2019 DUKE-UNC SYMPOSIUM ON VIRAL ONCOLOGY AND AIDS MALIGNANCY

Wednesday, December 4, 2019

9:00 a.m. – 3:00 p.m.

Continental breakfast at 8:30 a.m.

Great Hall, Trent Semans Center
Duke University Medical Center
Morning talks followed by lunch and poster session

Michael Lagunoff, PhD

Professor of Microbiology, University of Washington

"Metabolic Requirements for KSHV Latency"

Cary Moody, PhD

Associate Professor of Microbiology & Immunology, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill

"Regulation of the HPV Life Cycle by the DNA Damage Response"

Sankar Swaminathan, MD

Chief, Infectious Diseases Division, University of Utah Medical School

"Transcriptional Virus-Host Relationships of Gammaherpesviruses"

Free online registration required for attendance and poster session:
http://sites.duke.edu/dukevirology/

CFAR Center for AIDS Research DUKE UNIVERSITY & MEDICAL CENTER

UNIVERSITY OF NORTH CAROLINA CENTER FOR AIDS RESEARCH

Duke Cancer Institute

UNC LINEBERGER

DUKE CENTER FOR VIROLOGY

2019 Duke-UNC Symposium on Viral Oncology and AIDS Malignancy

Symposium Schedule

8:30 - 9:00 a.m.	Continental breakfast and poster set up
9:00 - 9:05 a.m.	Welcome and Introduction, **Micah Luftig**, Duke
9:05 a.m.	Introduction – **Dirk Dittmer**, UNC
9:10 - 10:05 a.m.	**Michael Lagunoff, Ph.D.**, Professor of Microbiology, University of Washington "*Metabolic Requirements for KSHV Latency*"
10:05 a.m.	Introduction— **Blossom Damania**, UNC
10:10 - 11:05 a.m.	**Cary Moody, Ph.D.**, Associate Professor of Microbiology and Immunology, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill "*Regulation of the HPV Life Cycle by the DNA Damage Response*"
11:05 - 11:25 a.m.	Coffee Break
11:25 a.m.	Introduction— **Micah Luftig**, Duke
11:30 - 12:25 p.m.	**Sankar Swaminathan, M.D.**, Don Merrill Rees Presidential Endowed Chair, Professor and Chief, Infectious Diseases Division, University of Utah School of Medicine "*Transcriptional Virus-Host Relationships of Gammaherpesviruses*"
12:25 p.m.	Closing Remarks— **Micah Luftig**, Duke
12:30 - 1:30 p.m.	Lunch
1:30 – 1:50 p.m.	Flash talks from selected posters
1:50 – 2:50 p.m.	Poster session
2:50 p.m.	Awards for best posters
3:00 p.m.	End of symposium—please take your poster down promptly

2019 Duke-UNC Symposium on Viral Oncology and AIDS Malignancy

Poster Numbers, Authors and Titles

1. **Katherine C. Barnett***, Julia M. Coronas-Serna, Wen Zhou, Michael J. Ernandes, Anh Cao, Philip J. Kranzusch, Jonathan C. Kagan, UNC-Chapel Hill, "Phosphoinositide Interactions Position cGAS at the Plasma Membrane to Ensure Efficient Distinction between Self- and Viral DNA"
2. **Dia C. Beachboard***, Moonhee Park, Madhuvanthi Vijayan, Dillon J. Fernando, Graham D. Williams, and Stacy M. Horner, Duke University, "RAB1B interacts with TRAF3 to promote antiviral innate immunity"
3. **Emmanuela N. Bonglack***, Joshua E. Messinger, Jana Cable, and Micah A. Luftig, Duke University, "Monocarboxylate Transporters 1 and 4 as Metabolic Regulators of EBV-Infected B Lymphocyte Homeostasis"
4. **Christine Daniels***, Esther Lee, Cindy Bowman, Celia LaBranche, Robert Edwards, Brian Watts, Gordon Joyce, David Montefiori, Barton Haynes, Kevin Saunders, Duke University, "HIV-1 Consensus Stabilized Envelope Immunogens Elicit Autologous Neutralizing Antibodies in Rabbits"
5. **Heather Froggatt***, Alfred Harding, Nicholas Heaton, Duke University, Activation screen identifies negative regulators of the type I IFN response "
6. **Margaret L. Gulley***, Sandra Elmore, Gaorav P. Gupta, Sunil Kumar, Joseph Ibrahim, Matthew Egleston, Ian Hoskins, Aaron Garnett, UNC-Chapel Hill, "A Method for Absolute Quantification of Tumor Markers & Viral Genomes by Massive Parallel Sequencing of Cell Free DNA"
7. **Hwang BJ***, Tsao LC, DeLeon G, Wei J, Yang XY, Lei G, Wang T, Morse MM, Lyerly HK, Hartman ZC, Duke University, "Overexpression of MAVS stimulates anti-tumor immunity and significantly reduces tumor growth of immune insensitive colorectal cancer in vivo"
8. **Yi-Yu Lin***, Ian Belle, Maria Blasi, Min-Nung Huang, Anne F Buckley, Wes Rountree, Mary Klotman, Andrea Cara, Donatella Negri, Duke University, "Skeletal muscle is an antigen reservoir in integrase-defective lentiviral vector-induced long-term immunity"
9. **Michelle Mac***, Dipendra Gautam, Bryan Johnson, and Cary Moody, UNC-Chapel Hill, "A Role of SETD2-mediated H3K36 Trimethylation in the Epigenetic Regulation of HPV31 Life Cycle"
10. **Michael J McFadden***, Alexa BR McIntyre, Nandan S Gokhale, Christopher E Mason, Stacy M Horner, Duke University, "Post-transcriptional regulation of antiviral gene expression"
11. **Ryan P. McNamara***, UNC-Chapel Hill, "Extracellular vesicles are utilized by KSHV to induce long-term endothelial cell reprogramming"
12. **Joshua E. Messinger***, Elizabeth Pavlisko, Joanne Dai, Lyla Stanland, Alexander Price and Micah Luftig, Duke University, "Identification of Host Biomarkers of Epstein-Barr Virus Latency IIb and Latency III"
13. **James Meyo*** Aubrey Bailey, Carson Mosso, Justin Landis, Kenny Dinnon, Kathleen Corcoran, Dirk Dittmer, UNC-Chapel Hill, "Exploring kinase inhibitors as a therapy for KSHV-associated cancers"
14. **Emily Moore***, Dana Jeffus, Alexis Davis, and Amy Adamson*, UNC-Greensboro, "Epstein-Barr virus lytic replication activates and is dependent upon MAPK-interacting kinase 1/2 in a cell-type dependent manner"
15. **Ashley N. Nelson***, Maria Dennis, Jesse F. Mangold, Katherine Li, Riley J. Mangan, George M. Shaw, Katharine Bar, Barton Haynes, M. Anthony Moody, Justin Pollara, Koen K.A. Van Rompay, Kristina De Paris, and Sallie R. Permar, Duke University. "A vaccination regimen to enhance immune responses associated with reduced risk of mother-to-child transmission of HIV"
16. **Sarah Quinlan***, Susan May, Ryan Weeks, Jennifer Luff, NC State University, "Comparative Analysis of Interferon Regulatory Factors (IRFs) and their impact on cutaneous papillomavirus (PV) function"
17. **Matthew T. Sacco***, Nandan S. Gokhale, Stacy M. Horner, Duke University, "The Mechanism of N6-Methyladenosine Deposition on Hepatitis C Viral RNA"
18. **Sara Selitsky***, Tamiwe Tomoka, Maurice Mulenga, Satish Gopal, Yuri Fedoriw, UNC-Chapel Hill. "Human Endogenous Retrovirus Expression in HIV-associated Diffuse Large B-cell Lymphoma"
19. **Sang-Hoon Sin***, Anthony B. Eason, Yongbaek Kim, Johann Schneider, Blossom Damania, and Dirk P. Dittmer, UNC-Chapel Hill, "Kaposi Sarcoma in whole KSHV genome transgenic mice"
20. **Daltry Snider***, Dia Beachboard, Madhuvanthi Vijayan, Christine Vazquez, Stacy Horner, Duke University, "A role for ufmylation in innate immunity"
21. **Lyla Stanland***, Hazel Ang, Kris Wood, Micah Luftig, Duke University, "Targeting the PI3K Pathway in Gastric Cancer"
22. **Tognasoli, Ana***; Greengrove, Eva; Adamson, Amy, UNC-Greensboro, "Epstein-Barr Virus as an Environmental Agent in Neurodegenerative Diseases: Lewy Bodies and Beyond"
23. **Joe Trimarco***, Nicholas Heaton, Duke University, "Optimizing a CRISPR screening system to identify regulators of hemagglutinin trafficking during influenza virus infection"
24. **Timothy N.Trotter***, Cong-Xiao Liu, Tao Wang, H. Kim Lyerly, Zachary C. Hartman, Duke University, "Dormant breast tumor cells avoid the adaptive immune system through regulation of the tumor microenvironment"
25. **Li-Chung Tsao***, Zachary Hartman, Herbert Kim Lyerly, Duke University, "CD47 blockade augmentation of Trastuzumab antitumor efficacy dependent upon antibody-dependent-cellular-phagocytosis"
26. **Joshua J. Tu***, David R. Martinez, Amit Kumar, Jesse F. Mangold, Riley J. Mangan, Ria Goswami, Elena Giorgi, Jui-Lin Chen, Michael Mengual, Ayooluwa O. Douglas, Holly Heimsath, Joshua Eudailey, Giovanna Hernandez, Papa Kwadwo Morgan-Asiedu, Celia LaBranche, David C. Montefiori, Kevin Wiehe, Feng Gao, and Sallie R. Permar, Duke University, "Maternal plasma broadly neutralizing antibodies drive the selection of neutralization-resistant viruses that initiate HIV infant infection"
27. **Katherine Willard***, Ashley Barry, Jeff Bailey, Ann Moormann, Micah Luftig, Duke University, "Recent Epstein Barr virus strains isolated from endemic Burkitt's lymphoma patients display a spontaneous lytic phenotype"
28. **Graham D. Williams***, Nandan S. Gokhale, Daltry L. Snider, and Stacy M. Horner, Duke University, "mRNA Cap 2`-O-methylation by CMTR1 regulates innate immune responses"
29. **Tia Morgan*** and Cary Moody, UNC-Chapel Hill. "The Role of the ATR DNA Damage Response Pathway During the Life Cycle of HPV31"

1

Katherine C. Barnett*, Julia M. Coronas-Serna, Wen Zhou, Michael J. Ernandes, Anh Cao, Philip J. Kranzusch, Jonathan C. Kagan

University of North Carolina, Chapel Hill

Phosphoinositide Interactions Position cGAS at the Plasma Membrane to Ensure Efficient Distinction between Self- and Viral DNA

The presence of DNA in the cytosol of mammalian cells is an unusual event that is often associated with genotoxic stress or viral infection. The enzyme cGAS is a sensor of cytosolic DNA that induces interferon and inflammatory responses that can be protective or pathologic, depending on the context. Along with other cytosolic innate immune receptors, cGAS is thought to diffuse through the cytosol in search of its DNA ligand. Herein, we report that cGAS is not a cytosolic protein but rather localizes to the plasma membrane via the actions of an N-terminal phosphoinositide-binding domain. This domain interacts selectively with PI(4,5)P2, and cGAS mutants defective for lipid binding are mislocalized to the cytosolic and nuclear compartments. Mislocalized cGAS induces potent interferon responses to genotoxic stress, but not viral infection. These data establish the subcellular positioning of a cytosolic innate immune receptor as a mechanism that governs self-nonself discrimination.

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Dia C. Beachboard*, Moonhee Park, Madhuvanthi Vijayan, Dillon J. Fernando, Graham D. Williams, and Stacy M. Horner

Duke University

RAB1B interacts with TRAF3 to promote antiviral innate immunity

The antiviral innate immune response activates a signaling cascade that induces type I and type III interferons (IFNs). This signaling, which is highly regulated, is initiated by pattern recognition receptors, such as RIG-I, which senses viral RNA and then signals to the adaptor protein, MAVS. This adaptor protein then recruits additional signaling proteins, including TRAF3 and TBK1, to form a signaling complex that results in the activation of IRF3 for the transcriptional induction of IFN. Here, we show that the GTPase trafficking protein RAB1B positively regulates the RIG-I sensing pathway to promote induction of IFN-β and the antiviral response. Over-expression of RAB1B increases RIG-I-mediated signaling to IFN-β, while deletion results in reduced signaling of this pathway. Additionally, this loss of RAB1B results in a dampened antiviral response, as Zika virus infection is enhanced in the absence of RAB1B. Importantly, we identified the mechanism of RAB1B action by determining that it interacts with TRAF3 to facilitate TRAF3 interaction with the nucleic acid sensing adaptor MAVS. Thus, we identified RAB1B as a regulator of TRAF3 to promote the formation of innate immune signaling complexes in response to nucleic acid sensing.

3

Emmanuela N. Bonglack*, Joshua E. Messinger, Jana Cable, and Micah A. Luftig

Duke University

Monocarboxylate Transporters 1 and 4 as Metabolic Regulators of EBV-Infected B-Lymphocyte Homeostasis

Epstein Barr Virus (EBV) is a highly prevalent pathogen which infects >90% of adults worldwide. Primary infection by EBV stimulates B-cell proliferation, controlled by a robust immune response. In immune-suppressed persons however, EBV can give rise to various lymphoproliferative disorders. To date, no targeted therapies exist to treat such diseases highlighting a need to investigate disease-propagating mechanisms. In vitro, EBV-immortalized lymphoblastoid cell lines (LCLs), are an established model for studying oncogenesis. Our laboratory previously published that metabolic stress is a barrier to EBV-driven B-cell transformation. However, how EBV regulates host metabolic genes is ill-understood. We have identified via RNA-Sequencing and gene microarray that Monocarboxylate transporters 1 and 4 (MCT1/4) are upregulated following EBV infection, and inhibition assays suggest they may be crucial for infected B-cell homeostasis. These plasma membrane-resident lactate transporters are intracellular pH regulators, and frequently associated with poor cancer prognosis making them attractive therapeutic targets. Queried ChIP-Seq. data suggests the EBV latency proteins EBNA-LP and LMP1, and the host transcription factors c-Myc, NRF1, HIF-1α, and NFκB, regulate MCT1/4 at different stages following infection. Our goal is to establish MCT1/4 as viable therapeutic targets in EBV-associated cancers, as well as determine how and why are upregulated in these contexts.

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Christine Daniels*, Esther Lee, Cindy Bowman, Celia LaBranche, Robert Edwards, Brian Watts, Gordon Joyce, David Montefiori, Barton Haynes, Kevin Saunders

Duke University

HIV-1 Consensus Stabilized Envelope Immunogens Elicit Autologous Neutralizing Antibodies in Rabbits

HIV-1 Envelope (Env) is the sole target for neutralizing antibodies. Env from different clades is genetically diverse, and exists on HIV-1 virions oscillating between varying degrees of “open” and “closed” conformational states. The diversity of Env sequence and presence of various Env states hinders vaccine elicitation of broadly-reactive neutralizing antibodies. We hypothesized that vaccination with a closed state Env with a sequence representative of all diverse HIV-1 isolates followed by epitope-focused boosts, will elicit neutralizing antibodies to conserved epitopes. Here, we generated two stabilized group M consensus envelope trimers using different stabilization approaches and a synthetic glycopeptide that mimics the epitope of V3-glycan targeting antibodies. Visualization by negative stain electron microscopy confirmed formation of native-like trimers. The stabilized Env trimers were antigenic for a panel of 24 broadly neutralizing antibodies with minimal reactivity to non-neutralizing antibodies. Three immunizations with each Env trimer elicited antibodies capable of neutralizing tier 1 virus and autologous virus in 9 of 10 rabbits. Subsequent boosts with the V3-glycan glycopeptide displayed on ferritin nanoparticles resulted in elicitation of serum antibodies that recognize V3-glycans and display moderate sensitivity to mutations at this site. Thus, we engineered two soluble native-like Envs in the closed state that minimized trimer instability, reduced exposure of non-neutralizing epitopes, synthesized a V3-glycan epitope-focused nanoparticle and elicited neutralizing antibodies in rabbits. The neutralizing antibodies induced with group M consensus envelope in this study provide a foundation for maturing neutralizing antibody responses to engage multiple group M HIV-1 isolates.

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Heather Froggatt*, Alfred Harding, Nicholas Heaton

Duke University

Activation screen identifies negative regulators of the type I IFN response.

Using a reporter for the type I interferon response, a screen was performed using the CRISPR SAM system to identify potential negative regulators of this antiviral pathway. Ten genes were identified as significant hits and potential negative regulators of the type I interferon response. After selecting for interferon-induced genes and validating significant hits, one known interferon stimulated gene (ISG) was found to be sufficient for downregulation of the interferon response. This gene, without a previously known role in the interferon response, is necessary to prevent the overexpression of certain ISGs and control influenza virus replication.

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Margaret L. Gulley*, Sandra Elmore, Gaorav P. Gupta, Sunil Kumar, Joseph Ibrahim, Matthew Egleston, Ian Hoskins, Aaron Garnett

University of North Carolina, Chapel Hill

A Method for Absolute Quantification of Tumor Markers & Viral Genomes by Massive Parallel Sequencing of Cell Free DNA

Massive parallel sequencing technology is increasingly used to measure somatic mutations and viral loads in plasma DNA. A problematic aspect is that somatic mutations and viral loads are typically quantified as a fraction relative to wild type human DNA, yet wild type levels vary with diverse biologic and pre-analytic interferences. We devised a novel strategy to quantify target analytes in 'copies per mL of plasma' after normalizing for read counts of spiked DNAs. To accomplish this, five synthetic DNAs (called EndoGenus spikes) were added to plasma before library preparation (modified ArcherDX LiquidPlex 28). By normalizing to the fractional recovery of EndoGenus spike reads, numerical values for each disease marker were reportable in units of 'copies per mL'. To show how well this system operates, replicate assays were performed on 40 mock plasmas having 23 engineered mutations, and on 21 natural plasmas. Reads for all five EndoGenus spikes were recovered (mean 322 and 451 copies/mL in mock and natural plasmas, respectively). Normalizing read counts for the proportional recovery of spikes helped control for variables in the multi-step protocol, reducing the CV in replicate tests from 34% to 22% for mutations, and from 25% to 7% for viral loads. In conclusion, the EndoGenus system is useful for evaluating efficiency of the total test system and for precisely quantifying target molecules. This system may benefit patients being monitored for disease burden while also tracking emerging subclones.

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Hwang BJ**, Tsao LC, DeLeon G, Wei J, Yang XY, Lei G, Wang T, Morse MM, Lyerly HK, Hartman ZC

Duke University

Overexpression of MAVS stimulates anti-tumor immunity and significantly reduces tumor growth of immune insensitive colorectal cancer in vivo

While the use of immune checkpoint blockade (ICB) has gained significant traction as a viable therapy cancers, many more cancers remain refractory to these immunotherapies due to their highly immunosuppressive tumor microenvironment (TME). This is perhaps best exemplified in colorectal carcinomas (CRCs) where ~90% of CRCs (non-MSI) are unresponsive to ICBs. Secondary analyses of ICB responders suggest that the presence of T-cells and a functional interferon signal transduction pathway within cancer cells is likely required for PDL1 ICB efficacy, as mutations in this pathway confer resistance to PD1/PDL1 therapies. Our studies have indicated that this lack of response may be due to mutations and loss of genes that mediate the innate immune responses in tumor cells, such as MAVS (Mitochondrial Antiviral Signaling Protein), which we found were significantly reduced in colorectal cancer. Thus, we hypothesize that stimulation of the MAVS pathway would elicit strong interferon signaling capable of reversing immunosuppression to allow for successful immune mediated tumor regression.

To circumvent issues associated with single ligand stimulation, we exploited the bow-tie structure of the MAVS through its overexpression as a novel genetic means to elicit ligand free innate immune signaling in the TME. We found that overexpression of MAVS elicited robust stimulation of Type I and II interferon pathways, along with a unique interferon gene signature (through transcriptomic analysis of infected primary cells). Using MAVS inducible-expressing colorectal cell lines, we found that MAVS expression stimulated significant anti-tumors responses in vivo through its stimulation of adaptive tumor-specific immune responses in anti-PDL1-resistant models. Moreover, in 20% (4 out of 20) of MAVS induced cancers, we observed complete tumor regression as well as an immunological memory response (in 100% of regressed mice) through tumor re-challenge experiment.

Utilizing MAVS expressing viral (adeno) vectors, we could demonstrate significant induction of interferon responses among different cells in the tumor microenvironment, including tumor cells, fibroblasts, as well as dendritic cells. Intra-lesional injection of tumors using these vectors elicited profound anti-tumor responses against primary tumors, as well as, an abscopal effect against syngeneic implanted tumors. However, we found that MAVS expression significantly upregulated PDL1 in infected cells, suggesting that MAVS vectors may synergize with PDL1 ICB.

In conclusion, our data demonstrates the importance of innate immunity activation by MAVS pathway in generating anti-tumor effects and immune “hotspots” in colorectal tumor microenvironment, which may synergize with PD1/PDL1 checkpoint blockade antibodies. Thus our findings suggest that a MAVS cancer gene therapy may offer an alternative approach to activate immunity in "immunologically cold" tumors.

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Yi-Yu Lin*, Ian Belle, Maria Blasi, Min-Nung Huang, Anne F Buckley, Wes Rountree, Mary Klotman, Andrea Cara, Donatella Negri

Duke University

Skeletal muscle is an antigen reservoir in integrase-defective lentiviral vector-induced long-term immunity

The development of vaccines eliciting protective and durable immune responses remains a global health priority. We previously developed Integrase-Defective Lentiviral Vectors (IDLVs) as antigen delivery system for inducing strong and prolonged immunity in animal models. Here, we evaluated the association between the persistence of antigen expression in vivo and the long-term immunity induced by IDLV. Following a single intramuscular (IM) or subcutaneous (SC) injection of IDLV delivering GFP as a model antigen in mice, we evaluated antigen expression, cellular infiltration at the site of injection and persistence of antigen-specific T cell response by IFNγ ELISpot at early and late time points. IM immunization with IDLV-GFP results in durable antigen expression in the muscle up to 90 days. Conversely, antigen expression is detectable in the skin only at early time points after SC immunization. The GFP-specific T cell response was more persistent in IM compared to SC injected mice. Interestingly, GFP positive muscle cells co-express MHC-I in vivo. In vitro, we demonstrate that primary myoblasts and myocytes are able to present the antigen to GFP-specific T effector cells through MHC-I but with different sensitivity to Fas-dependent CTL killing activity. Overall these data indicate that muscle cells may serve as antigen reservoir for maintaining the long term immunity induced by IDLV.

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Michelle Mac*, Dipendra Gautam, Bryan Johnson, and Cary Moody

University of North Carolina, Chapel Hill

A Role of SETD2-mediated H3K36 Trimethylation in the Epigenetic Regulation of HPV31 Life Cycle

High-risk types of human papillomavirus (HPV) are associated with multiple human cancers, most notably cervical cancer as well as an increasing number of head and neck cancers. HPV-associated cancers are mainly driven by the activity of the viral E6 and E7 oncoproteins. The HPV genome is histone-associated and is organized a manner similar to that of cellular chromatin. HPV chromatin is subjected to histone post-translational modifications, and several epigenetic regulators have been implicated in HPV replication. SETD2 is the sole methyltransferase in mammals responsible for trimethylation of lysine 36 of Histone H3 (H3K36me3), a mark of active transcription. SETD2-mediated H3K36me3 recruits effector proteins to regulate multiple cellular processes, including homologous recombination repair and alternative splicing, that are also important during the HPV life cycle. In recent studies, we have defined a critical role for SETD2-mediated H3K36me3 in the epigenetic regulation of the life cycle of the high-risk type HPV31. We have found that HPV positive cells have elevated SETD2 levels through an E7-dependent increase in protein stability, and SETD2 depletion leads to defects in productive viral replication and splicing of late viral RNAs. SETD2 activity is required to maintain H3K36me3 enrichment at the 3' end of the early region of HPV31 genome, suggesting that SETD2 may regulate the viral life cycle by recruiting H3K36me3 effectors to viral chromatin. Interestingly, we have found that activation of ATM DNA damage kinase, which is required for productive viral replication, promotes H3K36me3 maintenance on viral chromatin and processing of late viral RNAs. Together, our findings identify a significant role for SETD2-mediated H2K36me3 in epigenetic regulation of HPV life cycle. Current studies are focused on identifying H3K36me3 effectors that are recruited to viral chromatin as well as delineating the mechanism by which E7 increases SETD2 protein stability.

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Michael J McFadden*, Alexa BR McIntyre, Nandan S Gokhale, Christopher E Mason, Stacy M Horner

Duke University

Post-transcriptional regulation of antiviral gene expression

The type I interferon (IFN) response elicits the production of hundreds of interferon-stimulated genes (ISGs) to establish an antiviral cellular state. However, our understanding of type I IFN response regulation at the post-transcriptional level is limited. The RNA base modification N6-methyladenosine (m6A) is a post-transcriptional regulatory feature that has recently been shown to regulate viral infection by acting on either viral or cellular RNA. Here, we investigated the role of m6A in regulating the type I IFN response. We found that a subset of ISGs, including IFITM1, are translationally regulated by the m6A methyltransferase complex of METTL3 and METTL14 (METTL3/14) and that the transcripts of these ISGs are modified by m6A. Specifically, IFITM1 expression is enhanced by the m6A reader YTHDF1 in an m6A binding-dependent fashion, and m6A site ablation in IFITM1 impairs its translation. Importantly, we found that METTL3/14 enhances the antiviral activity of the type I IFN response. Therefore, these studies identify a class of post-transcriptionally regulated ISGs whose expression is supported by METTL3/14 and m6A to enhance antiviral innate immunity.

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Ryan P. McNamara*

University of North Carolina, Chapel Hill

Extracellular vesicles are utilized by KSHV to induce long-term endothelial cell reprogramming

Extracellular communication is critical for organismal homeostasis, and thus presents itself as a major network for viruses to usurp for disease pathogenesis. A principle mechanism that cells use to communicate with their surroundings is through extracellular vesicles (EVs), which package contents such as proteins, nucleic acids, and metabolites and deliver them to nearby cells. The tumorigenic Kaposi's Sarcoma-Associated Herpesvirus (KSHV) incorporates viral micro RNAs (miRNAs) into EVs secreted from infected cells, which we term KSHV-EVs. We have identified that chronic exposure of naïve endothelial cells to KSHV-EVs at physiological levels altered their transcriptome, activated select signaling pathways, and enhanced cell proliferation. This was all accomplished without de novo infection of cells. Importantly, these changes in recipient cell physiology did not activate innate immune/inflammatory regulators such as the interferon pathway or cGAS/STING. Our results demonstrate that KSHV can actively modify the local environment using EVs. We collectively propose that tumorigenic viruses such as KSHV hijack the extracellular communications network through EVs to establish a niche favorable for viral spread and tissue transformation, without tripping immune alarms. Current and future objectives are focused on the development of a fully tractable system for EV biogenesis, isolation, and in vivo delivery using KSHV as a model system. Through a virology-based approach, we hope to further characterize molecular mechanisms by which EVs influence recipient cell physiology, as well as how this signaling axis contributes to overall pathogenesis and tumorigenesis.

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Joshua E. Messinger*, Elizabeth Pavlisko, Joanne Dai, Lyla Stanland, Alexander Price and Micah Luftig

Duke University

Identification of Host Biomarkers of Epstein-Barr Virus Latency IIb and Latency III

Deciphering the molecular pathogenesis of virally induced cancers is challenging due, in part, to the heterogeneity of both viral gene expression and host gene expression. Epstein-Barr virus (EBV) is a ubiquitous herpesvirus prevalent in B-cell lymphomas of immune-suppressed individuals. EBV infection of primary human B cells leads to their immortalization into lymphoblastoid cell lines (LCLs), serving as a model of these lymphomas. In previous studies, reports from our laboratory have described a temporal model for immortalization with an initial phase characterized by expression of Epstein-Barr nuclear antigens (EBNAs), high levels of c-Myc activity, and hyperproliferation in the absence of the latent membrane proteins (LMPs), called latency IIb. This is followed by the long-term outgrowth of LCLs expressing the EBNAs along with the LMPs, particularly NFκB-activating LMP1, defining latency III. However, LCLs express a broad distribution of LMP1 such that a subset of these cells express LMP1 at levels similar to those seen in latency IIb, making it difficult to distinguish these two latency states. In this study, we performed mRNA sequencing (mRNA-Seq) on early EBV-infected latency IIb cells and latency III LCLs sorted by NFκB activity. We found that latency IIb transcriptomes clustered independently from latency III independently of NFκB. We identified and validated mRNAs defining these latency states. Indeed, we were able to distinguish latency IIb cells from LCLs expressing low levels of LMP1 using multiplex RNA-fluorescence in situ hybridization (RNA-FISH) targeting EBV EBNA2 or LMP1 and human CCR7 or MGST1. This report defines latency IIb as a bona fide latency state independent from latency III and identifies biomarkers for understanding EBV-associated tumor heterogeneity.

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James Meyo*, Aubrey Bailey, Carson Mosso, Justin Landis, Kenny Dinnon, Kathleen Corcoran, Dirk Dittmer

University of North Carolina, Chapel Hill

Exploring kinase inhibitors as a therapy for KSHV-associated cancers

Kaposi sarcoma-associated herpes virus (KSHV) is a DNA tumor virus that causes Primary Effusion Lymphoma (PEL) and two other human cancers. KSHV has a latent and lytic phase that are both essential to tumor maintenance. The lytic phase is short-lived, spontaneous and characterized by expression of all viral genes to facilitate virion production. The Replication and Transcription Activator (RTA), is the only protein that is both necessary and sufficient to induce the lytic cycle. RTA is a viral transcription factor that has many post-translational modifications, including phosphorylation. Interestingly, cellular kinases that target RTA have not been well described previously. The objective of this research is to identify the kinases that target RTA and explore the effectiveness of kinase inhibitors in perturbing RTA activity during lytic reactivation.

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Emily Moore*, Dana Jeffus, Alexis Davis, and Amy Adamson*

University of North Carolina, Greensboro

Epstein-Barr virus lytic replication activates and is dependent upon MAPK-interacting kinase 1/2 in a cell-type dependent manner

Epstein-Barr Virus (EBV) is a prevalent human herpesvirus. Infection with EBV is associated with several diseases, such as Burkitt's Lymphoma, nasopharyngeal carcinoma, and gastric carcinoma. EBV lacks the machinery necessary for synthesis of viral proteins, and therefore must exploit major cell signaling pathways for cap-dependent translation. The Akt/mammalian target of rapamycin complex 1 (mTORC1) and mitogen-activated protein kinase (MAPK) pathways stimulate downstream targets to promote biogenesis. Previous research showed that under rapamycin-mediated inhibition of mTORC1, EBV lytic protein production was altered in a cell-type specific manner, suggesting that EBV differentially activates or utilizes these pathways in B versus epithelial cells. Here we correlated activation of the mTORC1 pathway in relation to EBV lytic replication and discovered that activation of MAPK-interacting kinase 1/2 (Mnk1/2) was strongly associated with EBV lytic replication. Mnk1/2 promotes cap-dependent translation by phosphorylating the initiation factor eIF4E; increases in both proteins are associated with carcinogenesis. We found that during lytic replication, abundant phosphorylated Mnk1/2 was present in the nuclei of both B and epithelial cells; the difference was that phospho-Mnk1/2 co-localized with replicating EBV in epithelial cells but was present only in non-lytic B cells. Inhibition of mTORC1 by rapamycin intensified these effects. We further determined that Mnk1/2 activated lytic replication in epithelial cells yet acted as an inhibitor of lytic replication in B cells. The dependence of EBV lytic replication upon the activity of Mnk1/2, in a cell-type dependent manner, makes this kinase an interesting target for the control of EBV lytic replication.

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Ashley N. Nelson*, Maria Dennis, Jesse F. Mangold, Katherine Li, Riley J. Mangan, George M. Shaw, Katharine Bar, Barton Haynes, M. Anthony Moody, Justin Pollara, Koen K.A. Van Rompay, Kristina De Paris, and Sallie R. Permar

Duke University

A vaccination regimen to enhance immune responses associated with reduced risk of mother-to-child transmission of HIV

Progress towards the elimination of pediatric HIV infection via mother to child transmission (MTCT) is limited by several factors, including inconsistent access and adherence to maternal antiretroviral therapy (ART). The development of a maternal vaccine that synergizes with current ART prophylaxis could overcome challenges impeding achievement of an HIV-free generation. Both the epitope specificity of HIV envelope (Env)-specific antibody responses and autologous virus neutralization have been implicated in MTCT risk of HIV. Our goal was to evaluate the immunogenicity of a heterologous vaccine regimen to boost autologous HIV Env-specific antibody responses in Simian-Human Immunodeficiency Virus (SHIV)-infected, ART-suppressed rhesus macaques (RMs).

Twelve female RMs were infected intravenously with SHIV.CH505, and began a daily ART regimen at 12 weeks post-infection (wpi). At 2, 6, and 10 weeks of ART, RMs received either a HIV clade B/C gp120 vaccine (n=6) or a RSV vaccine (n=6) intramuscularly. ART was discontinued after 12 weeks. Binding and functional antibody responses were evaluated.

HIV Env gp120 vaccination not only induced robust IgG responses against the Env vaccine immunogen, but also enhanced the SHIV.CH505-specific IgG response. Additionally, the vaccinated animals exhibited enhanced V3-specific and soluble CD4-blocking IgG responses, and a rapid increase in tier 1 virus neutralization responses, all previously identified predictors of decreased MTCT risk. Yet, plasma autologous virus neutralization was similar between the two groups. Thus, vaccination of SHIV-infected RMs in the setting of ART can boost IgG responses against the original infecting antigen, SHIV.CH505, and Env-specific antibody responses previously associated with low risk of MTCT.

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Comparative Analysis of Interferon Regulatory Factors (IRFs) and their impact on cutaneous papillomavirus (PV) function

Cutaneous papillomaviruses (PVs) are oncogenic viruses that can cause severe, persistent skin infections that develop into non-melanoma skin cancers (NMSC), particularly in immunodeficient individuals. Viral persistence is key for cancer development, and PVs have evolved many mechanisms to dampen the immune system in order to avoid clearance. Persistent cutaneous PV infections that progress to metastatic cancer also occur similarly in immunodeficient dogs. Canine and human PVs share similar immunological characteristics and mechanisms of action within keratinocytes, their target cell for infection. Thus, dogs offer a spontaneous, natural animal model to study cutaneous PVs. We have shown that oncogenes E6 and E7 from a cutaneous canine PV are responsible for inhibiting the host interferon immune response in canine keratinocytes by interfering with expression of antiviral interferons (IFN) and interferon-stimulated genes (ISG), but the exact mechanism is unknown. We hypothesize that E6 and/or E7 inhibit interferon regulatory factors (IRF), which are necessary for the upregulation of IFN and ISGs during a viral infection. The pattern of IRFs responsible for this function has not been fully characterized in keratinocytes. Thus far, data suggests that IRF3 and IRF5 play a role in IFN and ISG expression, whereas IRF6 has no effect, and IRF1 and 7 are currently being investigated. We will then determine how these IRFs impact critical PV functions, including viral replication and transcription, and PV's effect on host tumor suppressor gene function, which is known to be suppressed by PV E6 and E7. Future investigations will examine mechanisms by which E6 and E7 proteins cause IRF dysfunction, progressing toward the long-term goal of developing IRF restorative treatments.

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The Mechanism of N6-Methyladenosine Deposition on Hepatitis C Viral RNA

The positive sense ssRNA virus hepatitis C (HCV) uses a variety of RNA regulatory mechanisms to carefully coordinate its lifecycle. Recently we have shown that the RNA base modification N6-Methyladenosine is present on the viral genome. Further, disruption of m6A sites within the viral RNA and depletion of the cellular m6A machinery both positively regulate the production of infectious viral particles. While methylation of cellular mRNAs occurs in the nucleus, HCV RNA is only present within the cytoplasm. Therefore, we hypothesize that HCV encodes a mechanism to recruit components of the m6A methylation machinery to the viral RNA. We found that the HCV E1 protein interacts with WTAP, a protein thought to be the central coordinator and targeting component of the m6A methylation complex. Importantly we also found that WTAP negatively regulations production of infectious viral particles. Currently we are engaged in mapping the interface of interaction between E1 and WTAP to engineer mutations that disrupt this interaction. Ultimately, our data suggest that E1 coordinates the recruitment of the m6A methylation machinery to viral RNA to facilitate m6A modification of the viral RNA and regulation of its replication.

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Human Endogenous Retrovirus Expression in HIV-associated Diffuse Large B-cell Lymphoma

Human endogenous retroviruses (HERVs) are viral elements integrated into the human genome that are associated with health and disease. We previously found that HERVs were negatively prognostic in The Cancer Genome Atlas RNA-seq which was composed of ~10,000 tumor samples from 32 cancer types (Smith et al. JCI, 2018). HERVs are up-regulated by HIV and some anti-retroviral therapies may cause a decrease in HERV expression; specifically assessed in the HERV-K family. The association of HERVs, HIV, and anti-retroviral therapy (ART) have not been assessed in Diffuse Large B-cell Lymphoma (DLBCL) despite its association with HIV infection.

We assessed RNA-seq samples from 36 pre-treatment cases of DLBCL (22 HIV+/14 HIV-) from the Kamuzu Central Hospital Lymphoma Study in Lilongwe, Malawi. Of 22 HIV+ DLBCL, 12 were ART naïve, and 10 were ART experienced at diagnosis. We quantified HERV expression using a method we previously reported, hervQuant. HERVs were up-regulated in HIV+ and ART-naïve patients compared to HIV-, and down-regulated in HIV+ and ART-experienced patients compared to those without HIV ($p=0.02$, ANOVA). Notably, 500 of 2,579 (19%) expressed HERVs were associated negatively with prognosis (nominal $p<0.05$ Cox Proportional Hazard Regression). We found that HERVs were positively correlated with gene expression modules related to transcription/translation and inflammation, and negatively correlated with cell differentiation and tumor formation. This unique cohort allowed us to investigate the relationship between HIV, ART, and HERVs in the setting of DLBCL and uncover unique biological associations that may give us insight into this ubiquitous class of viruses.

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Kaposi Sarcoma in whole KSHV genome transgenic mice

Kaposi's sarcoma-associated herpesvirus (KSHV) is a causal agent for Kaposi's sarcoma (KS). In addition, the virus is associated with lymphoproliferative diseases such as primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD). Animal models provide priceless tools for basic research and drug development. Yet, they only exist in limited form for KSHV, since this herpesvirus only infects humans. We generated a transgenic mouse (d.197) that carries the entire ~140,000bp viral genome. 15% of the mice develop angiosarcoma (AS) within 100 days. By pathology murine AS resembled human KS. Almost all of the KSHV genes were expressed in AS, as well as in multiple other organs. Endothelial cell (EC) markers were expressed in AS, establishing the EC as the cell of origin for AS. Complete blood count (CBC) suggest that d.197 mice also suffer life-threatening anemia. The pAKT, pmTOR, pS6, p4EBP1, VEGFA, VEGFR2, and pERK, were robustly expressed in AS indicating mTORC1 and VEGF signaling contribute to the phenotype.

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A role for ufmylation in innate immunity

The antiviral innate immune responses that induce type-I interferon (IFN) and IFN-stimulated genes (ISGs) are often the first line of defense against viral pathogens. These responses are tightly coordinated to provide host protection and prevent prolonged inflammation. To define novel regulators of innate immune signaling through the RIG-I/MAVS cascade, we previously uncovered new factors that relocalize to sites of innate immune signaling upon RNA virus infection. One of these new factors is UFL1, the E3 ligase for a relatively unexplored post-translational modification, ufmlyation, which is a ubiquitin-like modification in which ubiquitin-fold modifier 1 (UFM1) is added to lysine residues of target proteins. While the ufmylation pathway is known, relatively little is known about the functional importance of this post-translational modification, especially in antiviral innate immunity. We found that UFL1 relocalizes to sites of immune signaling complex formation and interacts with protein complexes that contain the essential innate immune adaptor protein, MAVS. Additionally, we have shown that overexpression of multiple components of the ufmylation conjugation machinery positively regulate IFN induction during antiviral innate immunity. Further, we found that loss of UFL1 leads to decreased activation of the signaling proteins TBK1 and IRF3. Finally, we have identified new targets of ufmylation during the antiviral response. Further studies will focus on the mechanisms by which ufmylation modulates the innate immune response and the impact of ufmylation of proteins on antiviral activity.

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Targeting the PI3K Pathway in Gastric Cancer

The PI3K/mTOR/AKT signaling pathway plays a crucial role in cell proliferation and survival, protein synthesis, metabolism and differentiation. The PIK3CA gene is the second most commonly mutated gene in cancer following TP53, and is mutated in 80% of EBV-positive gastric cancers and 20% of gastric cancers independent of EBV infection status. Although PIK3CA mutant tumors do not show a different prognosis than PIK3CA wild type tumors, cellular dependence on the PI3K/mTOR/AKT pathway represents a potential target for therapeutics. In this study, we utilized a potent PIK3CA inhibitor, BYL719, across a panel of cancer cell lines to identify sensitivities of multiple tissue types to single agent and combination therapies that target the PI3K pathway. We completed a targeted oncogene CRISPR knockout screen in the presence of BYL719 to identify synergistic interactions that may lead to novel drug combinations. Lastly, we explored differential cellular dependencies that were present in EBV positive gastric cancer cell lines when compared to EBV negative cell lines.

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Epstein-Barr Virus as an Environmental Agent in Neurodegenerative Diseases: Lewy Bodies and Beyond

Epstein- Barr virus (EBV) is a human herpesvirus that infects over 90% of the world's population, entering epithelial cells causing infectious mononucleosis, to later lay dormant in B cells. EBV can infect astrocytes and microglia, albeit through poorly defined pathways, and it can control the Mechanistic Target of Rapamycin (mTORC1) pathway to promote its viral lytic cycle. mTORC1 is involved in many neuronal functions, including cell survival and autophagy, thus highly relevant for neurodegenerative diseases. EBV has been implicated in nervous system (NS) conditions, including viral encephalitis associated with reversible Parkinsonism and recently multiple sclerosis, but interactions between EBV and the brain are still widely undescribed. Braak's dual hit hypothesis for initiationd of Parkinson's disease (PD) and Lewy Bodies proposes an environmental agent that induces cellular changes in the gut and/or nasopharyngeal tract, with consequences for the NS. In this context, we hypothesize that EBV has the potential to affect neuronal cell function by manipulating mTORC1, either via direct infection or due to viral protein interference, abetting neurodegenerative diseases or cellular disfunction. To test this hypothesis, we infected the Sh-sy5y neuroblastoma cell line, differentiated into dopaminergic neurons (DAs), and we have confirmed infection via quantitative PCR. Our results suggest that since EBV can enter differentiated DAs, the virus is a feasible candidate to initiate cellular changes, as predicted by Braak. We are currently studying infection progression using a 10-day time-course measuring not only infection levels, but also viral transcript production and alterations to mTORC1 and other autophagy related proteins.

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Optimizing a CRISPR screening system to identify regulators of hemagglutinin trafficking during influenza virus infection

Influenza viruses present a large burden on public health resulting in 10 million hospitalizations and nearly 200,000 deaths each year globally. Our lab is interested in understanding the host-pathogen interactions associated with influenza infection to inform the development of antiviral therapeutics. Here, we seek to better understand cellular factors that control the trafficking of the IAV hemagglutinin (HA) protein to the plasma membrane during infection. HA must be localized to the plasma membrane of infected cells for the production of infectious virus. During infection, HA is translated on the ER as a transmembrane protein and then trafficked through the secretory pathway utilizing lipid rafts for apical membrane targeting. Terminal glycosylation of the HA protein is necessary for successful trafficking of the HA protein to occur and glycosylation inhibitors have shown to be effective in restricting translocation of IAV glycoproteins to the plasma membrane. Using CRISPR-screening technology, we can individually activate or knockout genes to observe phenotypic effects on the trafficking of HA during infection. We used an mNeon-HA reporter virus to deliver HA to cells transduced with the GecKO v2 library of sgRNAs. We observed that infected cells expressed both mNeon and HA regularly in untransduced A549 cells. In cells transduced with GecKO library A, we observed the formation of a small population of cells that were less capable of trafficking HA to the plasma membrane. Using this optimized system, we will conduct a screen to determine host factors that promote IAV HA trafficking to the plasma membrane.

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Dormant breast tumor cells avoid the adaptive immune system through regulation of the tumor microenvironment

Many tumors respond to early treatment with complete remission only to recur, sometimes decades later, in a distant metastatic site. However, why the immune system is ineffective at eliminating disseminated/dormant tumor cells before growing into new metastatic lesions is currently unknown. In the current study, mammary fat pad (MFP) injections (n=8/group) revealed that proliferative tumors rapidly grow in the absence of an adaptive immune system in SCID mice but tumor growth is significantly reduced in syngeneic Balb/c mice ($p<0.0005$). Surprisingly, the dormant tumors grow at the same rate in Balb/c and SCID mice and persist post implantation up to 12 weeks and beyond in both, suggesting that these tumors are able to avoid the effects of the adaptive immune system despite very slow/minimal growth. To determine potential mechanisms of this differential response, MFP implanted tumors in Balb/c mice were evaluated 5 weeks post implantation (n=5/group). Interestingly, the dormant tumors contained significantly more Tregs than proliferative tumors (25.2% vs 12.6%; $p=0.0009$), as well as a lower CD4:CD8 ratio (0.45:1 vs 2.74:1; $p=0.032$) and enhanced PD-L1 expression on infiltrated myeloid cells (MFI = 1080.75 vs 1729.25; $p = 0.0146$). Furthermore, analysis of tumor cells revealed that dormant cells express significantly more immuno-modulatory cytokines and matricellular proteins including IL-10, TGFβ, tenascin-c, and periostin. Taken together, our data illustrates that dormant tumors survive and persist long term despite the immune system, likely by creating a immunosuppressive ECM and cytokine network.

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CD47 blockade augmentation of Trastuzumab antitumor efficacy dependent upon antibody-dependent-cellular-phagocytosis

The HER2-specific monoclonal antibody (mAb), Trastuzumab, has been the mainstay of therapy for HER2+ breast cancers (BC) for ~20 years. However, its therapeutic mechanism of action (MOA) remains unclear, with antitumor responses to Trastuzumab remaining heterologous and metastatic HER2+ BC remaining incurable. Consequently, understanding its MOA could enable rational strategies to enhance its efficacy. Using both novel murine and human versions of Trastuzumab, we found its antitumor activity dependent on Fcγ-Receptor stimulation of tumor-associated-macrophages (TAM) and Antibody-Dependent-Cellular-Phagocytosis (ADCP), but not cytotoxicity (ADCC). Trastuzumab also stimulated TAM activation and expansion, but did not require adaptive immunity, natural killer cells, and/or neutrophils. Moreover, inhibition of the innate immune ADCP checkpoint, CD47, significantly enhanced Trastuzumab-mediated ADCP, TAM expansion and activation, resulting in the emergence of a unique hyper-phagocytic macrophage population, improved antitumor responses and prolonged survival. In addition, we found tumor-associated CD47 expression was inversely associated with survival in HER2+ BC patients and that human HER2+ BC xenografts treated with Trastuzumab+CD47 inhibition underwent complete tumor regression. Collectively, our study identifies Trastuzumab-mediated ADCP as a significant antitumor MOA that may be clinically enabled by CD47 blockade to augment therapeutic efficacy.

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Maternal plasma broadly neutralizing antibodies drive the selection of neutralization-resistant viruses that initiate HIV infant infection

Despite the worldwide availability of antiretrovirals (ARV), ~180,000 pediatric HIV infections occurred in 2017. Thus, new strategies to prevent MTCT of HIV-1, such as a maternal HIV vaccine, are needed. To design immune-based treatments, it is necessary to understand the role of maternal neutralizing antibodies (nAbs) in potentially driving the genetic bottleneck of infant transmitted founder (T/F) viruses. Four HIV in utero or peripartum transmitting mother-infant pairs from the North American Women Infant Transmission Study (WITS) cohort and the Malawian Center for HIV/AIDS Vaccine Immunology 009 (CHAVI009) cohort were studied. Mothers 0601 and 9105 transmitted viruses to their infants (0616 and 9112 respectively) that were differentially sensitive to a panel of bnAbs. 0616 infant T/F and its closest maternal variant were resistant to V2 glycan-targeting bnAbs PG9, PG16, and PGT145 while the other maternal viruses were sensitive to these bnAbs. No V2-glycan targeting neutralization was detected in the maternal plasma, making the mechanism for selective transmission of the V2-glycan resistant variant unclear. The 9112 infant T/F virus was resistant to the V3 glycan-targeting bnAb PGT128 while the tested maternal viruses were not. A substantial fraction of the maternal plasma neutralizing activity in this case mapped to the V3-glycan bnAb epitope, suggesting that V3-glycan bnAb activity may select for V3-glycan bnAb resistance in the transmitting virus. These findings suggest that maternal plasma bnAb responses during natural infection may not be sufficient for preventing MTCT of HIV-1 and may drive the selection of neutralization-resistant infant T/F viruses.

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Recent Epstein Barr virus strains isolated from endemic Burkitt's lymphoma patients display a spontaneous lytic phenotype

Epstein Barr virus (EBV) is a large dsDNA gamma-herpesvirus that infects the majority of adults. In most people, the virus maintains a latent, asymptomatic infection in memory B cells. However, under certain conditions, like immunosuppression, the virus can lead to various malignancies such as endemic Burkitt's lymphoma (eBL). eBL is the most prevalent childhood cancer in Africa and is nearly 100% associated with EBV infection. Recently, we generated cell lines from Kenyan eBL patient tumors which enables us to study the diversity of currently-circulating EBV strains. Interestingly, EBV isolated from latently infected eBL cells display a spontaneous lytic phenotype that is atypical for herpesviruses. Our data indicate that this spontaneous lytic phenotype is encoded within the viral genome. Here, we explore viral gene expression and protein dynamics of these novel spontaneously lytic strains compared to their strictly latent counterparts. Ultimately, this work will determine viral genetic elements responsible for the production of this spontaneous lytic phenotype.

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mRNA Cap 2`-O-methylation by CMTR1 regulates innate immune responses

Responses to viral infection induce type-I interferon (IFN) signaling and a subsequent transcriptional burst that is tightly regulated to provide host protection but prevent damage from prolonged inflammation. The mechanisms by which post-transcriptional processing of host mRNA affect IFN responses and the translation of IFN-stimulated genes (ISGs) is less defined. Here we find that the multiple RNA viruses replicate more robustly in cells depleted of CMTR1. the nuclear host cap 1 2'O methyltransferase, CMTR1, a known ISG (previously ISG95), We then show CMTR1 is required for translation of specific classes of ISGs known to restrict virus infection, dependent on STAT1 signaling. Depletion of CMTR1 reduces the protein levels of many antiviral ISGs without affecting transcript levels. Intriguingly, the protein levels of some but not all ISGs correlates with CMTR1 abundance, indicating the existence of distinct classes of ISGs that are either translated through CMTR1 and cap-dependent processes or those that are translated independently of Cap 1 status Finally, deletion of a component of the interferon stimulated host Cap 0 translational inhibitor complex IFIT3 reduces translational dependence of multiple transcripts on CMTR1 activity. Together we establish the importance of the upregulation of the host cap 2'O methyltransferase, CMTR1, in response to IFN for limiting virus replication and establishing the cell-intrinsic innate immune response at a post-transcriptional level.

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The Role of the ATR DNA Damage Response Pathway During the Life Cycle of HPV31

Persistent infection with HPV types termed “high-risk” is associated with multiple human cancers, including cervical cancer. While HPV vaccines successfully prevent infections, they provide no protection against pre-existing HPV infections. HPV genomes are maintained as episomes at a low copy number in undifferentiated epithelial cells. Upon epithelial differentiation, the productive phase of the life cycle is activated, resulting in viral genome amplification and production of mature virions. Differentiating epithelial cells normally exit the cell cycle; however, HPV pushes differentiating cells back into the cell cycle largely through E7’s ability to target the cell cycle regulator Rb for degradation. Unscheduled cell cycle entry results in replication stress (RS) and DNA damage, leading to activation of the DNA damage kinases ATR and ATM, as well as their downstream effector kinases, Chk1 and Chk2, respectively. ATR and Chk1 activation mitigates RS by maintaining high levels of the E2F1 transcription factor. E2F1 drives expression of RRM2, a component of the ribonucleotide reductase complex that is necessary for de novo dNTP synthesis. We previously found that the ATR/Chk1/RRM2 pathway is necessary for HPV31 replication.

- Our preliminary data suggests that RRM2 mRNA levels and protein stability are increased in HPV positive cells in an E7-dependent manner through ATR activation.
- Also, preliminary data suggests that there is an increase of DNA damage during ATR inhibition.
- In addition, we have found HPV utilizes ATR activity to maintain high levels of factors involved in homologous recombination repair (e.g. BRCA1, Rad51), which we have previously shown is necessary for productive viral replication.

Although HPV requires ATR activity for viral replication, it is unclear if activation of the ATR/Chk1/E2F1/RRM2 pathway promotes viral persistence by protecting cells during RS. Further understanding of how the ATR DNA damage response pathway contributes to HPV infection will provide insight into mechanisms of viral persistence, a major risk factor for development of HPV-associated cancers.