
50th Anniversary Howard Temin Nobel Prize Symposium

Friday, 12 September 2025

Discovery Building, 330 N. Orchard St., University of Wisconsin – Madison

Poster Abstracts

Posters will be displayed throughout the meeting

Presenters will be at their posters from 12:40 – 1:30

Posters will be displayed on stands numbered with the abstract page number

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KLF4-PARP1 interaction regulates AAV genome localization and gene expression

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Adeno-associated virus type 2 (AAV2) is a small DNA virus that forms the basis of recombinant AAV (rAAV) vectors used in gene therapy. Wild-type AAV2 genomes target nuclear areas rich in DNA damage response (DDR) factors, while rAAV vectors do not, which could explain differences in transcriptional activity between AAV and rAAV and the need for high therapeutic doses. Understanding how AAV2 targets DDR sites is therefore essential for improving rAAV design. I studied two DDR host factors, KLF4 and PARP1, as regulators of AAV2 genome localization. Motif analysis and chromatin immunoprecipitation (ChIP) revealed KLF4 binding near viral promoters, and mutating this site (AAV2ΔKLF4p5) reduced expression of Rep68/78 and disrupted both KLF4 and PARP1 binding to the genome. Inhibition of PARP1 with Olaparib, along with V3C-seq and ImmunoFISH, further supported PARP1's role in genome localization. Ongoing studies using CRISPR-mediated PARP1 knockouts and mutants will test whether PARP1's enzymatic activity and its interaction with KLF4 are necessary for this process. These findings support a model where KLF4 recruits PARP1 to AAV2 genomes, aiding their localization to DDR foci. This work provides a mechanistic framework for viral persistence and offers strategies to engineer rAAV vectors with improved nuclear targeting and transcriptional activity.

Comparative structural insights into the immature HIV capsid lattice

William G. Arndt, Jiaxin Wei, Dalton Piottter, Huixin Yang, Wei Zhang, and Louis M. Mansky

University of Minnesota - Twin Cities

One of the key aspects of HIV replication that has remained underexplored has been virus particle assembly. The Gag protein – the major retroviral structural protein – is crucial for driving particle assembly. The capsid (CA) domain is the key determinant for Gag multimerization by forming an incomplete spherical lattice consisting of capsid hexamers. Despite strong homology between the HIV-1 and HIV-2 capsid sequences, previous studies have shown the HIV-2 lattice is more homogenous and complete compared to the HIV-1 lattice. While the HIV-1 CA lattice has been well characterized by high resolution structural analysis, the HIV-2 lattice has been poorly characterized. Here, we sought to use cryo-electron microscopy (cryo-EM) to determine high-resolution structures of the immature lattice. Using single particle analysis (SPA), we have determined a reconstruction of the HIV-2 immature lattice at 2.9 Å resolution. The cryo-EM structural analysis has revealed a highly conserved capsid hexamer structure between the HIV-1 and HIV-2 lattices. Despite the high conservation, there are subtle differences in the intra-hexamer six-helix bundle (6HB) between these viruses. Bevirimat (BVM), a HIV-1 maturation inhibitor that binds to the six-helix bundle, has been shown to have limited antiviral activity against HIV-2. We determined a 3.1 Å resolution structure of BVM bound to the immature HIV-2 CA lattice, suggesting the loss of BVM antiviral activity against HIV-2 is due to an unknown resistance mechanism rather than a loss of drug binding. Further, addition of purified HIV-2 protease to HIV-2 assembled in the presence of BVM shows no inhibition in processing despite BVM binding. Taken together, these observations imply that subtle differences in the immature CA lattice can impact particle morphology, lattice stability, and susceptibility to antivirals.

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Single-cell analysis of systemic immune response to sequential radiation and immune checkpoint blockade in metastatic colorectal cancers

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Immune checkpoint blockade (ICB) has shown significant clinical efficacy in mismatch repair deficient colorectal cancers (CRCs). However, an effective means to use these therapies for mismatch repair proficient (pMMR) CRC has yet to be developed. To enhance the efficacy of ICB for patients with oligometastatic pMMR CRC, we performed a clinical trial evaluating the sequential use of stereotactic body radiotherapy (SBRT), pembrolizumab, surgical resection, and then adjuvant pembrolizumab (NCT02837263). The primary endpoint of 1-year recurrence-free survival (1 yr RFS) was met in 60% of trial participants compared to a historical control of 50%. From this trial, 13 subjects could have blood samples collected for peripheral blood mononuclear cell isolation pretreatment and after operative management. We hypothesize that systemic immune response changes upon combined treatment and between responders and non-responders. We performed scRNA-Seq on these samples to compare the circulating immune cell populations with the clinical outcomes for these subjects. Despite of small sample size, integrated bioinformatics analysis identified (i) pre-existing CD8⁺ T cells were observed with treatment response ($p = 0.031$) (ii) combined RT and ICB induces changes in myeloid cell plasticity, including reducing pro-inflammatory monocytes ($p = 0.038$) and conventional cDC2 ($p = 0.030$) in subjects who without recurrence after a year and increasing pDCs ($p = 0.024$) in most patients. CD8⁺ T cells from those recurrence-free subjects showed terminally differentiated effector phenotypes, expressing *CX3CR1*, *GZMB*, and *KLRG1* before treatment. cDC2s expressed interferon activity markers, including *STAT1*, *IFI44*, and *IRF7*, that presented a more activated state. CD8⁺ T cell abundance remained stable, with no significant changes before and after treatment. In contrast, myeloid cells showed greater variability, reflecting unique polarization states through differences in cytokine (*CCL3*, *CXCL8*), interferon (*ISG15*, *IFI44*), and complement (*C1QA*, *C1QB*) associated genes. Future work will increase sample size in independent patient cohorts to elucidate the extent to which circulating phenotypes are also identified in tumors. Overall, this study demonstrates pre-existing T cell abundance and changes in myeloid cell phenotypes are associated with clinical outcomes from combined immunotherapy, which have the potential to serve as predictive biomarkers for therapeutic response.

HIV-1 virological synapse formation enhances infection spread by dysregulating Aurora Kinase B

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HIV-1 spreads efficiently through virological synapses (VSs), cell-cell interfaces induced by interaction of HIV-1 envelope protein on infected cells and the CD4 receptor on uninfected target cells. Using mass spectrometry, we identified changes in the levels and phosphorylation of many cellular proteins in HIV-1-infected cells after co-culture with CD4+ cells. We identified alterations in numerous cellular pathways, including striking effects on genes regulating the cell cycle. Notably, the key cell cycle regulatory kinase Aurora kinase B (AURKB) showed a dramatic decrease in kinase activity. We found that AURKB specifically inhibits HIV-1 Env-mediated cell fusion and cell-to-cell transmission. This suppression is specific to HIV-1 Env and alters Env's membrane fusion activity. We found that, in T cell lines and primary T cells, HIV-1 envelope-CD4 engagement prevents this suppression by causing premature relocalization of AURKB to nuclear centrosomes but does not alter the cell cycle state of the infected cell. Thus, we identified cellular signaling pathways altered by co-culturing HIV-1+ and CD4+ T cells, novel effects of HIV-1 on cell cycle regulatory kinases and a previously unknown role for AURKB at the plasma membrane. The results have significant implications for understanding HIV-1 and cell processes and for controlling HIV-1.

Identification and characterization of cellular proteins that interact with HIV-1 RNA

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Human immunodeficiency virus is the causative agent of AIDS. This retrovirus integrates into the human genome and maximizes its coding capacity by generating over 20 different viral transcripts, broadly broken into unspliced (US), partially spliced (PS), and completely spliced (CS) classes of RNAs, each playing distinct roles in viral replication. In addition, HIV viral transcripts have unique 5' leader sequences with specific functions. Elucidating the host protein 'interactomes' for each variant viral transcript is crucial to understanding virus-host interplay. Previously we described utilizing Hybridization purification of RNA-protein complexes followed by Mass spectrometry (HyPR-MS) for the isolation of the interactomes for the US, PS, and CS transcript classes (*Knoener et al. eLife 2021;10:e62470*). Here, we describe expansion of this technique in combination with fluorescent reporter viruses to expand our understanding of the cellular interactome for specific HIV-1 transcript variants and track these interactions in the cell. This study validates HyPR-MS for discovery of specific viral splice variant protein interactomes and provides an unprecedented resource of factors and pathways likely important to HIV-1 replication.

Complement receptor 1 and Notch 2 mediate entry of Kaposi sarcoma-associated herpesvirus into human B cells

Beniah Brumbaugh, Mitchell Hayes, and Bill Sugden

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Kaposi sarcoma-associated herpesvirus (KSHV) causes several B cell lymphoproliferative disorders yet consistently infects only ~1-2% of primary B cells, regardless of donor. This suggests KSHV has a narrow tropism for a rare B cell subset. Identifying these cells could reveal the cellular origin of KSHV-associated Primary Effusion Lymphoma. We are pursuing two approaches to identify this rare B cell subset: 1) using surface mediators, Complement Receptor 1 (CR1) and Notch 2, to isolate primary B cells permissive to KSHV infection; and 2) isolating primary B cells bound by an mNeon glycoprotein-tagged KSHV. Using targeted CRISPR/Cas9 screening, we identified five candidate entry receptors for KSHV: CR1, Notch 2, EphA7, Siglec-7, and XK. We validated CR1 and Notch 2 as necessary for KSHV infection using knockout and cDNA complementation experiments. Virus binding assays confirmed significantly reduced binding in CR1 and Notch 2 knockout cells. Data mining of tonsillar scRNAseq data shows ~5% of tonsils co-express CR1 and Notch 2, supporting the hypothesis that these surface molecules are co-expressed on the small fraction of primary B cells which KSHV infects. These findings demonstrate that CR1 and Notch 2 are surface mediators of KSHV infection in B cell lines. We are now testing whether CR1 and Notch 2 mediate infection in primary B cells and can be used to isolate and characterize the 1-2% of primary B cells permissive to KSHV.

Overcoming paclitaxel resistance by preventing focusing of multipolar spindles

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One out of every eight women in the United States will be diagnosed with breast cancer in her lifetime. Anti-microtubule therapies are one of the leading therapeutic options available. Paclitaxel (PTX), a microtubule-stabilizing agent, is standard-of-care breast cancer treatment that is considered highly effective. However, with a response rate of approximately 50%, there is a pressing need to circumvent resistance. For decades paclitaxel and other microtubule poisons were believed to be efficacious by causing mitotic arrest and subsequent cell death. We recently showed that concentrations of paclitaxel in primary breast cancers are too low to cause mitotic arrest; instead, these low concentrations cause cells to undergo an abnormal mitosis with mitotic spindles that aberrantly contain >2 spindle poles (termed multipolar spindles; Fig 1B). Division of the 2 sets of replicated chromosomes into >2 cells cause intolerable levels of chromosome missegregation and daughter cell death, resulting in paclitaxel efficacy. Resistance to paclitaxel arises when cells focus paclitaxel-induced multipolar spindles into normal bipolar spindles, which dramatically reduces chromosome missegregation (Fig 1C). The mechanism of focusing has yet to fully be elucidated. However, my preliminary data suggests three key players are the plus-end directed motor protein HSET (encoded by the KIFC1 gene), the mitotic kinase Aurora A, and the microtubule-associated protein TACC3.

CENcyclopedia: dynamic landscape of kinetochore architecture throughout the cell cycle

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The kinetochore, an intricate macromolecular protein complex located on chromosomes, plays a pivotal role in orchestrating chromosome segregation. It functions as a versatile platform for microtubule assembly, diligently monitors microtubule binding fidelity, and acts as a force coupler. Comprising over 100 distinct proteins, many of which exist in multiple copies, the kinetochore's composition dynamically changes throughout the cell cycle, responding to specific timing and conditions. This dynamicity is important for establishing functional kinetochores, yet the regulatory mechanisms of these dynamics have largely remained elusive. In this study, we employed advanced quantitative immunofluorescence techniques to meticulously chart the dynamics of kinetochore protein levels across the cell cycle. These findings offer a comprehensive view of the dynamic landscape of kinetochore architecture, shedding light on the detailed mechanisms of microtubule interaction and the nuanced characteristics of kinetochore proteins. This study significantly advances our understanding of the molecular coordination underlying chromosome segregation.

Contributions of small DNA tumor viruses to oncoprotein-induced replication stress

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Tumor viruses cause ~15% of human cancers worldwide, posing a significant public health burden. Merkel Cell Polyomavirus (MCPyV) is causally associated with 80% of Merkel Cell Carcinoma (MCC) cases, where integration facilitates continued expression of viral oncoproteins, called tumor (T) antigens. Human Papillomavirus (HPV) is the etiological agent of at least 5% of cancers worldwide, contributing significantly to anogenital and oropharyngeal cancers. High-risk HPVs produce viral oncoproteins E6 and E7. Both MCPyV and HPV oncoproteins drive carcinogenesis through inactivation of tumor suppressor pathways, exacerbate replication stress, and impair host DNA repair. **In this study, we are testing the hypothesis that MCPyV and HPV viral oncoproteins alter host replisomes to increase their oncogenic potential.** Utilizing a quantitative unbiased screening methodology called Isolation of Proteins On Nascent DNA (iPOND), we are characterizing proteins at actively replicating DNA (replisomes) to identify factors differentially recruited in keratinocytes expressing MCPyV T antigens or high-risk HPV oncogenes. Putative targets for dysregulation will be identified in HPV-infected cervical cancer cells and patient-matched normal cells. These studies will enable us to identify tumorigenic pathways and processes manipulated by each virus that could serve as targets for novel therapies for patients with HPV-and MCPyV-associated cancers, as well as in virus-associated cancers more broadly.

Visualizing the trafficking of HIV-1 genomic RNA by super-resolution microscopy

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HIV-1 harbors a dimerized genomic RNA (gRNA) within a single virion. Although the proper packaging of dimerized gRNA is essential for HIV-1, the spatiotemporal regulation of this process within host cells remains largely unexplored. Fluorescence microscopy, while a powerful tool for investigating the spatiotemporal dynamics of cellular targets, is limited by a resolution of approximately 250 nm laterally and 600 nm axially, posing challenges for the detailed study of HIV-1 gRNA. To overcome these optical limitations, we have recently developed an optimized Expansion Microscopy technique, termed 12x 3D-ExM, which enables lateral resolution as fine as 20 nm using a standard confocal microscope. We are currently optimizing this method to study the dynamics of HIV-1 gRNA dimerization and its interaction with Gag polyprotein in a host cell.

Is EBV Gene Expression Dose-Dependent on DNA Copy Number?

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Epstein-Barr virus (EBV) is a ubiquitous human tumor virus causally associated with malignancies, including Burkitt lymphoma (BL). We identified cellular genes that, when inactivated, support BL cell growth after partial eviction of EBV using CRISPR/Cas9 screens. Knocking out all three members and three effectors of the epigenetic silencing complex HuSH improved cell survival after forced loss of EBV genomes. The Human Silencing Hub (HuSH) complex inhibits the expression of endogenous retroelements and viruses, including herpes simplex virus 1. We measured 20-60% increases in the expression of three latent genes in the BL cell line Oku1 following CRISPR/Cas9 knockout of the HuSH complex. In EBV-transformed B cells, viral genome copy number exists as a distribution. When a single cell is cloned, its progeny return to their parental distribution. We hypothesized that EBV gene expression is dose-dependent on genome copy number such that cells with a higher number of genomes have higher levels of transcription. In HuSH- Oku1 cells, we measured 15-45% reductions in the average number of EBV genomes per cell. However, EBV-transformed B-cells, separated using fluorescence-activated cell sorting, expressing the top 5% of latent protein LMP1, contain only 25% more EBV genomes on average than cells expressing the bottom 5%.

Identification of anti-tumoral neutrophil phenotypes induced by immunotherapies in head and neck cancer

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Only ~20% of recurrent and metastatic head and neck squamous cell carcinoma (HNSCC) patients respond to immune-checkpoint blockade (ICB) therapy. A major roadblock is the immunosuppressive nature of the tumor microenvironment. Increased infiltration of neutrophils in blood and tumors of HNSCC patients is associated with worse prognosis and ICB therapy resistance. Depleting neutrophils, however, with or without ICB treatment, has not inhibited tumor growth in mice bearing syngeneic MOC2 tumors, which are phenotypically analogous to immunologically “cold”, aggressive HNSCC tumors. We hypothesize that this is due to the heterogeneity of tumor-infiltrated neutrophils with both pro- and anti-tumoral phenotypes. This study uses multi-omics single-cell profiling, combining RNA and protein, to identify pro- and anti-tumoral neutrophil states in MOC2 tumors treated with recently reported neutrophil-related therapies. Specifically, we tested anti-CD40 agonist, TNF-alpha, and both in combination with cetuximab, an anti-tumor antibody (designated Neutrophil Activating Therapy, NAT) that has been recently reported in other cancers. Cetuximab (an anti-EGFR mAb) was used for MOC2-huEGFR (M2h), an HNSCC cancer model transduced with human-EGFR. We found this treatment induced new neutrophil subsets, defined by scRNA-Seq bioinformatics analysis. One subset has recently reported interferon-stimulated phenotypes, which we found in the anti-CD40 alone, and NAT combinatorial treatment groups; these two groups also showed the best anti-tumor efficacy. Gene signature of this subset is also found in scRNA-Seq of infiltrating neutrophils in our previous K17-KO MOC2 model, which shows spontaneous tumor regression in the absence of therapy. In addition, we identified a neutrophil subset associated with migration-related transcription factors only in NAT and TNFa treatment groups, which might explain why NAT is superior to anti-CD40 alone. Our findings thus far demonstrate the presence of neutrophil heterogeneity, with some phenotypes associated with anti-tumor responses, including a potentially new phenotype, in a murine head and neck cancer model induced by a combination of immunotherapies. Ongoing and future work includes identifying specific synergistic mechanisms of the anti-tumoral neutrophil subpopulations induced by anti-CD40 and TNF-alpha treatment and if these phenotypes are also seen in ICB-treated HNSCC patients who respond to their therapy. We anticipate our characterization of anti-tumoral neutrophil phenotypes induced by NAT in M2h tumors may provide insights into how more effective immunotherapy regimens beyond ICB can be designed to confer tumor clearance and prolonged survival in patients with head and neck cancer.

Combined inhibition of MEK and HDACs improves the cytotoxicity of CD4 and CD8 T cells in NRAS;ASXL1-driven acute myeloid leukemia mice

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Acute myeloid leukemia (AML) is a heterogeneous malignant blood cancer that occurs mainly in the elderly. Its treatment outcome is greatly influenced by leukemia-driving mutations. Oncogenic *NRAS* mutations are associated with both AML progression and multi-drug resistance and treatment failure in AML. We have previously developed a NRAS; ASXL1-driven secondary AML (NA-AML) mouse model, which is characterized by a suppressive immune microenvironment resulting in reduced leukemia killing activities of T cells. Targeting hyperactive RAS/MEK signaling via trametinib (T, a MEK inhibitor) attenuates the exhaustion of CD8 T cells and prolongs the survival of NA-AML mice. To further boost the efficacy of T, we performed a re-purpose screen of ~2,500 drugs, either approved by FDA for treating various human diseases or currently under clinical evaluation, in the absence or presence of T. We identified that quisinostat (Q), a 2nd generation of HDAC inhibitor, potently inhibited the growth of both mouse and human NA leukemia cells and significantly synergized with T in vitro. In addition, combined T and Q (TQ) downregulated AP-1 transcription factors and expression of immune checkpoint ligands (PD-L1/L2, CD80, and CD86) while upregulated expression of MHC I/II in mouse NA-AML cells.

We further validated our results in NA-AML mice. Vehicle (Veh) -treated mice rapidly succumbed to AML and died within 4-5 weeks post-treatment. T alone and Q alone slowed down AML progression and prolonged the survival of leukemia mice, while all TQ-treated mice remained alive ~22 weeks post-treatment. Surprisingly, TQ only provided moderate survival benefits in immunodeficient NSG mice engrafted with NA-AML cells, suggesting an important role of cytotoxic T cells in TQ-mediated AML alleviation. We then analyzed CD4 and CD8 T cells from moribund vehicle-treated mice and age-matched drug-treated mice. TQ decreased the exhaustion of CD4 and CD8 T cells and expanded the activated effector/memory T (Tem) cells. Single-cell RNA-Seq analysis showed increased expression of gene signatures associated with cytotoxicity of Tem cells, including CXCR3 and CXCR5. Consistent with these findings, leukemia: T cell co-culture experiments demonstrated significantly enhanced cytotoxicity of TQ-treated CD4 and CD8 T cells, which appeared to be mediated by increased expression of MHC I and II in AML cells. We are currently pursuing the underlying molecular mechanisms. Our data provide a strong rationale to develop immunotherapies via epigenetic modulation of both leukemia and leukemia-associated T cells.

Residual Infections after Spontaneous Regression of Rapid-onset Squamous Cell Carcinoma Following Papillomavirus Infections of the Base of the Tongue in Immunocompetent Mice

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Squamous cell carcinomas (SCCs) induced by the mouse papillomavirus MmuPV1, which are generally diagnosed 4-6 months post-infection, can be found as soon as 2 weeks post-infection. SCCs developed within 2 weeks in immune-competent, adult FVB (FVB/Ntac) mice at sites prone to human papillomavirus (HPV)-induced tumors and cancers in human patients, including the base of the tongue. The presence of HPV16 oncogene E5 increases the frequency of rapid-onset cancer development. Just 2-8 weeks after MmuPV1 infection, however, these rapid-onset cancers at the base of the tongue regress in a T-cell-dependent manner; while control mice exhibit regression of lesions and cancers between 2 and 4 weeks post-infection, lesions and cancers in T-cell depleted mice do not regress. While cancers and dysplastic lesions are cleared by the immune system, immune-privileged Lgr5+ stem cells continue to harbor MmuPV1. These mouse data suggest that residual infections can remain following regression of clinical disease, suggesting the possibility of latent disease at specific cells within the base of the tongue, which in humans is a site of papillomavirus associated cancers.

Integrated single-cell and spatial analysis of immune cell interactions in response to immune checkpoint blockade

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Approximately 15-20% of head and neck cancer squamous cell carcinoma (HNSCC) patients respond favorably to immune checkpoint blockade (ICB). Previous single-cell RNA-Seq (scRNA-Seq) studies identified immune features, including macrophage subset ratios and T-cell subtypes, in HNSCC ICB response. However, the spatial features of HNSCC-infiltrated immune cells in response to ICB treatment need to be better characterized.

Here, we perform a systematic evaluation of cell interactions between immune cell types within the tumor microenvironment using spatial omics data, using complementary techniques from both 10X Visium spot-based spatial transcriptomics and Bruker CosMx single-cell spatial omics with RNA gene panel, including 435 ligands and receptors. In this study, we used integrated bioinformatics analyses to identify cellular neighborhoods of co-localizing cell types in single-cell spatial transcriptomics and proteomics data. In addition, we used both publicly available scRNA-Seq and in-house spatial RNA-Seq data to identify spatially constrained Ligand-Receptor interactions in Responder patients.

With 522,399 single cells profiled with both RNA and protein from 26 patients, in addition to spot-resolved spatial RNA-Seq from 8 patients treated with ICB, together with bioinformatics analysis of publicly available single-cell and bulk RNA-Seq, we have identified a spatial and cell-type specific context-dependency of myeloid and T cell interaction difference between Responders and Non-Responders. We defined further cellular neighborhood and the sources of chemokine CXCL9/10-CXCR3 interactions in Responders, emerging targets in ICB, as well as CXCL16-CXCR6, CCL4/5-CCR5, and other underappreciated and potential markers and targets for ICB response in HNSCC. In addition, we have contributed a rich data resource of cell-cell Ligand Receptor interactions for the immunotherapy and HNSCC research community.

Our work provides a comprehensive single-cell and spatial atlas of immune cell interactions that correlate with response to ICB in HNSCC. We showcase how integrating multiple technologies and bioinformatics approaches can provide new insights into potential immune-based biomarkers of ICB response. Our results suggested refining future studies using preclinical animal models in a more context-specific manner to elucidate potential underlying mechanisms that lead to improved ICB responses.

Development of an Rta/Zta Induction Reporter Virus (RZIR-EBV) to Optimize Lytic-Induction Therapy of EBV-Associated Cancers

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Epstein-Barr virus (EBV)-associated cancers depend on EBV latency genes for continued growth and survival, making disruption of latency a promising therapeutic target. “Lytic induction therapy” combines small molecules capable of disrupting EBV latency with anti-viral drugs to increase tumor cell toxicity. Though well tolerated in clinical trials, current induction agents display low efficacy. Therefore, the full potential of this approach will require the development of second-generation induction agents that reactivate a greater proportion of tumor cells.

The latent/lytic switch is controlled by two viral transcription factors, Rta and Zta. Activators of the Rta and Zta promoters have been identified, but these approaches could not capture regulatory mechanisms governing viral reactivation in EBV-positive tumors. To overcome these limitations, a novel EBV reporter virus was created to discover agents that induce the strongest activation of Rta and Zta. The Rta Zta Induction Reporter (RZIR-EBV) replaces the Rta and Zta loci with green and red fluorescent proteins, respectively. This reporter additionally remains in the context of the full virus, maintaining endogenous regulatory mechanisms. The RZIR-EBV is being used to discover optimal inducers of Rta and Zta within the EBV genome and identify induction agents exhibiting the greatest synergy for clinical translation.

Single-cell delineation of HIV-1 strain-specific gene activities using live-cell imaging and dual reporter sensor cells (DRSCs)

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HIV-1 genomes exhibit marked heterogeneity both within and between hosts resulting in consequences for pathogenesis and treatment efforts. Methods such as sequencing that are currently used to study strain differences have limitations in that they only rarely predict phenotype and often yield false positives due to the detection of non-functional virus genomes. Existing cell-based platforms allow for detection of actively infectious virus but only provide limited information relevant to strain- or subtype-specific viral attributes. Herein, we describe the design and validation of a multivariate dual reporter sensor cell (DRSC) system that can be used to not only detect and quantify HIV-1 viruses but also directly assess and define multiple viral features including activation rates of viral gene expression, onset of virion assembly, downregulation of host antiviral factors, and virus co-receptor preference (X4- vs. R5-tropism). We show that our DRSC platform can detect strain specific gene activities attributable to the Vif and Vpr accessory proteins, provide information on HIV-1 Rev, Tat, and Gag function, and screen for drug blocks to the processes driven by these proteins. DRSCs illustrate the capacity of multivariate imaging and multi-purpose biosensors to be used for detection of HIV-1 and studies of viral genetic variability responses to antiviral strategies.

Using Mechanisms of AAV to Engineer an Improved rAAV Dual-Vector System

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Adeno-associated virus (AAV) is a single-stranded DNA virus in the *Parvoviridae* family, which is modified to serve as recombinant AAV (rAAV) gene therapy vectors. Some major limitations have restricted its widespread use in viral gene therapy, one of which is the limited transgene size. Therefore, a better understanding of AAV is necessary to develop rAAV vectors that can accommodate larger transgenes. I hypothesize that sequences that drive AAV concatemer formation are sufficient to rearrange rAAV vectors to generate large transgenes. To characterize these head-to-tail junctions, we amplified AAV rearrangements and performed TOPO cloning followed by Sanger sequencing and independently performed high-throughput Illumina sequencing. Leveraging this knowledge, I designed a dual-vector rAAV reporter system where I inserted these AAV sequences. The first vector contains a promoter, the first portion of a green fluorescent protein (GFP) gene, and the inserted AAV sequence before the 3' viral telomere. The second vector contains another inserted AAV sequence just after the 5' viral telomere, along with the second portion of the GFP gene, followed by translational cis-regulatory elements. We will transduce these dual vectors into cells and test for recombination. This work can lead to the development of better viral gene therapy vectors that can treat more diseases and lower dosages required for patients.

Regulation of Adeno-Associated Virus and Recombinant AAV Localization in the Host Nuclear Milieu

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Adeno-Associated Viruses (AAV) are single-stranded DNA viruses that are modified to deliver therapeutic transgenes as recombinant AAV (rAAV) gene therapy vectors. However, the mechanisms governing AAV localization and formation of replication centers in the nuclear compartment remain unknown, which precludes efficient production of rAAVs in producer cells. In-silico analysis in our lab identified CCCTC Binding Factor (CTCF) as a binding element on the AAV genome that regulates efficient AAV gene expression and, potentially, the virus/vector chromatin landscape. We therefore hypothesize that CTCF may also regulate AAV's interactions with cellular DNA damage sites during productive phases of viral replication. Using a mutant AAV lacking CTCF binding sites, we monitored the formation of viral replication centers in U2OS osteosarcoma cells. Preliminary studies show that AAV replication centers contain CTCF and colocalize with cellular DNA damage sites. Additionally, we found that localization to DNA damage sites during replicative phases is associated with homologous recombination-associated cellular factors. Taken together, these studies suggest that CTCF may synergize with cellular DNA damage response proteins to form AAV replication centers.

Genetic Complementation Reveals Structure-Function Links in Nodavirus RNA Replication Complex Crowns

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Positive-strand RNA virus genome replication is compartmentalized in virus-induced membrane rearrangements, which for nodaviruses are spherular invaginations of the outer mitochondrial membrane. Our cryo-EM showed that these spherules are "crowned" by 24 copies of viral replicase protein A, whose C-terminal RNA polymerase domains assemble in two 12-mer rings stacked above the spherule neck. In each ring, protein A's N-terminal RNA capping and membrane binding domains adopt different conformations: in the apical ring, extending outward as membrane-anchored legs; in the basal ring, clustering into a floor gating the spherule neck. This floor's center contains a density implicated as the polymerase of a third protein A conformation, interacting with viral dRNA inside the spherule. Protein A's multiple conformations in the crown likely provide distinct RNA replication functions, and the 12 identical subunits in the two rings may provide redundancy and robustness to some functions. To test these possibilities, we performed genetic complementation analyses between mutants in protein A's polymerase, RNA capping and flanking domains. Successful complementation between null mutants in the polymerase and RNA capping active sites showed that these enzymatic domains operate as independent units functional in distinct protein A subunits of the multimeric crown. Thus, neither RNA polymerase nor RNA capping activities are required in all copies of protein A, nor are both required together in any single copy. Emerging results imply that the central polymerase actively copies the dRNA template into new (+)RNA genomes, which are then m7G capped by the lower 12-mer crown floor. Many or all capping domains in the floor appear to be functional, but complementation tests between distinct capping mutants show that several if not all steps in capping a given RNA must be performed within a single protein A subunit. While RNA polymerase and capping activity are not required in all subunits, most deletions in these domains cannot be complemented, indicating non-enzymatic requirements such as crown assembly. A C-terminal intrinsically disordered region can be deleted from some but not all protein A crown subunits. Several mutations outside of the polymerase catalytic center revealed further insights into which regions are needed for polymerase activity vs crown assembly, underscoring the power of complementation analyses to probe structure-function relationships.

Behavior of Highly Oncogenic HPV16 Variants is Cell Type Dependent

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Human papillomavirus type 16 (HPV16) is the most oncogenic HPV genotype, with variants of this genotype influencing cancer risk. In this study, we compared the viral DNA replication and RNA splicing profiles of two European variants A1 and W12 (3-99), and two Asian/African American variants D2 and D3, the latter two being more commonly found in cervical cancers. Using spontaneously immortalized human foreskin keratinocytes (NIKS) and normal human cervical epithelial cells (WICVX-3E) transfected with these genomes, we assessed replication fitness by Southern blotting to detect viral DNA copy number and analyzed RNA splicing patterns of the RNAs encoding the two major viral oncogenes, E6 and E7, by RT-PCR. In NIKS, the D2 and D3 variants demonstrated enhanced DNA replication compared to A1 and W12 variants, consistent with their association with more aggressive lesion progression to cancer. RT-PCR revealed distinct differences in splicing patterns among variants, with varying ratios of full-length E6/E7 and spliced E6*/E7 and E6**/E7 transcripts. In contrast, in WICVX-3E cells, viral DNA copy numbers and splicing patterns were similar across all variants. These results highlight the significance of the cellular environment in shaping HPV16 variant biology.

Nodavirus Protein A's Interdomain Elbow Controls RNA Replication Complex Formation and Function

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Positive-strand (+)RNA viruses replicate their RNA genomes within membrane-associated replication complexes (RCs) whose structure and function remain poorly understood. Recent atomic-resolution analysis of the Nodaviral RC revealed that viral RNA replication protein A, with N-terminal membrane binding/RNA capping and C-terminal polymerase domains, forms a “crown” of two stacked 12-mer rings. The lower crown is a ring of 12 polymerases with N-terminal RNA capping domains clustered inward to form a central floor. The upper crown mirrors the polymerase ring but projects N-terminal domains radially outward.

This crown structure provides a powerful basis for addressing key questions about RC formation and function. We identify a critical 17-aa “elbow” segment of protein A acting as a molecular switch controlling crown assembly and independent activation of RNA polymerase and capping functions. This rigid elbow is deeply embedded at the inter-subunit interfaces and acts as an inter-subunit interaction hub regulating Protein A assembly and conformation: docking each elbow in the lower crown drives the formation of the central floor, the stable foundation for the crown, while undocking elbows in the upper crown allows outward projection of N-terminal domains.

Extensive analysis of elbow mutants confirms the elbow's essential assembly role and crucial regulation of RNA replication. Striking complementation results reveal that diverse elbow interactions activate the adjacent RNA capping domain and, independently, the distal polymerase domain. Mutations throughout the elbow disrupt assembly of the replication crown. Together, these results establish the small, 17-aa elbow as the linchpin for crown assembly, controlling Protein A conformation and switching on viral RNA capping and polymerase activities, adding to the growing evidence that the crown floor is the site of viral RNA capping. This work underscores the sophisticated regulation used by (+)RNA viruses to coordinate RNA genome replication steps and informs future control strategies.

Spatial Omics Analysis of Neutrophil–T Cell Interactions in Head and Neck Squamous Cell Carcinoma

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We evaluated spatial mRNA and protein expression data in HNSCC samples from ICB responders and non-responders. We hypothesized that neutrophil interactions with CD8, Treg, and tumor cells correlate with ICB response. Neutrophil interactions with CD8+ and Treg cells were significantly more frequent in non-responders, whereas neutrophil colocalization with tumor cells was more prevalent in responders. These results indicate notable spatial patterns of neutrophil interactions that differ by ICB response in HNSCC.

Modeling Metastatic ER+ Breast Cancer Driven by FGFR Alterations in Rats

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Estrogen receptor–positive (ER+) breast cancer is the most common type of breast cancer, making up nearly 70% of cases. Many patients initially respond to hormone therapy and newer treatments like CDK4/6 inhibitors. However, about half eventually relapse, and only 27% survive five years once their cancer spreads.

When ER+ breast cancer spreads, it often metastasizes to the liver. Liver metastasis is a serious complication that is hard to treat, and there are no good models to study this process. To address this gap, we developed a new ER+ breast cancer model in a strain of rats that mimics what happens in humans. From these tumors, we generated a cell line called MG-1 to study how ER+ cancer grows, spreads, and responds to therapies.

When MG-1 cells are injected into the bloodstream, they readily colonize the liver. From these, we derived a liver-specific subline that is especially aggressive. Cells that grow in the liver become faster-growing, more invasive, and resistant to standard treatments. Even if these cells grow first as a mammary tumor, they can metastasize to the liver. This is the first ER+ model to show this liver-directed behavior. These aggressive cells also have higher levels of FGFR1 and FGFR2, which may help drive liver metastasis and therapy resistance. Reducing FGFR1 or FGFR2 decreases their invasive ability and restores sensitivity to standard treatments.

Our model provides a way to test FGFR inhibitors with current treatments to improve care for patients with ER+ breast cancer that spreads to the liver.

Interferon- β induction heterogeneity during Kaposi's sarcoma-associated herpesvirus infection

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Type I interferons (IFN) such as IFN- β are potent cytokines that defend mammals against viral infections, making their subversion by viruses is a key component of the virus-host arms race. Our lab previously found that Kaposi's sarcoma-associated herpesvirus (KSHV) uses host caspases to prevent IFN- β induction during lytic infection. Inhibiting caspases results in high levels of IFN- β production during lytic KSHV infection. Surprisingly, single cell transcriptomics revealed IFN- β was produced by fewer than 4% of infected cells even under this de-repressed condition. We used a reporter that enables fluorescence-activated cell sorting of IFN- β -high and low cells, allowing comparison of the activation of signaling proteins in the IFN- β induction pathway. We observed that the IFN- β transcription factor IRF3 is activated, regardless of IFN- β induction. In contrast, the activation or expression of the two other IFN transcription factors, NF- κ B and AP-1, correlated with IFN- β induction. Our results suggest that during viral infection, activation of IRF3 does not always result in IFN induction at the individual cell level, but that other factors, such as NF- κ B and AP-1, are limiting for type I IFN induction. We propose that IRF3 is downstream of infection sensing, while NF- κ B and AP-1 allow few cells to make IFN- β .

Identification of LANCL2 as a novel HIV-1 host cell-derived regulatory factor

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Recently, we identified post-translational modifications in HIV-1 Capsid (CA) and Matrix (MA) proteins known as dehydroamino acids (DHAAs) (PMID: 35192358). DHAAs are reactive alkenes that arise from dehydration of Ser, Thr, and Cys residues, and can form protein crosslinks. A class of mammalian enzymes known as LanC-like proteins (LANCL) were recently shown to suppress DHAA crosslinking reactivity through the formation of irreversible adducts with glutathione (PMID: 33932340). We therefore sought to determine if modulating the expression of either of the two most widely expressed human LANCL proteins, LANCL1 or LANCL2, would influence HIV-1 replication. Here we show that overexpression of LANCL2, but not LANCL1, enhances HIV-1 infectivity. Conversely, knockdown of LANCL2 impairs infectivity. We identified two isoforms of LANCL2: “long” LANCL2 (L-LANCL2), which is typically reported in the literature, and “short” LANCL2 (S-LANCL2), which lacks the N-terminal membrane targeting and nuclear localization motifs found in L-LANCL2. Interestingly, S-LANCL2 was significantly more potent at enhancing HIV-1 infectivity relative to L-LANCL2 without impacting HIV-1 promoter activity. We also mutated residues in the HIV-1 CA and MA proteins predicted to be modified with DHAAs making them resistant to LANCL2 enhancement, supporting the hypothesis that LANCL2 enhances HIV-1 by targeting virion DHAAs. Taken together, we propose a model wherein HIV-1 virion DHAAs impair virion infectivity and S-LANCL2 enhances HIV-1 virion infectivity through glutathionylation activity. Future studies will test this model, determine what activities within S-LANCL2 contribute to this enhancement, and determine if S-LANCL2 is a viable target for pharmacological intervention in the context of acute infection or viral latency reversal.

Autocorrelation-aware resampling improves spatial transcriptomics cell-cell interaction analysis

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Spatial transcriptomics enables increasingly high-resolution analyses of cell-cell interactions through the co-expression of ligands and their cognate receptors, accounting for the spatial constraints of signaling. Existing methods to identify gene-gene correlations estimate statistical significance via random permutations that do not accommodate autocorrelation structure commonly present in spatial datasets. By not properly accounting for spatial autocorrelation, statistical significance is misestimated in the majority of studies. We introduce STAAR (Spatial Transcriptomics Autocorrelation-Aware Resampling), a statistical method for testing gene-gene correlations in spatial data that maintains spatial gene-level autocorrelation in resampled datasets used to evaluate significance levels. STAAR utilizes spatial map patterns to decompose autocorrelation patterns. The associations between gene expression and autocorrelation patterns are then randomized to construct resampled datasets. We test the performance of our method in both simulated and Head and Neck cancer (HNC) 10X spot-based Visium data. We show that STAAR (i) maintains gene-level spatial autocorrelation from the original data and (ii) outputs a lower false positive rate compared to random permutations at varying degrees of gene-level spatial autocorrelation in simulated studies. In addition, we obtained similar results in the HNC datasets for both true-positive and false-negative cases, while identifying biologically relevant interactions that were missed by SpatialDM, a method that utilizes random permutations. Our method provides a more sensitive correlation testing approach grounded in spatial statistics which outperforms existing testing methods. Future developments will enable the identification of region-specific correlations that can be linked to changes in biological pathways, defining potential biomarkers and therapeutic targets.

CTCF regulates AAV and recombinant AAV lifecycle through changes in the viral chromatin landscape

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Successful viral infection requires efficient expression of viral genes, regulated by chromatin landscape or genome location in the nucleus. These steps are controlled by the major host architectural protein CCCTC-binding factor (CTCF). We have investigated how CTCF regulates the life cycle of the small single stranded DNA dependo-parvovirus Adeno-Associated Virus (AAV) to improve recombinant AAV (rAAV) gene therapy vectors. ChIP-qPCR analysis revealed that CTCF binds to the AAV genome upstream of the P19 promoter and regulates expression of the viral Rep68/78 gene. Although rAAV is the major platform for gene therapy, it expresses transgenes inefficiently. We have re-engineered rAAV expressing a GFP transgene to contain CTCF binding sites. CTCF binding dramatically increased rAAV transduction efficiency and GFP expression by 4-fold across multiple cell types. We monitored the impact of CTCF on AAV and rAAV-chromatin environment using CUT&RUN analysis. 4C-seq assays revealed that CTCF-bound AAV and rAAV genomes localize to distinct nuclear sites. We propose that CTCF facilitates viral localization within the human genome to sites that support expression either through association with active chromatin regions and/or tethering genomes to active regions. These new rAAV gene therapy platforms will lower the dose needed in the clinic and minimize toxicity.

Exploring the interplay of viral genomic RNA structure and protein biochemistry in coronavirus replication

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Coronaviruses (CoVs) are positive-sense single-stranded RNA (+ssRNA) viruses. CoVs contain uniquely large genomes ranging in size from 26 to 32 kb, which require sophisticated regulatory mechanisms to ensure genome replication efficiency (Woo et al. 2010). These mechanisms are largely carried out by viral non-structural proteins (nsps). Three of these nsps—nsp7, nsp8, and nsp12—form the core Replication Transcription Complex (core RTC). The core RTC is responsible for *de novo* (i.e., primer-independent) replication of the CoV +ssRNA genome. This process is hypothesized to be reliant upon interactions of the core RTC with specific RNA structures within the CoV gRNA terminal untranslated regions (UTRs) (Yang et al. 2015). The CoV UTRs contain conserved RNA secondary structures which have been implicated in various processes of the viral life cycle. However, the critical UTR sequences and/or structures that interact with the core RTC to mediate to initiate gRNA replication remain undetermined. We have developed an *in vitro* biochemistry pipeline to examine the impact of CoV gRNA secondary structures on the efficiency of core RTC-mediated RNA replication. Through this work, we seek to elucidate the key biomolecular interactions driving CoV *de novo* genome replication.

Hepatitis B Virus Localizes to Cellular Fragile Sites to Induce Replication Stress Signals and Exacerbate Cellular DNA Damage

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Hepatitis B Virus (HBV) is a leading cause of hepatocellular carcinoma, with 257 million chronically infected individuals worldwide. Although HBV-infected patients benefit from drug regimens that help to control chronic infection, they are rarely clinically cured of HBV. The HBV genome persists in the nucleus of infected hepatocytes in the form of a covalently closed circular DNA (cccDNA) molecule. This HBV reservoir serves as the template for continuous production of HBV proteins associated with chronic HBV infection, and eventually, hepatocellular carcinoma. However, despite playing a central role in the viral life cycle, little is understood about where cccDNA molecules localize, what makes them stable, and how they alter host genome stability. Perhaps because of this, there are few treatments that target cccDNA, which is critical for eradication of clinical HBV. Here, we show that HBV infection induces a cellular DNA damage response (DDR) that is initiated *after* the formation of cccDNA molecules and occurs downstream of cellular replication stress. We have developed a novel high-throughput assay that monitors the localization sites of the cccDNA molecules. These studies identified cellular binding sites associated with several host factors and cellular fragile sites associated with DDR signals as mediators of cccDNA localization. Our findings contribute to the understanding of how HBV cccDNA navigates the host nuclear environment, identifying functional targets for development of therapies against HBV infection and resulting liver cancer.

Investigating the connection between post-translational modifications of the DNA sensor cGAS and caspase-8 mediated inhibition of the type I interferon response during KSHV lytic

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Kaposi's sarcoma-associated herpesvirus (KSHV) suppresses the type I interferon (IFN) response, a key component of innate immunity, to replicate after reactivation from latency. Our lab has shown that KSHV downregulates type I IFN by manipulating caspase-8, a protease involved in apoptosis. Caspase-8 is activated in KSHV-infected lytic cells, and its activity blocks IFN induction and release. Genetic analysis revealed that the DNA sensor cyclic GMP-AMP synthase (cGAS), which detects cytoplasmic DNA, is the primary IFN-inducing receptor repressed by KSHV. While other caspases cleave cGAS, we found no evidence that caspase-8 does so directly. We hypothesize that caspase-8 cleaves a regulatory protein essential for cGAS activity. To test this, we are using proteomics to identify relevant targets. Preliminary analysis using an antibody that detects caspase cleavage motifs identified proteins from lytic BC3 cells. Several regulate post-translational modifications (PTMs) such as phosphorylation and ubiquitination, all known to impact cGAS. We are validating their cleavage and role in cGAS regulation. To examine PTM changes, we created a KSHV-infected cell line expressing cGAS fused to blue fluorescent protein. Additionally, we are optimizing proximity biotin labeling to identify caspase-8 interactors. This study will define how KSHV exploits caspase-8 to evade the type I IFN response.

Structural and functional characterization of HIV-2 resistance to Lenacapavir

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Human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) have diverged significantly, exhibiting approximately 40% amino acid difference in viral proteins including the capsid (CA) domain of Gag. Antiretroviral therapies that target HIV-1 are typically less effective against HIV-2 due to significant sequence and structural divergence. The HIV Gag CA domain is essential for early and late viral replication stages and is the target of Lenacapavir (LEN), a first-in-class HIV-1 CA inhibitor that binds both the immature and mature CA FG-pocket. Despite its potency against HIV-1, LEN is ~11–14-fold less effective against HIV-2. HIV-2 CA contains several amino acid substitutions relative to that of HIV-1 CA at critical LEN-interacting residues (i.e., Y50Q, Q54T, I69L, R70K, and V82R for the immature CA form; and Q41S, R70K, and P179Q for the mature CA form). Here, we have tested whether reduced LEN susceptibility of HIV-2 is due to sequence and/or structural differences compared to that of HIV-1. The structure of the mature HIV-2 CA core bound to LEN has been determined, which has revealed a similar binding mechanism for HIV-2 compared to that of HIV-1. Additionally, several mutants have revealed an increase in LEN susceptibility, including Q54T. The Y50Q mutant led to efficient particle production with reduced particle infectivity. Taken together, our current observations imply that architectural differences in HIV-2 CA explain differences in LEN susceptibility between HIV-1 and HIV-2.

Defining the role of Epstein-Barr virus (EBV) in Diffuse Large B-Cell Lymphoma (DLBCL) pathogenesis

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Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive lymphoma which can be challenging to study and treat due to its heterogenous nature. Recent genetic classifications of DLBCL include ~6 subtypes, but do not consider Epstein-Barr virus (EBV) status, despite 5-14% of DLBCL being EBV-positive and EBV's known role in lymphomagenesis. We analyzed published whole exome and RNA sequencing of DLBCL tumors (n=481) and identified 19 EBV-positive DLBCLs. EBV-positive DLBCL is significantly enriched within the BN2 subtype, which comprised ~1/3 of EBV-positive DLBCLs, and most of the remaining ~2/3 are distinct from currently defined subtypes. Several differentially mutated genes within the BN2 tumors were found to correlate with EBV status. These include subtype-defining genes and genes involved in oncogenic processes. This finding supports our hypothesis that EBV oncogenes can substitute for specific cellular mutations in DLBCL. Using cell line models and transcriptomic analysis of tumors, we demonstrated that EBV-positive DLBCL unexpectedly lacks the expression of the viral oncoprotein, LMP2A, a viral mimic of the B cell receptor. Additional characterization of EBV-positive DLBCL cell lines revealed limited correspondence with EBV-positive tumors, underscoring the need for additional cell line models for *in vitro* studies of EBV-positive DLBCL.

Investigating the role of MCPyV T antigens in skin cell neuroendocrine reprogramming during Merkel cell carcinoma development

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Merkel Cell Carcinoma (MCC) is a rare and aggressive neuroendocrine skin cancer with approximately 80% cases attributed to clonal integration of Merkel Cell Polyomavirus (MCPyV) and subsequent expression of viral T antigens: Small T (ST) and truncated Large T (tLT). Recent studies/evidences show that keratinocytes and fibroblasts are currently, the two most plausible host cell types for the origin of MCC and that MCPyV early viral proteins may direct infected cells towards a neuroendocrine lineage under certain conditions; however, our understanding of this process remains limited. Thus, in this study, we investigate the potential reprogramming capacity of the T antigens when expressed in human skin cells: NIKS (keratinocytes) and NHDF (fibroblasts). 2D monolayer and 3D organotypic raft systems were generated using NIKS or NHDFs transduced with MCPyV T antigens and bulk RNA was performed. Sequencing revealed that T antigens dysregulate host transcriptome in a cell-type-specific manner and transduced NIKS exhibited a transcriptome more similar to that of MCPyV (+) MCC than NHDF. RNA and protein validation were performed to confirm selected DEGs from the bulk RNA sequencing. In conclusion, our study highlights the reprogramming capacity of MCPyV T antigens in various skin cell types, furthering our current understanding of MCPyV (+) MCC development.

A novel role for Mad1 in cytokinesis via $\alpha 5$ integrin trafficking

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Mad1 is an essential component of the mitotic spindle assembly checkpoint. During interphase, Mad1 regulates the trafficking of $\alpha 5$ -integrin from the Golgi to the plasma membrane. Here, we show that depletion of Mad1 or $\alpha 5$ integrin induces cytokinesis failure in both non-transformed and cancer cell lines. A pool of both Mad1 and $\alpha 5$ integrin localizes to the midbody region. Though the cytokinetic furrow ingresses with normal timing in cells depleted of Mad1 or $\alpha 5$ integrin, it ultimately regresses, resulting in cytokinesis failure. We identify an ~300 amino acid internal fragment of Mad1 that is necessary and sufficient for recruitment of Mad1 to the Golgi apparatus. This fragment, which we term Mad1-Golgi, can rescue cytokinesis in cells depleted of Mad1, as can $\alpha 5$ integrin. The contribution of both Mad1 and $\alpha 5$ integrin is observed specifically under adherent growth conditions. These results demonstrate a previously uncharacterized role for Mad1 in cytokinesis by regulating the secretion of $\alpha 5$ -integrin from the Golgi apparatus.

Spatial Transcriptomic Analysis of Homotypic Versus Heterotypic Colon Cancers

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We previously developed a mouse model of colorectal cancer (CRC) in which two distinct epithelial cell clonotypes are marked by fluorescent reporters (referred to as B6.FCTG3KM). We found that heterogeneous tumors (composed of multiple clonotypes) are more aggressive than heterotypic tumors. In this study, we employ spatial analysis to identify the differences in gene expression patterns underlying these phenotypic differences.

Colon tumors from B6.FCTG3KM animals were staged by a pathologist (KAM) and categorized via immunofluorescent staining for red fluorescent protein (RFP) and green fluorescent protein (GFP). Spatial transcriptomics was performed on 10x Genomics' Visium with CytAssist platform, with RNA sequencing performed on the Illumina NovaSeqX to an average depth of 2.6×10^8 reads per sample. Differential expression (DE) analysis was performed using a fold change threshold of 1.5 and an adjusted p-value threshold of 0.05. Enrichment analysis was conducted using Enrichr.

Enrichment analysis of DE genes unique to the comparison of homotypic green tumors to heterotypic tumors identified multiple gene signatures involved in the recruitment and activation of innate immune cells, including Response to Type II Interferon ($p < 0.0001$, $q = 0.001$) and Macrophage Activation ($p < 0.0001$, $q = 0.001$).

Initial analyses identified differences in the innate immune response as potentially contributing to the phenotypic differences previously observed between homotypic green tumors and heterotypic tumors.

Machine Learning Prediction Models to Facilitate Equitable Anal Cancer Screening – Update!

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Medicine, and Medical Microbiology and Immunology

The recent ANCHOR study from 2024 definitively shows that treating precancerous high-grade intraepithelial lesions (HSIL) anal dysplasia prevents anal cancer. However, this study does not provide: (1) the best screening methods for anal cancer dysplasia and (2) the percentage of patients at risk for anal cancer who should be screened for anoscopy. While anoscopies are not limited at UW Health, they are not available for most patients who are at high risk for HSIL. It is unclear when and how anoscopies should be utilized.

While there are many parallels between anal cancer and cervical cancer, there are also many differences. Both cancer types are preceded and caused by an oncogenic HPV infection. Yet, the presence of high-risk HPV in the anus is roughly 10-fold higher in men who have sex with men. The presence of high-risk HPV is insufficient as a single time point to warrant invasive preventative action unlike with cervical cancer screening. Evidence from UW and other groups agree that abnormal immune parameters (particularly low CD4/CD8 ratios) precede most anal cancer by months to years. Furthermore, small immune abnormalities are linked to dysplasia, with a correlation between time of immune parameter abnormality and the worsening degree of dysplasia. There is a need for prediction models based on risk that includes more than age, but it is unclear if immune parameters are powerful enough to be used to alter clinical decision-making.

Comparative Analysis of Retroviral Matrix-Membrane Interactions in Virus Assembly

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Distinct differences exist in the assembly pathways and the virus particle morphologies among retroviral genera, which infer distinct virus-host cell interactions among retroviruses. For example, previous observations have identified pronounced variation in immature particle morphologies among retroviral genera. To gain greater insights into the variability in assembly pathways that impact immature particle biogenesis and morphology, we have conducted a comparative parallel analysis of matrix (MA) protein binding to the plasma membrane by using a quantitative dual color, z-scan fluorescence imaging assay in living cells with the MA proteins of Rous sarcoma virus (RSV, alpharetrovirus), Mason-Pfizer monkey virus (MPMV, betaretrovirus), human T-cell leukemia virus type 1 (HTLV-1, deltaretrovirus), walleye dermal sarcoma virus (WDSV, epsilonretrovirus), murine leukemia virus (MLV, gammaretrovirus), human immunodeficiency virus type 1 (HIV-1, lentivirus) and human foamy virus (HFV, spumavirus). Strikingly, this comparative parallel analysis identified the HTLV-1 and the MLV MA proteins as having strong membrane binding affinities relative to that of the RSV, MPMV, WDSV, HIV-1 and the HFV MA proteins. This observation implies a functional relationship between the HTLV-1 and MLV MA proteins compared to that of other retroviral MA proteins. Analysis of immature particle morphologies of HTLV-1 and MLV provides evidence for morphological similarities not observed with other retroviral genera. HTLV-1 and MLV Gag chimeras with MA domain swaps are being analyzed for their impact on Gag-membrane interactions, particle assembly, and particle morphology to gain further insights into the relationships in between deltaretroviral and gammaretroviral MA and Gag proteins in particle assembly and morphology. Taken together, these observations implicate relationships among retroviral genera that impact virus assembly and particle morphology.

Human Germinal center (GC) B cells infected with EBNA2-deleted EBV (Δ EBNA2 EBV) develop LMP2A-dependent diffuse large B cell lymphomas (DLBCLs) with type 2 Viral latency

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Epstein-Barr virus (EBV) infection is found in 5-15% of diffuse large B cell lymphomas (DLBCLs) in humans. The latent EBV protein, EBNA2, is required for EBV transformation of B cells in vitro. However, EBV+ DLBCLs often lack EBNA2 expression and instead may be driven by the EBV membrane proteins LMP1 (which activates NF- κ B signaling) and/or LMP2A (which mimics tonic B cell receptor signaling). There is currently no model system for studying how EBV causes DLBCLs with “type II” viral latency (EBNA2-neg, LMP1+, LMP2A+) in the absence of EBNA2 expression. Here we show that normal human tonsil GC B cells proliferate indefinitely on a CD40L/IL21-expressing feeder layer when infected with an EBNA2-deleted EBV mutant (Δ EBNA2 EBV), whereas uninfected GC B cells die within a few weeks. Furthermore, we show that cell lines derived from Δ EBNA2 EBV-infected GC cells form DLBCL-like tumors in NSG mice. Some of these tumors have type II viral latency (LMP1+/LMP2A+/EBNA-LP neg) whereas others have a viral protein expression pattern resembling “WP-restricted” latency (LMP1+, LMP2A-neg, EBNA-LP+). Cell lines derived from tumors with type II latency express plasma cell markers such as IRF4 and BLIMP1 whereas cell lines derived from tumors with WP-restricted latency express GC markers. Although LMP2A is not required for the ability of wild-type EBV to transform primary B cells in vitro, we find that LMP2A expression in Δ EBNA2 EBV-infected GC cells dramatically increases tumor aggressiveness in NSG mice. Using this novel model system, we have discovered that LMP2A promotes plasma cell differentiation of GC B cells (at least partially) by inhibiting BACH2 and Pax5 expression. This new model system is the first to show that EBV infection induces GC B cell lymphomas in vivo in the absence of EBNA2, and the first to demonstrate that LMP2A contributes to the aggressiveness and differentiation state of EBV-induced GC lymphomas when EBNA2 expression is turned off.

Virion Entry Pathway Determines HCMV Infection Outcome

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Herpesviruses, including human cytomegalovirus (HCMV), use lytic infections to spread, and latency to persist, in their host populations. Both lytic and latent infections require viral entry into cells. Upon engaging a receptor, enveloped virions (like HCMV) can enter cells either by endocytosis, or by fusion at the plasma membrane. We previously showed that HCMV naturally enters incompletely differentiated myeloid cells by endocytosis and establishes latency. During that entry process, virion tegument proteins remain endosome associated while viral capsids dock at nuclear pores and viral genomes enter the nucleus. Here we show that when HCMV enters those same latency model cells by fusion at the plasma membrane, the tegument proteins traffic to the nucleus, latency is not established, and lytic infection follows. Our work reveals that entry pathways not only initiate infections but can also influence their ultimate outcome (lytic or latent).

Imaging of Human Retrovirus Assembly in 3D with Deep Learning

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The mechanisms of human retrovirus assembly and release are complex and not fully understood but are crucial for infectious virus spread. Retrovirus assembly and release have been difficult to observe in real time by standard biochemical techniques as they are ill suited to reveal details of these biological processes. Furthermore, large fluorescence backgrounds and sample geometry have limited the application of live-cell fluorescence imaging approaches, particularly total internal fluorescence (TIRF) microscopy. TIRF microscopy illuminates the sample with an exponentially decaying field of a few hundred nanometers, limiting out-of-focus background fluorescence but complicating quantitative analysis and precluding observations away from the bottom plasma membrane (PM). Here, we describe a 3D imaging system using the double helix point spread function (DHPSF) that provides an extended depth of field and 3D location information to allow real-time, 3D imaging of retrovirus assembly at the plasma membrane (PM) away from the coverslip (i.e., top membrane). An analysis pipeline was developed by using deep learning to localize and infer intensity (and thus copy number) from individual nascent virions. This method was then used to measure the kinetics of assembly at both bottom and top PM, where release can be directly observed. We compare our observations to previously reported kinetics from TIRF microscopy, and describe long-term measurement of motion of nascent virions at the top PM. This experimental approach has broad applications in both virology as well as cell biology.

Deletion variants of SARS-CoV-2: Preliminary evidence for selection in COVID-19 patientsNan Jiang¹, Colin Dewey², John Yin^{1,3}¹ Wisconsin Institute for Discovery² Biostatistics and Medical Informatics³ Chemical and Biological Engineering

Defective viral genomes with internal deletions arise frequently during SARS-CoV-2 replication and can shape persistence, host immune responses, and viral evolution within patients. To systematically study defective viral genomes (DVGs), accurate detection is critical, yet multiplex-PCR sequencing often introduces false deletion signals. To overcome this obstacle, we developed and validated a bioinformatics pipeline that removes more than 99 percent of false positives while reliably capturing authentic deletion events. Applying this approach to sequencing data from persistently infected COVID-19 patients, we identified numerous deletions and assessed their frequency, length, and overlap with viral cis-acting signals. DVG frequencies fluctuated dynamically, with specific deletions appearing and disappearing over time, often coinciding with shifts in single-nucleotide variants, suggesting coupled evolutionary dynamics. Across patients, we observed a consistent enrichment of deletions that preserved both replication and packaging signals compared to random expectation, while deletions disrupting these signals were depleted. These findings provide preliminary evidence that SARS-CoV-2 deletion variants are shaped by selective pressures to retain essential genomic elements. Such constraints may underlie their persistence in patients and highlight their potential roles in viral evolution and disease outcomes.

BRD8 is a therapeutic vulnerability for overcoming resistance to dual ER/HER2 blockade therapy in HR+/HER2+ breast cancer

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Hormone receptor (HR)-positive, HER2-positive breast cancers often develop resistance to endocrine and anti-HER2 therapies due to the heterogeneous expression of estrogen receptor (ER) and HER2 and the crosstalk of these growth-promoting pathways. However, how anti-HER2 agents activate ER and other growth-promoting pathways remains unknown. Single-cell RNA sequencing of BT474 breast cancer cells identified Bromodomain Containing Protein 8 (BRD8), an acetyl-lysine reader protein in the histone acetylase EP400 complex, as a pivotal mediator to activate ER in response to neratinib treatment. BRD8 expression was rapidly induced by various anti-HER2 agents (neratinib, lapatinib, and trastuzumab), and its depletion disrupted the crosstalk between ER and HER2 signaling pathways and rendered HR+/HER2+ cells and PDxOs more sensitive to anti-HER2 agents. BRD8, ER, and ER target genes are co-induced by neratinib in single-nucleus RNA and ATAC sequencing of a patient-derived xenograft (PDX). SnATAC-seq also reveals that the activated genes share open chromatin regions enriched in ER, forkhead box (FOX), and ETS family transcription factors (TF) binding motifs. FOX family TFs are well-known for regulating estrogen signaling, and ETS proteins have been shown to promote tumorigenesis. Since EP400 enhances H2AZ deposition and acetylation on chromatin, we performed H2AZ and H2AZac ChIP-sequencing in the presence or absence of BRD8 and neratinib treatment. We found that, in response to neratinib treatment, BRD8 activates ER, FOX, and ETS target genes through modulating H2AZac deposition and chromatin decompaction. This finding coincides with RNA-sequencing where BRD8 promotes cell growth in an ER-dependent and -independent manner. In line with these findings, patients who responded poorly to the anti-HER2 therapies exhibited higher levels of BRD8 target gene signature as compared to the responders. Furthermore, BRD8 knockdown ablates the ER and HER2 signaling crosstalk and re-sensitizes neratinib-resistant HR+/HER2+ cells to neratinib. In summary, this work not only explains why ER signaling is activated upon anti-HER2 therapies but also identifies BRD8 as a druggable vulnerability for treating HR+/HER2+ breast cancer.

CTR9 Controls the Mammary Luminal Lineage and Susceptibility to Carcinogen/hormone-induced Breast Tumors

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Abstract

CTR9 encodes a core component of the human RNA polymerase II-associated factor complex (hPAFc). Loss-of-function (LOF) variants of CTR9 predispose individuals to rare myeloid malignancies and Wilms tumors, and ~0.1% of breast cancer patients harbor LOF mutations. While CTR9 supports estrogen receptor (ER)-positive breast cancer cell growth, its *in vivo* role in mammary gland biology remains unclear. scRNA-seq and immunofluorescence revealed CTR9 is highly expressed in luminal cells of mouse mammary glands and human breast tumors. To define its function, we generated a mammary gland-specific CTR9 knockout (KO) mouse. CTR9 loss enhanced duct branching and elongation during puberty and increased alveologenesis during pregnancy, coinciding with expansion of mammary stem cells. Following carcinogen and progestin treatment, CTR9 KO mice exhibited greater tumor susceptibility than wild-type counterparts. Early tumorigenesis analysis showed CTR9 depletion expanded stem and luminal progenitor populations, reduced mature luminal cells, increased proliferation (Ki67), and decreased ER/PR. KO glands also displayed elevated KRT15, reduced KDM5A, and increased H3K4me3, indicating impaired luminal differentiation via altered histone methylation. Collectively, our findings establish that CTR9 depletion blocks terminal luminal cell differentiation and predisposes mammary glands to carcinogen/hormone-induced tumorigenesis.