

Jenna Antonnuci (PhD student; Wu Lab)
1st Place poster presentation 2016 Symposium of Center for RNA Biology.

Jenna Antonnuci (PhD student; Wu Lab) Winner of poster presentation travel award 2016 College of Veterinary Medicine Research Day

Amanda Panfil, PhD (Post-doc; Green Lab)
Winner Oral Platform Presentation, 2016 College of Veterinary Medicine Research Day.

Miguel Lopez (PhD student; Yoder Lab)
Ohio State University Fellowship (2016-2017)

Zhihua Qin (PhD student; Wu lab)
Graduate Scholarship recipient from Chinese Scholar Council (2016-2020).

Shuliang Chen, PhD (Visiting Scholar; Wu lab)
Postdoctoral Scholarship recipient from Chinese Scholar Council (2016-2018).

Roopa Comandur (PhD student; Musier-Forsyth Lab)
Three-minute Thesis Competition, People's Choice Award, Ohio State and regional competition

Selected Upcoming Meetings

Cold Spring Harbor Laboratory "Retroviruses"
May 22-27, 2017, Cold Spring Harbor, NY

American Society for Virology
June 24-28, 2017, Madison, WI

29th Workshop on Retroviral Pathogenesis
August 27-31, 2017, Prague, Czech Republic

Selected Publications

Alwarawrah Y, Hughes P, Loiselle D, Carlson DA, Darr DB, Jordan JL, Xiong J, Hunter LM, Dubois LG, Thompson JW, Kulkarni MM, Ratcliff AN, **Kwiek JJ***, Haystead TA*. Fasnall, a selective FASN inhibitor shows potent anti-tumor activity in the MMTV Neu model of HER2+ breast cancer. *Cell Chemical Biology*, 2016 23:678-688. *Co-corresponding authors.

Antonucci J, St. Gelais C, de Silva S, Yount JS, Tang C, Ji X, Shepard C, Xiong Y, Kim B, **Wu L.** SAMHD1-mediated HIV-1 restriction in cells does not involve ribonuclease activity. *Nature Medicine*, 2016; 22: 1072–1074.

Bonifati S, Daly MB, St. Gelais C, Kim SH, Hollenbaugh JA, Shepard C, Kennedy EM, Kim DH, Schinazi RF, Kim B and **Wu L.** SAMHD1 controls cell cycle status, apoptosis and HIV-1 infection in monocytic THP-1 cells. *Virology*, 2016; 495: 92–100.

Cantara WA, Hatterschide J, Wu W, **Musier-Forsyth K.** RiboCAT: A new capillary electrophoresis data analysis tool for nucleic acid probing. *RNA*, 2016 Nov 7. pii: rna.058404.116

Cantara WA, Olson ED, **Musier-Forsyth K.** Analysis of RNA structure using small-angle X-ray scattering. *Methods*, 2016 Oct 21, pii: S1046-2023(16)30394-2.

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Jones, ND, Lopez Jr MA, Hanne J, Peake MB, Lee J-B, Fishel R, **Yoder KE**. 2016. Retroviral intasomes search a target DNA by 1D-diffusion. *Nature Communications*, 2016 7:11409.

Kawatsuki A, Yasunaga J,Mitobe Y, **Green PL**, Matsuoka M: HTLV-1 bZIP factor protein targets the Rb/E2F-1 pathway to promote proliferation and apoptosis of primary CD4+ T cells. *Oncogene* 2016, 35(34):4509-4517.

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Kodigepalli KM, Li M, Liu S-L, **Wu L**. Exogenous expression of SAMHD1 inhibits proliferation and induces apoptosis in cutaneous T-cell lymphoma-derived HuT78 cells. *Cell Cycle*. 2016; Dec 8: 1-10.

Kohnken R, Kodigepalli KM, Mishra A, Porcu P, and **Wu L**. MicroRNA-181 contributes to downregulation of SAMHD1 expression in CD4+ T-cells of Sèzary syndrome patients. *Leukemia Research*. 2017; 52: 58-66. ePub on Nov. 17, 2016.

Li C, Wang HB, Kuang WD, Ren XX, Song ST, Zhu HZ, Li Q, Xu LR, Guo HJ, **Wu L***, Wang JH*. Naf1 regulates HIV-1 latency by suppressing viral promoter-driven gene expression in primary CD4+ T cells. *J Virol* 2017; 91 (1). ePub Dec 16, 2016 (*co-corresponding authors).

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Markosyan RM, Miao C, Zheng Y-M, Melikian GB, **Liu S-L***, Cohen FS*. Induction of Cell-Cell Fusion by Ebola Virus Glycoprotein: Low pH Is not a Trigger. *PLoS Pathogens*. 2016 12(1): e1005373. *co-corresponding authors

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Selected Publications - continued

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Panfil AR, Dissinger NJ, Landes K, Fernandez S, **Green PL**: Functional Comparison of HBZ and the Related APH-2 Protein Provide Insight Into HTLV-1 Pathogenesis. *J Virology* 2016 Jan 27;90(7):3760-72. doi: 10.1128/JVI.03113-15.

Patel D, Antwi J, Koneru PC, Serrao E, Forli S, Kessl JJ, Feng L, Deng N, Levy RM, Fuchs JR, Olson AJ, Engelman AN, Bauman JD, **Kvaratskhelia M**, Arnold E. A new class of allosteric HIV-1 integrase inhibitors identified by crystallographic fragment screening of the catalytic core domain. *J Biol Chem* 2016 291(45):23569-23577.

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Turner AN, Maierhofer C, Funderburg NT, Snyder B, Small K, Clark J, Bazan JA, Kwiek NC, **Kwiek JJ**. High levels of self-reported prescription opioid use by HIV-positive individuals. *AIDS Care*, 2016. Dec;28(12):1559-1565.

Yoder, KE*, Bundschuh R. Host double strand break repair generates HIV-1 strains resistant to CRISPR/Cas9. *Scientific Reports*, 2016 6:29530.(*corresponding author)