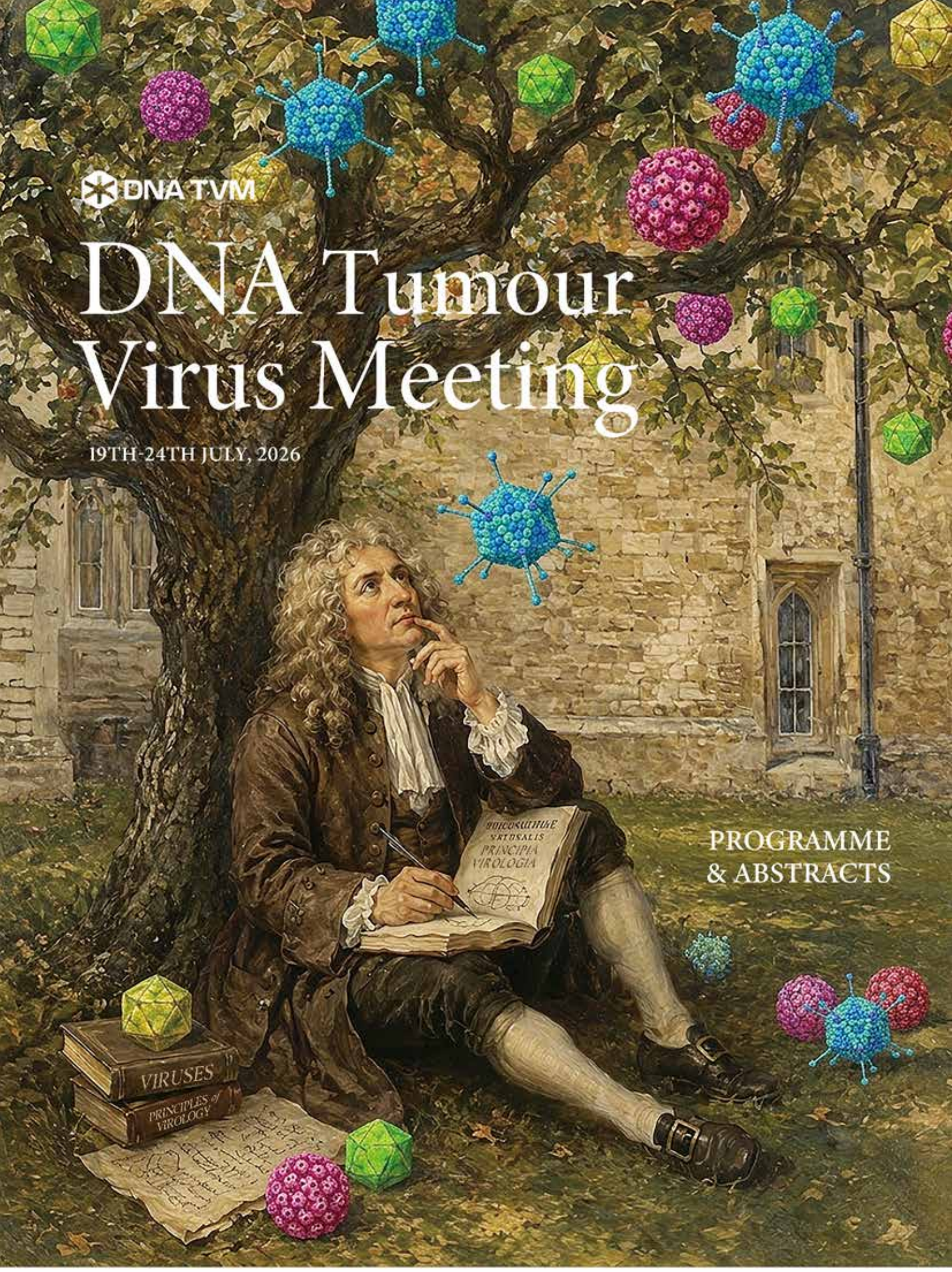

CHURCHILL COLLEGE

UNIVERSITY OF CAMBRIDGE

DNA TVM

DNA TVM

19-24th July 2026
Cambridge, UK

Churchill College,
University of Cambridge
Storey's Way
Cambridge, CB3 0DS, UK

Organised by the Doorbar Lab
Department tof Pathology, University of Cambridge

We gratefully acknowledge sponsorship provided by

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Organising Committee

Local Organising Committee

Amy Davies & Freya Pan

Ademola Aiyenuro, Enrique Boccardo, Weizhuo Chen, Nagayasu Egawa, Heather Griffin, Mai Miyazaki, Konstanze Schichl, Lara Termini, Lu Zhang,

John Doorbar

Department of Pathology, University of Cambridge

Scientific Organising Committee

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Department of Cancer and Genomic Sciences, University of Birmingham

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Centre for Cancer Immunology, University of Southampton

Andrew MacDonald

Astbury Centre for Stuctural Molecular Biology, University of Leeds

Sheila Graham

MRC Centre for Virus Research, University of Glasgow

Colin Crump, Enrique Boccardo, Nagayasu Egawa, Lara Termini, John Doorbar

Division of Virology, Department of Pathology, University of Cambridge

Meeting Chairs

Lawrence Banks, Paola Blanchette, Enrique Boccardo, Colin Crump, James DeCaprio, John Doorbar, Koenraad Van Doorslaer, Tim Fenton, Nicole Fischer, Sheila Graham, Lou Laimins, Paul Lambert, Alison McBride, Karl Munger, Michelle Ozbun, Joanna Parish, Megan Spurgeon, Frank Stubenrauch, Vjeko Tomaic, Elizabeth White, Thomas Zheng

Plenary Speakers

Benjamin Simons and Nicole Fischer

Acknowledgement

Logo	Joshua Doorbar
Theme Graphics	Amy Davies & Freya Pan
Abstract Book Layout	Lu Zhang & Freya Pan

Important Note

The abstracts published in this meeting book should not be cited in bibliographies. Material described herein should be treated as personal communication and cited only with the consent of the author.

General Information

Meeting Location
DNA TVM 2026 will take place at Churchill College, University of Cambridge, Storey's Way, Cambridge, CB3 0DS

Lecture Location
Wolfson Hall, Churchill College

Wifi
Free WiFi access is available throughout the Conference Centre. Please check in with the reception desk for wifi code ticket upon arrival.

Welcome Reception
Served in Buttery/Concourse/Jock Colville Lawn (weather dependent)

College Bar
- To be open from 2pm – 11 pm daily (12am Gala Dinner evening) for delegates
- Pizzas, snacks, and drinks can be purchased from the bar as required – card payment only

Dining Hall
- Breakfast is available for those staying at Churchill, from 07:30 - 09:30
- Buffet lunch is available for all delegates, from 12:00 - 14:00
- Buffet dinner is available for all delegates and guests, from 17:45 - 19:10

Your name badge is required for admission to the meeting and dining hall, please wear it at all times.

Technical Services

All speakers who have been assigned morning talks should upload their presentations to the AV team using a USB stick the day before their talk. For those with afternoon talks, speakers should upload their presentation during the morning session or at the start of lunch on the day of their presentation. AV will be open during the welcome reception for those who have talks on the evening of 19th July.

Oral Presentations

- Oral abstracts in this meeting book are presented in chronological session order.
- Plenary lectures have been allocated 60 minutes, including 10 minutes for questions.
- Speakers have been allocated slots of 12 minutes - 10 minutes for presentation plus 2 minutes for questions.

Poster Presentations

- Abstracts in this meeting book are arranged in alphabetical order.
- Posters should be displayed for the duration of the meeting and can be put up upon arrival. Please remember to take them down before the start of the final session on Friday 24th July.

Useful Resources

1. Emergency Numbers

In an emergency, if you or someone else is in danger of significant harm, dial 999.
If you are worried about a non-life-threatening medical concern, call 111 and speak to a fully trained advisor.
For less urgent health needs, contact a local pharmacist.

2. Pharmacy

Superdrug, Pharmacy and Health Clinic	59 Sidney St, Cambridge CB2 3HX
Boots, Pharmacy	28 Petty Cury, Cambridge CB2 3ND

3. Hospitals

Addenbrooke's Hospital	Hills Rd, Cambridge CB2 0QQ

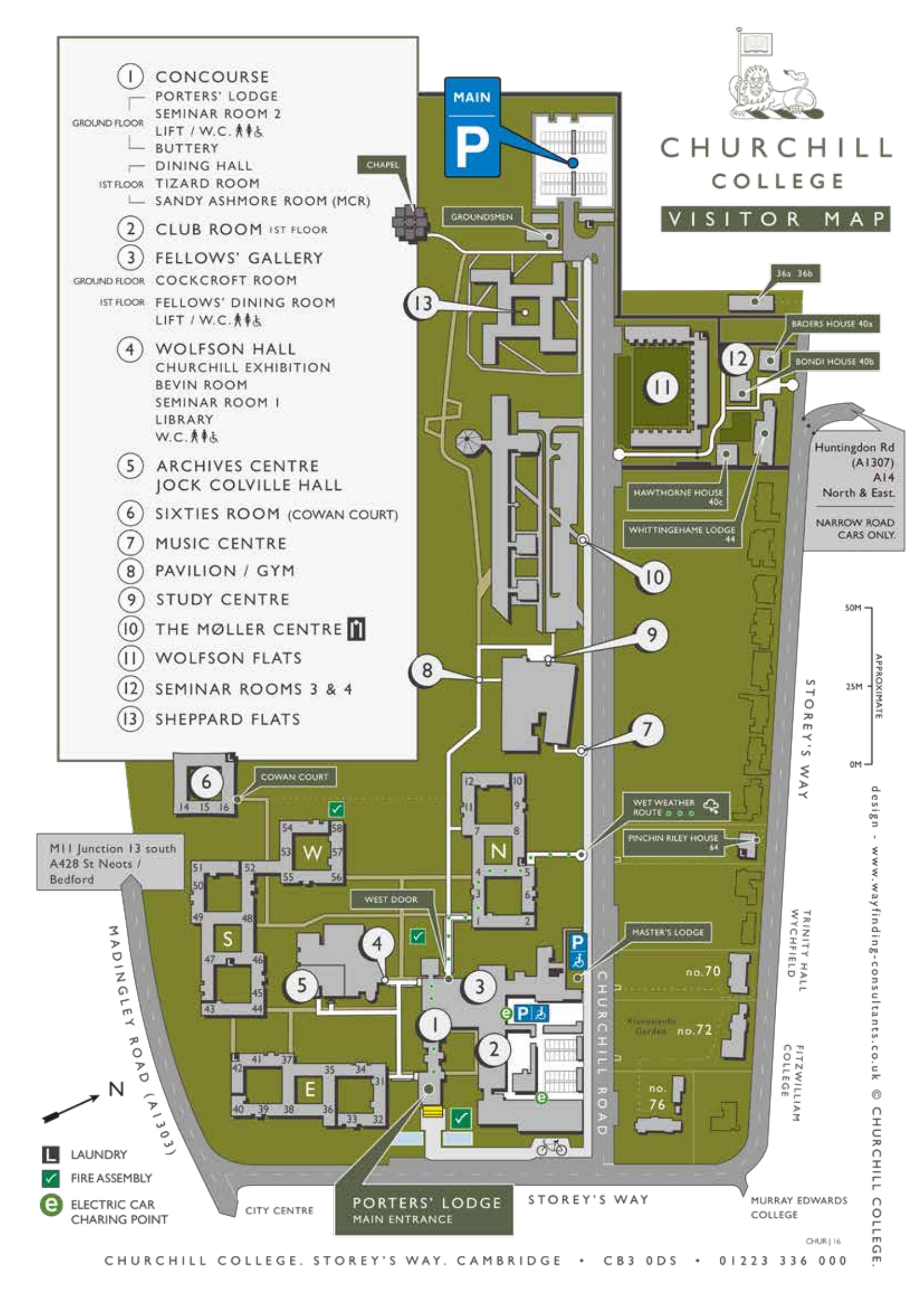
In an emergency, please dial 999.

4. Emergency Dental Care

If you have an emergency dental problem in Cambridge, such as severe pain, swelling, or a broken tooth, you should call the NHS at 111.
You can also visit an Urgent Care Centre - Brookfields Community Dental Service (Brookfields Health Centre, 94 Seymour St, Cambridge CB1 3DQ), accessible via 111.
If you experience severe swelling (affecting breathing/swallowing), uncontrolled bleeding, or serious trauma to the face or jaw, go to A&E or call 999.

5. Banks

Lloyds Bank	3 Sidney St, Cambridge CB2 3HQ
Barclays	9-11 St Andrew's St, Cambridge CB2 3AA
Santander	60 St Andrew's St, Cambridge CB2 3RR


CHURCHILL COLLEGE
VISITOR MAP
1 CONCOURSE
PORTERS' LODGE
SEMINAR ROOM 2
GROUND FLOOR LIFT / W.C.
BUTTERY
DINING HALL
1ST FLOOR TIZARD ROOM
SANDY ASHMORE ROOM (MCR)
2 CLUB ROOM 1ST FLOOR
3 FELLOWS' GALLERY
GROUND FLOOR COCKCROFT ROOM
1ST FLOOR FELLOWS' DINING ROOM
LIFT / W.C.
4 WOLFSON HALL
CHURCHILL EXHIBITION
BEVIN ROOM
SEMINAR ROOM 1
LIBRARY
W.C.
5 ARCHIVES CENTRE
JOCK COLVILLE HALL
6 SIXTIES ROOM (COWAN COURT)
7 MUSIC CENTRE
8 PAVILION / GYM
9 STUDY CENTRE
10 THE MØLLER CENTRE
11 WOLFSON FLATS
12 SEMINAR ROOMS 3 & 4
13 SHEPPARD FLATS
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Meeting Schedule

Sunday 19th July

14:00 - onward **Registration**
Located in the Concourse adjacent to the College Buttery

15:30 - 17:00 **Welcome Reception**
Buttery or Jock Coville Lawn depending on weather

17:00 - 19:00 **Session I: Replication**
Chairs: Alison McBride and Frank Stubenrauch
Abstract 1-8

19:00 - onwards **Dinner & World Cup Final**

Monday 20th July

08:45 - 10:15 **Session II: Virus-Host Interactions**
Chairs: Elizabeth White and Nicole Fischer
Abstract 9-14

10:15 - 10:45 **Break**

10:45 - 12:30 **Session II: Virus-Host Interactions (Continued)**
Abstract 15-21

12:30 - 14:00 **Lunch**

14:00 - 15:40 **Session III: Immune Responses**
Chairs: Paul Lambert and Enrique Boccardo
Abstract 22-28

15:40 - 16:10 **Break**

16:10 - 17:45 **Session III: Immune Responses (Continued)**
Abstract 29-34

17:45 - 19:30 **Dinner**

19:30 - 20:45 **Plenary Lecture I**
Benjamin Simons, University of Cambridge
Stem Cell Dynamics: Unlocking Barriers to Tumour Development

20:45 - 22:00 **Poster Session I**

Tuesday 21st July

08:45 - 10:20 **Session IV: Oncogenesis 1**
Chairs: Karl Munger and Vjeko Tomaic
Abstract 35-41

10:20 - 10:50 **Break**

10:50 - 12:30 **Session IV: Oncogenesis 1 (continued)**
Abstract 42-48

12:30 - 14:00 **Lunch**
13:00 - 14:00 *Optional Session: NCI Funding and Opportunity Discussion + Panel Q&A*
Speakers: Vignesh Gunasekharan

14:00 - 15:45 **Session V: Virus Entry & Infection Mechanisms**
Chairs: Michelle Ozbun and Colin Crump
Abstract 49-56

15:45 - onwards **Afternoon free (Posters available for viewing)**

Wednesday 22nd July

08:45 - 10:15 **Session VI: Life Cycle**
Chairs: Lou Laimins and James DeCaprio
Abstract 57-63

10:15 - 10:45 **Break**

10:45 - 12:30 **Session VI: Life Cycle (Continued)**
Abstract 64-70

12:30 - 14:00 **Lunch**

14:00 - 16:00 **Session VII: Gene Expression**
Chairs: Shella Graham and Thomas Zheng
Abstract 71-79

16:00 - 17:15 **Poster Session II**

17:00 - 18:30 **Dinner**

18:30 - 19:45 **Plenary Lecture II**
Nicole Fischer, University Medical Center Hamburg-Eppendorf
Advancing Human Polyomavirus Research: From Viral Biology to Disease

19:45 - 20:00 **Break**

20:00 - 22:00 **Session VIII: Oncogensis 2**
Chairs: Lawrence Banks and Paola Blanchette
Abstract 80-88

Thursday 23rd July

08:45 - 10:15 **Session IX: Pathogenesis**
Chairs: Megan Spurgeon and Tim Fenton
Abstract 89-95

10:15 - 10:45 **Break**

10:45 - 12:15 **Session IX: Pathogenesis (Continued)**
Abstract 96-101

12:15 - 13:45 **Lunch**

13:45 - 15:25 **Session X: Mechanistic Understanding for Therapy**
Chairs: Joanna Parish and Koenraad Van Doorslaer
Abstract 102-108

15:25 - 15:55 **Break**

15:55 - 17:15 **Session X: Mechanistic Understanding for Therapy (Continued)**
Abstract 109-114

18:30 - 19:30 **Gala Dinner Reception**

19:30 - onwards **Gala Dinner**

Friday 24th July

07:30 - onwards **Breakfast and Departure**

Poster Session I Abstract numbers: 117, 123, 124, 125, 127, 129, 132, 133, 137, 139, 140, 141, 142

Poster Session II Abstract numbers: 115, 116, 118, 119, 120, 121, 122, 126, 128, 130, 131, 134, 135, 136, 138, 143

Session Programme

SESSION I: REPLICATION

Chairs: Alison McBride and Frank Stubenrauch

Abstract 1-8

1. **The Spatial and Functional Architecture of HPV Replication Factories**
   C. Redmond, D. Chen, W. Zheng, J. Chang, A.A. McBride

2. **Viral inhibition of the anaphase promoting complex enhances replication by elevating nucleotide pools**
   O.J. Chen, C.Y. Feng, R.J. Ladak, M.A. Crosby, M.S.T. Russo, Y. Wang, P. Blanchette, Y. Liang, D.M. Sharon, M. Moustafa-Kamal, I. Gamache, N. Sonenberg, D. Avizonis, J.G. Teodoro

3. **Restoration of HR Limits HPV-Positive Cellular Viability**
   Christopher D. Collins, Dhruthi Suresh, Keira Ylvisaker, Marcel Morgenstern, Drew Jordahl, Katie Overmyer, Josh Coon, Megan E. Spurgeon, Kavi Mehta

4. **Targeting Cisplatin-Induced RAD51 Filaments Uncovers a Vulnerability in HPV-Positive HNSCC**
   Marie-Anne Bérubé, Julien Dessapt, Line Balisatire, Élise G. Lavoie, Andréanne Blondeau, Amélie Fradet-Turcotte

5. **Defining the Polymerase milieu employed by Human Papillomavirus (HPV) across the viral lifecycle and viral interaction with the host replication machinery**
   Dhruthi Suresh, Marcel Morgenstern, Christopher D. Colins, Gopishankar Thirumoorthy, Denis Lee, Micheal Leser, Kristie Lindsey Rose, Paul Lambert, Joshua Coon, Kevin Lang, Alison McBride, Megan E. Spurgeon, Kavi P.M. Mehta

6. **ARGLU1 is a Negative Regulator of the Adenoviral Replicative Cycle**
   A. Koul, L. Fulham, N. Akkerman, D. Graves, E. Kaul, K. Khadija, P. Pelka

7. **APOBEC3-mediated suppression of archetype BKPyV rearrangements and replication in urothelial cells**
   Helena Vogel, Gabriel J Starrett

8. **APOBEC3B inhibits HPV genome replication independently of its deaminase activity**
   Iwao Kukimoto, Seiichiro Mori, Kousho Wakae

SESSION II. VIRUS-HOST INTERACTIONS

Chairs: Elizabeth White and Nicole Fischer

Abstract 9-21

9. **Identifying mechanisms of antiviral enzyme APOBEC3A expression in tonsil epithelium using organoid models**
   B.P. Sharpe, I. Reddin, G. Thomas, E. King, T.R. Fenton

10. **Detection and structural characterization of HPV16 E2 variants in Tunisian precancerous and cancerous cervical lesions**
M. Ardhaoui, B. Gayed, K. Ouerhani, E. Ennaifer, R. Belhadj-Rhouma

11. **E2 activation of the DNA damage response creates targetable vulnerabilities in HPV infections and cancers**
Apurva T. Prabhakar, Claire D. James, Allison Q. Nguyen, Phoebe V. Bridy, Xu Wang, Ryan J. Zoldork, Jenny D. Roe, Austin J. Witt, Srimanya S. Panidepu, Molly L. Bristol, Krista Dalton, Andreas Wieland, Ahmed Diab, Anthony C. Faber, Elliot J. Androphy, Devraj Basu, Paul F. Lambert, Megan E. Spurgeon, Iain M. Morgan

12. **The Polyomavirus Middle T-Antigen Protein Forms a Discrete Nanocluster Complex in the Outer Cell Membrane**
Stephen M Dilworth

13. **YAP Activation Disrupts MCPyV – Driven Tumor Maintenance and Requires mTOR Signaling for Cellular Survival**
C. Mazziotta, F. Bahar, J.A. DeCaprio

14. **Using a germ-free mouse model to study the role of the cervicovaginal microbiome in human papillomavirus infection**
C. Schiff, C. Salas-Quinchucua, M. E. Spurgeon

15. **E2F1-3 activate MCPyV LT/ST transcription and viral replication**
N.J.H. Salisbury, S. Amonkar-Patil, A. Roman, D.A. Galloway

16. **NF-κB reprograms MCPyV early transcription to suppress viral replication**
N.J.H. Salisbury, S. Amonkar-Patil, A. Roman, D.A. Galloway

17. **HPV16, but not HPV18, represses chemokine signalling in tonsil epithelia through targeted modulation of host cell chromatin**
Leanne Jones, Jack Ferguson, Pavan Thind, Sally Roberts, Jo Parish

18. **HPV18 mutes cGAS-STING signalling in the presence of increased cellular cGAMP levels**
S. Shivpuri, D. P. Kosanke, I. G. Tobey, S. K. Campos, K. Van Doorslaer

19. **MmuPV1 E6 and IKKα - There is More to E6R130A Than Meets the Eye**
L. Zhang, K. Duxbury, Z. Morris, J.C. Romero-Masters

20. **Development of a two-colour fluorescence stability assay to identify novel regulators of HPV16 E7 stability**
Louisa M Wootton, Megan Wagstaff, Benjamin P Towler, Helfrid Hochegger, Rhys G Morgan, Ethan L Morgan

21. **Creation of a novel and inducible knock-in mouse skin model to analyze the functional role(s) of JC virus ORF4 protein, in keratinocytes, using HSV as a surrogate virus**
A.S. Saribas, M. Safak

SESSION III. IMMUNE RESPONSES

Chairs: Paul Lambert and Enrique Boccardo

Abstract 22-34

22. Targeting NSD2 to Modulate Tumor Immunogenicity in HNSCC According to HPV Status

Lavinia Ghiani, Marta Tagliabue, Simona Citro, Francesco Chu, Farkhondeh Ghoryani, Mirko Doni, Francesco Bonacina, Fausto Maffini, Mohssen Ansarin, Susanna Chiocca

23. Detection of traces of EBNA2 in the Microenvironment of Pediatric Classical Hodgkin Lymphoma Reveals Its Immunomodulatory Role

Mangiaterra TS, Lindl KE, Jiménez OE, Attardo A, García Lombardi M, Soria M, De Matteo E, Chabay PA

24. Immunogenetic Determinants of Viral Persistence in Breast Tumorigenesis: An In Silico HLA Binding Affinity Analysis Immunomodulatory Role

A. Salman Muhammad, B. Khan Hayat

25. Immune-mediated viral clearance and cellular mechanisms of immune memory in MmuPV1 infection

Lam Khue Pham, Donghwan Jeon, Ella T. Ward-Shaw, Andrea Bilger, Denis Lee, Rong Hu, Allison Geiger, Paul F. Lambert

26. Disruption of Host Innate Immune Response by HAdV-C5 via Human Ubiquitin E3 Ligase UBR4

L. Fulham, N. Casiano, J. Rempel, R. Helwer, P. Pelka

27. The Balance Between Immune Surveillance and Viral Immune Evasion Drives the Persistence of Distinct HPV Lifecycle States

A. Davies, F. Pan, A. Aiyenuro, K. Schichl, W. Chen, F. Inturrisi, N. M. Diwan, T. Omar, S. Sayed, J. Lukorito, N. Mugo, N. Egawa, H. Griffin, S. de Sanjose, M. Schiffman, M. Chung, J. Doorbar

28. Elucidation of a B cell specific inflammatory pathway that resembles the response to EBV infection

M. A. M. Hill, S. J. Kim, F. A. Dick

29. Modulation of Intrinsic Immunity by Merkel Cell Polyomavirus in Skin Organoids

L.M. Röpke, C. Schmidt, V. Brinschwitz, C. Conze, S. Krasemann, L. Allweiss, M. Dandri, A. Hansen, A. Grundhoff, S. Albertini, N. Fischer

30. EBV inhibits antiviral immunity through the generation of MDSCs in systemic CAEBV disease and EBV-associated HLH

Zhaoqin Zhang, Paul Collins, Sara Anisi, Alex Glover, Matthew Blake, Sophie Chander, Claire Shannon-Lowe

31. Breaking the balance: Human papillomavirus E7 interrupts IL-18 inflammatory signaling in head and neck cancers

A.M. Evans, W. Anderson, A.H. Karimi, W. Al Jawhri, J.S. Mymryk

32. Characterization of the immune microenvironment at the sites of cervical HIV/HPV co-infection
F. Pan, A. Davies, A. Aiyenuro, K. Schichl, F. Inturrisi, N. M. Diwan, T. Omar, S. Sayed, J. Lukorito, N. Mugo, N. Egawa, S. Sanjosé, M. Schiffman, M. Chung, J. Doorbar

33. Assessing HPV type specific immune interactions influencing persistence in tonsil epithelia
Grace Akinyi Odongo, Shweta Manoharan, Jack Ferguson, Sally Roberts, Jo Parish

34. Viral genome state and host immunity in HPV-driven cervical cancer: insights from a longitudinal cohort in India
Keshav Saini, Muneeb Pervez, Manpreet Kaur, Iman Dandapath, Andreas Wieland, Lawrence Banks, Rafi Ahmed, Murali-Krishna Kaja, Nilanchali Singh, Anmol Chandele

SESSION IV. ONCOGENESIS 1

Chairs: Karl Munger and Vjeko Tomaic

Abstract 35-48

35. HPV E6/E7 promotes TBX3 oncogenic activity through CDK5-mediated phosphorylation
Sharon Prince, Carly Burmeister, Michael Myers, Lawrence Banks

36. Understanding oncogene-induced replication stress in tumorigenesis using HPV disease model
Christy S Varghese, James A Scarth, Molly A Guscott, Nisha Gangadharan, Jason Parsons, Michael A Boemo, Sarah E McClelland, Eva Petermann, Joanna L Parish

37. Host genetic landscape of HPV E6/E7-driven immortalization and transformation
Danny Papini, Leah Soriaga, Kathryn E Woods, Kyoungwha Pang, Chukwudumebi Okonkwo, Jayoung Choi, Zeyu Zhu, Suji Kim, Alina Xiaoyu Yang, Amalio Telenti, Karl Munger, Elizabeth A White, Seungmin Hwang

38. mEEP, a novel syngeneic model of HPV+ oropharyngeal carcinoma
Kimberly Manning, Anna Macmanus, Angelyn Campillo, Lovely Raghav, Erqianqian Ye, Devraj Basu, Sydney Shaffer, Ahmed Diab

39. HPV16 E7 Reprograms Glutamine Metabolism to Promote Metabolic Plasticity in HPV-Associated Cancers
V. Georgiou, V. Andreou, I. Shanlitourk, K Strati

40. Lipid reprogramming of stratified squamous epithelium by the high-risk HPV E6 and E6/E7 oncoproteins
Megan E Spurgeon, Taylor Lange, Alexandra Baty, Helena Li, Paul F. Lambert, Kenneth D.R. Setchell, Susanne I. Wells, Xueheng Zhao

41. APOBEC mutagenesis in cancers associated with DNA tumor viruses
Reuben Harris

42. Non-CpG and CpG methylation in Merkel cell carcinoma
Ilies Benotmane, Lisa Boxer, Jaehyuk Choi, Kenneth Tsai, James A DeCaprio, Gabriel Starrett

43. NEDD8-dependent Proteasomal Degradation of p53 by the Beta-HPV49 E6 Oncoprotein is Independent from E6AP, MAML1 and MDM2
Meret Beyer, Tina Rehm, Leonie Brosch, Thomas Iftner, Frank Stubenrauch

44. Biochemical Activity of HPV16 E6 and E7 Variants Does Not Correlate with Cancer Association
Miranda Grace, Harshita Beeravolu, Ahmad Alhamshari, Josipa Skelin, Si-Young Kiessling, Andrew Bohm, Elizabeth A. White, Karl Munger

45. YAP1/HMGCS1 signaling axis regulates Clamp Loader Rad17-dependent lipid droplet formation in cervical cancer
Mengda Niu, Shiyuan Hong

46. Inactivation of the RB1 and PTPN14 cooperatively enables the carcinogenic activity of the human papillomavirus E7 oncoprotein
Pushkal Sinduvadi Ramesh, Angelo A. Nicolaci, Leah E. Graham, Jennifer M. Binning, Karl Munger, Elizabeth A. White

47. HPV E6/E7 promote the interaction of TBX3 with its oncogenic partners, Hsc70 and nucleolin, in cervical cancer
Carly Burmeister, Lawrence Banks, Michael Myers, Sharon Prince

48. Toll-like Receptor 3 (TLR3) and E7-induced lnc-FANCI-2 in Cervical Cancer
Lulu Yu, Haibin Liu, Vladimir Majerciak, Zhi-Ming Zheng

SESSION V. VIRUS ENTRY & INFECTION MECHANISMS

Chairs: Michelle Ozbun and Colin Crump

Abstract 49-56

49. Investigating the Trafficking Mechanisms of Merkel Cell Polyomavirus (MCPyV)
Isabel Amaya, Andrew Ampomah, Alan Marshall, Chelsey Spriggs

50. Unravelling the Role of CFTR in BK polyomavirus Infection
Gemma Swinscoe, Margarita-Maria Panou, Michelle Antoni, Eleni-Anna Loundras, Matthew Welberry-Smith, Andrew Macdonald

51. A Forward Genetics Platform for Papillomavirus Research
Yuka Takeo, Sydney Hirsch, Daniel DiMaio

52. The human papillomavirus L2 protein in the capsid is an ensemble of related structures poised to exit the virus particle
Changin Oh, Huaxin Yu, Patrick Buckley, Frank Horrigan, Kashif Mehmood, Jeongjoon Choi, Jun Liu, and Daniel DiMaio

53. Functional elements of the Human Papillomavirus L2 protein act sequentially from its C-terminus during virus entry
Jeongjoon Choi, Changin Oh, Lisa M. Petti, Bayan Galal, Daniel DiMaio

54. Genetic complementation studies of Human PapillomaVirus type 16 minor capsid protein L2 mutations that prevent infection
Z. Guan, J. Terrell, R.B.S. Roden

54a. Interplay Between Promyelocytic Leukemia Nuclear Bodies and Phospholipases C Facilitates Early Events in the HPV16 Lifecycle
A. Kushwaha, TR. Keiffer, M. Bienkowska-Haba, M. Represa-Perez, M. Sapp, K. Zwolinska

55. Sp140L is a Novel Restriction Factor of Herpesvirus Infection
Wiyada Wongwiwat, Jana Cable, Micah Luftig, Robert White

56. Mutant mania! Generating E1A HAdV mutants to investigate the role of E1A-Protein Kinase A interactions during HAdV infection
K. Palmateer, J. Mymryk

SESSION VI. LIFE CYCLE

Chairs: Lou Laimins and James DeCaprio

Abstract 57-70

57. The development of a kidney organoid model of JC Virus infection
Si Jie Tang, Stephen Leon-Icaza, Irene Cortese, Colin Crump

58. Unraveling Merkel Cell Polyomavirus Infection Dynamics and Persistence in Human Hair-Bearing Skin Organoids Using Multi-omics
Silvia Albertini, Manja Czech-Sioli, Lisann M. Roepke, Claudia Schmidt, Veronika Brinschwitz, Emanuel Wyler, Markus Landthaler, Sanamjeet Virdi, Patrick Blümke, Susanne Krasemann, Lena Allweiss, Maura Dandri, Christian Conze, Carola Schneider, Rudolf Reimer, Arne Hansen, Adam Grundhoff and Nicole Fischer

59. Cellular origin of cervical reserve cells: primary target for HPV infection and the onset of cervical neoplasia and dysplasia
Heather Griffin, Ademola Aiyenuro, Aslam Shiraz, Norman Shreeve, Mercedes Jimenez-Linan, Nagayasu Egawa& John Doorbar

60. Exploring the functional significance of the interaction between the HPV capsid protein L2 and the oncogenic transcription Factors TBX2 and TBX3 in the HPV life Cycle
Mr MGE Goldschagg, Dr SF Khan, Dr L Banks, A/Prof G Schäfer, Prof S Prince

61. Investigating the interplay between SV40 chromosome architecture and activity
M. Lano, B. Milavetz

62. Host chromatin surveillance reshapes hepatitis B viral gene expression and interplay with endogenous retroelements
Xiaxuan Zhu, Senko Tsukuda, Daisy Jennings, James Docker, Rebecca V. Berrens, James M. Harris and Jane A McKeating

63. Cloning and Analysis of an Epstein-Barr virus Strain from an LCL Established with virus from Kenyan Breast Milk
A. Li, L. Granger, W. Wongwiwat, A. Bayoumy, R. Rochford, R.E. White

64. NFX1-123 is required for keratinocyte growth, differentiation, and HPV 16 infection maintenance
Maura A. Dankoski, DeShawn Thompson, Kevin M. Quist, Rachel A. Katzenellenbogen

65. Understanding Immune-Mediated Resolution of HPV-Related Disease and the Control of Subclinical Infections
W. Chen, H. Griffin, N. Egawa, A. Aiyenuro, A. Davies, K. Schichl, F. Pan, N. Diwan, T. Omar, F. Inturrisi, S. Sayed, J. Lukorito, N. Mugo, M. Schiffman, S. de Sanjosé, M. Chung, J. Doorbar

66. Stochastic Modeling of Epithelial Homeostasis in persistent HPV16 and HPV11 infection
A. Giaretta, Y. Chen, G. Pillonetto, N. Egawa, B. D. Simons and J. Doorbar

67. Mitochondrial ROS levels are enhanced by the adenovirus E4orf4 protein and play a role in viral replication and virion stability
Amir basis, Hanan Taha, Rakefet Sharf and Tamar Kleinberger

68. Merkel cell polyomavirus small T antigen regulates the progression of the viral life cycle by promoting the maturation of late transcripts
Masahiro Shuda, Jeffery Nurdin, Amanda E. Moore

69. Investigating the spatial localisation of the HPV16 late protein, L1 and HPV16 virions in organotypic raft cultures
Anna Kirk, Andrew Stevenson, Swetha Vijayakrishnan, Sheila V Graham

70. One virus, two pathways – Lytic and nonlytic cell egress
U.F. Greber, C. Bircher, M. Suomalainen

SESSION VII. GENE EXPRESSION

Chairs: Sheila Graham and Thomas Zheng

Abstract 71-79

71. Deregulation of high-risk HPV in cervical precancer centres around the crypt entrance epithelial niche
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72. HPV18 regulates alternative splicing of E6/E7 through PRMT1-dependent m6A methylation of viral mRNA
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73. The role of N6-methyladenosine in regulating the expression of HPV capsid transcripts
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74. The mammalian DREAM complex regulates gene expression and DNA damage repair to impact longevity
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75. PPARα-activated transcriptome of HPV16(+) oropharyngeal cells
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76. Degradation of Mettl3 and Mettl14 by high-risk E6 stabilizes RNA:DNA hybrid formation and alters the epitranscriptome of HPV positive cells
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81. KAT5 (TIP60) Is Required for ST- MYCL- Driven Chromatin Activation and Oncogenic Transcription in Merkel Cell Carcinoma
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82. Dissecting the role of genomic instability in human papillomavirus (HPV)-driven cancer initiation
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84. HPV Status Defines MBD2-Dependent Regulation of Cellular Plasticity in Head and Neck Cancer
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85. Investigating Anti-Retroviral Therapy-Induced Stemness and Epithelial Mesenchymal Transition Shifts That May Enhance Susceptibility to HPV16 Driven Oropharyngeal Carcinogenesis
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86. Integrative structural analysis of the interaction between the onco-protein E7 of human papilloma virus 16 and the clathrin adaptor protein 2
F. Lapenta, Bharti, S. di Marco, S. Covaceuszach, P. Pompach, N. Kavcic1, A. Magistrato, S. Onesti, N. Skoko, L. Banks

87. SIRT1 inhibition disrupts replication-checkpoint control and reveals a p53-p21 vulnerability in HPV-driven cancers
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90. MicroRNA-Mediated Regulation of Matrix Metalloproteinase Inhibitors in HPV-Transformed Cells
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92. Single-cell RNA sequencing reveals potential tumor-associated macrophage-mediated immunosuppression in equine papillomavirus type 2-associated squamous cell carcinoma
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93. Beyond the Infected Cell: Reframing HPV Pathogenesis Through the Microenvironment
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94. HPV Infection at the Cervical Transformation Zone: Reserve Cell Biology, and the Risk of HSIL Recurrence
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107. Integrative multi-omics reveals HPV-driven ganglioside metabolism as a candidate for drug repurposing
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119. Comparing the ability of HPV oncoproteins to promote basal epithelial identity
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120. Defining the Role of Epithelial Wounding in Persistent Papillomavirus Infections
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129. HPV8-E6 drives coordinated transcriptional and epigenetic reprogramming of keratinocytes
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136. Phosphorylated HPV 16 E7 Perturbs Vangl1 Trafficking to Drive Polarity Loss
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Cheshta Shandilya[1,2], Ifeoluwa D. Gbala[1], Michael Myers[3], Lawrence Banks[1]

Abstracts

The Spatial and Functional Architecture of HPV Replication Factories

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The final stage of HPV replication produces very high levels of viral genomes to be packaged in viral particles. At this stage, viral DNA replication shifts to a recombination-dependent replication (RDR) mechanism that requires the host DNA damage and repair response (DDR), and enables the virus to amplify its genomes in differentiated cells no longer in S-phase. This productive replication occurs in nuclear foci that recruit many factors required for DNA replication, the DDR, chromatin packaging, and transcription. By mimicking DNA damage, the virus induces an influx of factors that promote efficient viral genome amplification.

In addition to studying wild type HPV replication factories that form in infected, differentiated keratinocytes, we studied factories generated by an HPV16 E8^E2 null or reduced expression E8^E2 mutant. These viruses generate enlarged replication factories, even in the absence of differentiation and within these foci we can detect the native HPV16 E1 and E2 proteins and the E8^E2 protein using antibodies generated in-house or from colleagues.

We performed a comprehensive analysis of HPV replication factories using high-resolution confocal microscopy, fluorescence in situ hybridisation (FISH), immunofluorescence, and nucleotide pulse-labelling approaches to construct a three-dimensional model of their functional architecture. These methods enable estimation of focus formation rates and characterisation of their temporal progression. We identify cellular proteins associated with DNA damage sensing, repair and signalling, DNA replication, transcription, histone modification, and innate immunity, and define their spatial and functional relationships with respect to viral components.

Viral inhibition of the anaphase promoting complex enhances replication by elevating nucleotide pools

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The anaphase promoting complex/cyclosome (APC/C) is a large, ubiquitin ligase and a central regulator of cell cycle progression. By targeting key substrates for degradation during mitosis and G1 phase, the APC/C coordinates metabolic fluctuations that occur during the cell cycle. A diverse range of viruses including Papillomavirus, adenovirus, herpesvirus, poxvirus, retrovirus and annellovirus have all been shown to encode proteins that bind and inactivate the APC/C; however, a molecular understanding for these interactions has never been demonstrated. Here, we use chicken anemia virus (CAV), a small single-stranded DNA virus encoding only three proteins, to demonstrate the importance of viral APC/C inhibition during replication. We show that the Vp3 protein of CAV inhibits the APC/C, causing a dramatic mitotic arrest during infection. Mutant virus lacking Vp3 are defective for replication and can be rescued by APC/C inhibition. Metabolomic profiling during CAV infection revealed that Vp3 expression mediates a broad increase in nucleotide pools. Moreover, viral inhibition of the APC/C resulted in stabilization of enzymes required for nucleotide biosynthesis. These findings suggest that the APC/C is a general target of many viruses to elevate nucleotide levels and facilitate viral genome replication.

Restoration of HR Limits HPV-Positive Cellular Viability

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Human papillomaviruses are the etiological agents of virtually all cervical cancers, as well as most head and neck cancers and other anogenital cancers. Low-risk types also cause benign warts and recurrent respiratory papillomatosis. In high-risk types, presence of the ~8kb extrachromosomal element, oncogenes E6 and E7, and replication proteins E1 and E2 influence and activate host DNA damage responses and induce genomic instability. Not only do HPVs and encoded genes induce genomic instability, but they counterintuitively require DDR activation and components of the replication stress response and host replication proteins (replisome) for their productive lifecycle and cellular viability. Low-risk types are also implicated in altering host DNA replication protocols.

Previous studies indicate that HPVs recruit replication stress factors to the viral genome, diluting them from human DNA. Additionally, HPV oncoproteins are thought to impose a functional homologous recombination (HR)-deficient state by altering BRCA1 in both mucosal and cutaneous types, yet viral genome amplification requires intact HR activity, revealing stage-specific exploitation of the same pathway dismantled by the virus during transformation.

iPOND (isolation of proteins on nascent DNA) captures replisome-associated proteins at the replication fork by labeling nascent DNA with EdU, followed by click chemistry-mediated biotin conjugation and streptavidin pulldown. Coupled to mass spectrometry, iPOND provides an unbiased snapshot of replisome composition and fork-associated repair factors under any condition of interest. Using an optimized method of iPOND in patient-derived matched HPV negative and positive cervical epithelium, we observe substantial dysregulation and loss of host replication factors at actively replicating human DNA including BRCA1 and BRCA-adjacent factors. Interestingly, restoring HR activity both genetically and pharmacologically disrupts HPV positive cellular viability in competition assays. Single-molecule DNA combing assays demonstrate that HPV and oncogenes markedly increase DNA synthesis rates, with genetic restoration of HR processes wholly rescuing normal rates. Surprisingly, this also occurs in epithelia harboring the HPV episome. These data establish a model in which HPV usurps the host replisome by suppressing HR activity at replication forks. Ongoing in vivo xenograft cancer models will define whether pharmacological and genetic restoration of this pathway can selectively reduce HPV-driven tumor growth and proliferative capacity.

Targeting Cisplatin-Induced RAD51 Filaments Uncovers a Vulnerability in HPV-Positive HNSCC

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DNA interstrand crosslinks (ICLs) are highly cytotoxic lesions that covalently link the two strands of the DNA double helix. Their detection and repair typically rely on the Fanconi anemia (FA) pathway, which coordinates lesion recognition and unhooking. Here, we report that FA pathway components are largely dispensable for the survival of human papillomavirus (HPV)-positive head and neck squamous cell carcinoma (HNSCC) cells treated with the ICL-inducing agent cisplatin. A genome-wide CRISPR-Cas9 screen revealed this unexpected independence from canonical FA-mediated repair. Consistent with prior observations in keratinocytes (DOI: 10.1371/journal.ppat.1007442), ectopic expression of HPV16 E6 and E7 in HPV-negative HNSCC cells is sufficient to confer FA-independent growth under ICL stress. Despite FA pathway dispensability, HPV-positive HNSCC cells remain dependent on the DNA repair factor FIRRM. The FIRRM/FIGNL1 complex regulates the cellular response to ICL-induced DNA double-strand breaks by promoting RAD51 disassembly following strand invasion, suggesting that HPV-positive cells rely on RAD51-dependent pathways for ICL tolerance.

To define this mechanism, we analyzed DNA damage signaling following ICL induction. Using confocal microscopy and immunofluorescence, we observed robust accumulation of RAD51 foci in HPV-positive cells, a repair intermediates that usually accumulates downstream of ICL processing. RAD51 accumulation, as well as single-stranded DNA formation, is dependent on EXO1, indicating a DNA end resection–driven repair mechanism. Functionally, HPV-positive HNSCC cells exhibit increased sensitivity to RAD51 depletion or pharmacological inhibition of RAD51 filament compared to HPV-negative counterparts. Strikingly, while RAD51 activity supports survival under cisplatin treatment, excessive RAD51 stabilization induced by small molecules or depletion of FIRRM/FIGNL1 is highly toxic in HPV-positive cells. This finding highlights RAD51 filament as a toxic intermediate in HPV-positive cancer cells.

Together, these findings demonstrate that HPV oncogenes rewire the ICL response toward an FA-independent, RAD51-dependent pathway. This establishes RAD51 filament dynamics as a key determinant of ICL tolerance in HPV-positive HNSCC and reveals a potential therapeutic vulnerability.

Defining the Polymerase milieu employed by Human Papillomavirus (HPV) across the viral lifecycle and viral interaction with the host replication machinery

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Human Papillomaviruses (HPVs) are small, ubiquitous, double-stranded DNA viruses that are the etiological agents of cervical, anogenital, nasopharyngeal, and oropharyngeal cancers. HPV infects the basal epithelium via microabrasions caused by sexual contact and maintains its copy number through viral maintenance during cellular division. Eventually, an infected basal cell commits to differentiation, moving upwards towards the stratified epithelium, where the virus amplifies to thousands of copies per cell, a process known as viral amplification. Previous studies indicate that the virus shifts from theta replication during viral maintenance to rolling-circle replication or recombinant-dependent replication (RDR) during viral amplification. The HPV life cycle itself activates the human replication stress response, recruits host factors to the viral genome, and induces genomic instability, including DNA double-strand breaks (DSBs). The mechanism through which this occurs, and which factors HPV uses to hijack the human replisome, is largely unknown.

Using a systematic and unbiased proteomics-based approach, we are defining the polymerases that PVs use for replication through the differentiation-dependent lifecycle and how subversion of these polymerases influence mutagenesis in the host. Our studies indicate that polymerases such as polymerase α (Polα) are readily recruited to HPV genomes both during viral maintenance and amplification. Literature recently described a potent and specific small-molecule inhibitor (CD437) of Pola. Nanomolar concentrations of this inhibitor induces a robust replication stress response in HPV+ cells. Further, treatment of episomal cell lines influences both viral maintenance and viral amplification. Treatment of HPV-16 (+/-) primary-matched patients demonstrates hypersensitivity to low levels of Pola inhibitor in both novel live-competition assays and viability assays in HPV+ cells, indicating potential therapeutic potential. Several translesion synthesis (TLS) polymerases, notably polymerase ζ (Polζ), polymerase κ (Polκ), and polymerase η (Polη), have also been shown to affect DNA synthesis, differentiation-dependent amplification, maintenance, and mutagenic potential in HPV+ cells. Further investigation aims to understand the specific roles of these polymerases in viral replication and how HPV genes recruit polymerases and host DNA damage response proteins.

ARGLU1 is a Negative Regulator of the Adenoviral Replicative Cycle

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We have previously reported that human adenovirus E1A interacts with ARGLU1, a small disordered cellular protein. The consequences of this interaction for virus replication were unclear. E1A is the first protein produced during adenovirus infection. Via protein-protein interactions, E1A modifies the cellular environment to create optimal conditions for viral replication.

To better understand the molecular mechanisms driving viral infection, we further investigated the functional consequences of the interaction between ARGLU1 and E1A. ARGLU1 interacts with E1A directly as determined by a GST-pulldown assay with recombinant proteins. Importantly, ARGLU1 was found to act as a transcriptional repressor when it was localized to viral promoters. Repression was driven by enhanced promoter-proximal RNA polymerase II pausing.

Significantly, ARGLU1-induced repression of viral promoters is likely an unintended consequence of ARGLU1 binding to E1A, as a mutant of E1A (dl1102), unable to bind to ARGLU1, did not show reduced viral gene expression. Furthermore, ARGLU1 was found to colocalize with E1A in infected cell nuclei.

Finally, the binding of E1A to ARGLU1 appears to reduce DNA damage repair in bleomycin-treated and adenovirus-infected cells, suggesting that E1A binding to ARGLU1 is important for DNA damage response inhibition. Overall, this study underscores the complexity of virus-host interactions and reveals a novel role for ARGLU1 during adenoviral infection.

APOBEC3-mediated suppression of archetype BKPyV rearrangements and replication in urothelial cells

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BK Polyomavirus (BKPyV) establishes a persistent urinary infection with intermittent, asymptomatic shedding. In transplant recipients, BKPyV can generate highly replicative variants with a rearranged NCCR causing disease such as nephropathy and hemorrhagic cystitis. The mechanism of BKPyV reactivation/rearrangement remains unclear. However, the innate, immune APOBEC3A/B (A3) cytosine deaminases may be involved as BKPyV shows evolutionary scars from A3 activity and A3-mediated mutations have been observed in disease. Previously A3 expression and knockdown has not been shown to affect rearranged BKPyV replication. To test the involvement of A3 enzymes in archetype replication, we conducted a 100-day serial passage of archetype and mock infected immortalized human urothelial cells and their corresponding A3A/B-KO derivatives. BKPyV replication only occurred the A3A/B-KO cells and corresponded to a diverse set of rearrangements in the NCCR suggesting that A3s are suppressing rearrangement and subsequent replication. To determine the underlying mechanism, we reintroduced stable expression of A3A, A3B, or their respective catalytic mutants into our A3A/B-KO cells. Even under overexpression conditions no significant difference in rearranged BKPyV replication was observed. For archetype, possible low frequency APOBEC3-mediated mutations could be identified by ultra-deep sequencing shortly after uncoating in cells with active deaminases. However, it is unclear if these low frequency mutations are directly deleterious to virus replication. Another possibility is that A3 enzymes directly block DNA binding proteins involved in generating NCCR rearrangements, which we are assessing using a reporter pseudovirus. Overall, we have identified that A3 enzymes can restrict archetype BKPyV rearrangements and replication in urothelial cells, and ongoing work is determining if this observation is deamination-dependent and occurring in replicating or transcribing DNA compartments.

APOBEC3B inhibits HPV genome replication independently of its deaminase activity

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Objective: HPV16 E6 induces the expression of the host DNA-editing enzyme APOBEC3B (A3B) at the transcriptional level. We previously analyzed HPV genome mutations in human cervical lesions and demonstrated that APOBEC3 introduces mutations into the HPV genome (Hirose et al., 2018, J Virol, doi: 10.1128/JVI.00017-18). Although it is hypothesized that APOBEC3 may inhibit HPV replication by introducing mutations into the viral genome, this has not been experimentally verified. The aim of this study was to investigate the effect of A3B expression on HPV genome replication.

Methods: To induce HPV16 genome replication, we transfected expression plasmids for the HPV16 replication proteins E1/E2 into U2OS cells stably harboring a circular HPV16 genome and investigated the effect of co-transfection with an A3B expression plasmid on HPV16 replication levels. The amount of replicated HPV16 genomic DNA was quantified by qPCR. Mutations introduced into the HPV16 genome were analyzed using next-generation sequencing. The interaction between A3B and E1/E2 was examined by co-immunoprecipitation in HEK293 cells.

Results: A3B inhibited E1/E2-dependent HPV16 genome replication in U2OS cells by approximately 50%. Furthermore, an A3B mutant (E255A) lacking deaminase activity inhibited HPV16 DNA replication to the same extent as wild-type A3B. Sequence analysis of the HPV16 genome replicated in the presence of A3B revealed no increase in C-to-T or G-to-A mutations characteristic of A3B mutagenesis. When HA-tagged A3B and E2 were expressed in HEK293 cells and the cell extract was immunoprecipitated with an HA antibody, E2 was co-precipitated with A3B. Furthermore, E255A co-precipitated with E2 as similarly as wild-type A3B. On the other hand, no interaction between A3B and E1 was detected.

Discussion: It is proposed that A3B inhibits HPV replication by binding to E2 and thereby blocking some E2 functions. Alternatively, E2 may inhibit the deaminase activity of A3B by binding to it, thereby limiting the anti-viral activity of A3B.

Identifying mechanisms of antiviral enzyme APOBEC3A expression in tonsil epithelium using organoid models

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The apolipoprotein B mRNA-editing enzyme, catalytic polypeptide (APOBEC) family enzymes are polynucleotide cytidine deaminases that act on single-stranded nucleic acids. They are induced by type I interferons during viral infection and introduce DNA mutations and RNA edits in foreign nucleic acids, including those of oncogenic viruses (e.g. HPV, HBV, HIV, EBV). However, other mechanisms exist for their induction: APOBEC3A is a potent mutator of cancer genomes, with over 50 % of cancers displaying evidence of APOBEC mutagenesis.

While its mutagenic potential of APOBEC3A has been well demonstrated in cancer contexts, the role of these enzymes in normal physiology is poorly understood due to the lack of suitable in vitro models. A robust understanding of physiological roles also enables a better understanding of mechanisms of APOBEC dysregulation in cancer.

In the tonsil, immune-rich crypt epithelial cells consistently express APOBEC3A through an unknown mechanism. We modelled the healthy tonsil epithelium using epithelial organoids derived from healthy tonsil biopsies, and phenotyped the organoids using wholemount immunofluorescence. We demonstrate that APOBEC3A is prominently expressed in a subset of differentiating keratinocytes. This is consistent with our previous findings of squamous transcriptional programmes driving APOBEC3A expression in healthy keratinocytes and head and neck tumour cells (DOI:10.1038/s44318-024-00298-9). However, the findings do not completely account for ubiquitous expression in tonsil epithelial cells, nor the crypt specificity, suggesting an immune-derived signal may be partly responsible. Future efforts aim to identify the immune signal(s) responsible for APOBEC3A expression in healthy tonsil crypts using these established models. This may provide insights into how APOBEC mutational activity is induced in tumours, as well as providing better models for oncogenic viruses of the tonsil (HPV) and other squamous epithelia.

Detection and structural characterization of HPV16 E2 variants in Tunisian precancerous and cancerous cervical lesions

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Human papillomavirus type 16 (HPV16) is the most prevalent high-risk genotype associated with cervical carcinogenesis. Although the oncogenic roles of E6 and E7 are well established, the E2 protein is a key regulator of viral replication, transcription, and genome maintenance. Sequence variation within E2, particularly in the DNA-binding domain (DBD), may alter its regulatory functions and contribute to lesion progression. The objective of this study was to characterize HPV16 E2 sequence diversity in Tunisian cervical lesions and to evaluate the structural and docking-based impact of selected recurrent variants on the E2–DNA interaction.

Computational approaches, including multiple sequence alignment, sequence variation analysis, protein structural modelling, and protein–DNA docking, were employed to identify recurrent HPV16 E2 variants in Tunisian cervical lesions and to evaluate their potential structural and functional impact on the E2 DNA-binding region.

Comparative analysis identified 76 variable nucleotide sites and 28 distinct haplotypes among 50 Tunisian HPV16 E2 sequences. Structural analysis and protein–DNA docking of the Tunisian HPV16 E2 variants revealed that recurrent substitutions cluster within functionally relevant regions of the protein, particularly near the C-terminal DNA-binding domain hotspot and, to a lesser extent, within the upstream regulatory portion of the analysed E2 fragment. Notably, three prioritized variants, T310K, Q342H, and D344N, were identified as structurally relevant candidates. Docking analyses suggested that Q342H and D344N generated more favourable E2–DNA interaction profiles than the selected wild-type reference model, whereas T310K, because of its proximity to the DNA interface, may influence local protein–DNA recognition through positional and conformational effects. Together, these findings support the existence of a recurrent Tunisian E2 DNA-binding hotspot and suggest that naturally occurring E2 variation may alter DNA-binding and, consequently, regulatory activity relevant to cervical lesion progression.

This study provides a lesion-annotated overview of HPV16 E2 diversity in Tunisian cervical samples and identifies recurrent E2 variants within the C-terminal DBD as promising candidates for further structural and functional investigation.

E2 activation of the DNA damage response creates targetable vulnerabilities in HPV16 infection and cancer models

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During the HPV16 life cycle E2 activates the DNA damage response (DDR) via displacement of CIP2A from TOPBP1. This facilitates CIP2A-mediated inhibition of PP2A leading to ATM phosphorylation and activation of the DDR. E2 DDR activation is dependent upon TOPBP1 interaction and occurs in multiple keratinocyte models of the HPV16 life cycle. Importantly, this DDR activation is also observed in in vivo MmuPV1 lesions and in HPV16 positive oral cancer models that retain E2 expression and episomal viral genomes. Keratinocyte models immortalized only with the viral oncogenes E6 and E7, or that have integrated genomes, do not have an active DDR during differentiation and similarly, tumors with integrated viral genomes do not have an active DDR. This demonstrates that while E6 and E7 can induce DNA damage, they are insufficient to induce and sustain an active DDR. DDR activation occurs early during epithelial differentiation, and is maintained in cancers that arise through "escape" from differentiation while retaining E2 expression and episomal viral genomes.

The DDR activated by E2 generates a strong activation of ATM signaling with reduced ATR signaling. This promotes homologous recombination via ATM (required for replication of the viral genome) and continued cell proliferation by suppressing ATR-mediated cell cycle arrest. Here we will describe the DDR activation mechanism and demonstrate that E2 expression generates sensitivity to multiple reagents that inhibit DDR signaling and repair pathways. Overall, these findings identify a novel HPV16 signaling pathway that generates therapeutically advantageous vulnerabilities in HPV infections and in a sub-set of cancers that retain E2 expression, which includes the majority of HPV16 positive oropharyngeal cancers.

The Polyomavirus Middle T-Antigen Protein Forms a Discrete Nanocluster Complex in the Outer Cell Membrane

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Viral proteins have evolved to interact with host cell proteins in a similar but uncontrolled manner to the way these proteins are normally regulated. This means the study of viral proteins has provided many insights into how these cell proteins function. For example, both tyrosine kinases and PI3Kinases were discovered through studies of the middle T-antigen (MT) encoded by the mouse polyoma virus. MT acts as an analogue of a permanently active growth factor receptor (GFR), to create growth signals in cells. MT exerts this function by assembling a large multi-protein complex at the cell membrane, similar to a receptor tyrosine kinase. Amongst the proteins bound to MT are the core dimer of protein phosphatase 2A, a SRC-family kinase, PI3K, PLCγ-1, ShcA, YAP and members of the 14-3-3 protein family.

MT is an example of a Tail Anchored (TA) membrane protein that is inserted into the ER by recognition of a 22 amino acid hydrophobic region near its C-terminus. An HA tag added to the MT C-terminus is exposed on the outside of cells and forms discrete clusters on the cell surface. Mutants in the hydrophobic region were isolated that prevent this MT clustering, still associate with all of MTs known binding proteins but don't transform cells. This indicates these cell surface macromolecular signaling complexes are probably required for MT activity. Similar cell surface nanoclusters are observed with growth factor receptors, suggesting that signaling from GFRs is similarly dependent upon the assembly of these nanoclusters.

Tyrosine containing peptide sequences have previously been added before to the MT C-terminal half to analyze the roles of these sequences within the ShcA sequence. Here, we have found that small extra protein domains can also be added to the flexible C-terminal region in MT without affecting transforming activity. To study the role of the MT membrane nanoclusters, fluorescent proteins or proximity ID tags have been added into the MT sequence and transformed cells isolated. These have been used to demonstrate the movement of these clusters in living cells, and to identify novel proteins in these clusters that may be involved in GFR signaling.

YAP Activation Disrupts MCPyV – Driven Tumor Maintenance and Requires mTOR Signaling for Cellular Survival

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Merkel cell polyomavirus (MCPyV)-positive Merkel cell carcinoma (MCC) maintains a neuroendocrine transcriptional program with low Hippo pathway activity, suggesting that YAP suppression is required for tumor maintenance. Here, we test the hypothesis that YAP activation is intrinsically incompatible with the MCPyV-driven oncogenic state and define the mechanisms enabling transient cellular adaptation.

Re-expression of YAP1 in MCPyV-positive MCC cells induces rapid growth arrest and cell death and is accompanied by a marked reduction in MCPyV T antigen transcripts and protein, indicating disruption of the viral oncogenic program. To identify pathways that permit survival under these conditions, we performed a genome-wide CRISPR-Cas9 knockout screen, which identified all three components of the GATOR1 complex (DEPDC5, NPRL2, NPRL3) as top hits. Loss of GATOR1 enhances survival following YAP activation, implicating mTORC1 signaling as a key mediator of this adaptive response.

Mechanistically, YAP activation reduces GATOR1 protein levels and drives mTORC1 activation, as evidenced by increased phosphorylation of p70S6K and 4EBP1. Nutrient modulation experiments further demonstrate that mTORC1 activity is required to sustain viability in YAP-activated cells. Pharmacologic inhibition of mTOR reduces cell survival, with the ATP-competitive inhibitor Torin1 exerting a stronger effect than rapamycin, particularly in the context of YAP activation.

These findings support a model in which YAP activation disrupts the MCPyV-dependent transcriptional state, creating a cellular stress that necessitates mTORC1 signaling for survival. GATOR1–mTOR thus functions as a buffering axis that enables transient tolerance to YAP-induced reprogramming but is insufficient to fully rescue viability.

Together, this work defines a functional incompatibility between Hippo pathway activation and MCPyV-driven tumor maintenance and identifies mTOR signaling as a critical adaptive response, revealing a context-specific vulnerability in virus-positive MCC.

Using a germ-free mouse model to study the role of the cervicovaginal microbiome in human papillomavirus infection

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Despite the availability of highly effective Human Papillomavirus (HPV) vaccines, high-risk HPV infections contribute to >5% of the global cancer burden and are the etiological agents of cervical, anogenital, and oropharyngeal cancers. As obligate intracellular pathogens, both viral and host factors are necessary for HPV infection and persistence. Recent studies suggest that cervicovaginal microbiota are modulators of HPV infection outcomes in the female reproductive tract (FRT); however, these findings are predominantly epidemiological and/or clinical, highlighting the need for experiments in a tractable model to define the fundamental mechanisms behind microbe-virus-host interplay during HPV infection.

We have generated gnotobiotic FVB/N mice to develop a germ-free mouse papillomavirus (MmuPV1) infection model to investigate how the cervicovaginal microbiome (CVM) promotes and/or protects against mucosal papillomavirus infection and associated cancer progression. Following intravaginal infection with sterile MmuPV1 virus, we show that germ-free FVB/N mice support robust viral replication in the stratified squamous epithelium. At 2- and 4-weeks post infection, viral load was significantly higher in germ-free mice compared to conventional controls, suggesting that the CVM is protective against MmuPV1 establishment. Ongoing studies will determine the role of the CVM in later stages of infection and throughout neoplastic disease and cancer progression.

Histological analysis of uninfected FRTs revealed variable epithelial thickness in conventional mice consistent with cycling estrous stages. In contrast, germ-free mice displayed intermediate and relatively uniform epithelial thickness suggesting impaired estrous cycling. This observation may implicate microbiome-mediated hormone regulation as a key mechanism underlying papillomavirus infection. Ongoing studies are examining estrous cycle dynamics, viral genome integration, and immune response to define how the microbiome shapes papillomavirus infection, persistence, and progression to cancer.

E2F1-3 activate MCPyV LT/ST transcription and viral replication

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Merkel cell polyomavirus (MCPyV) expresses Large and Small Tumor antigens (LT/ST), which are splice variants that drive viral replication and tumorigenesis. One of LT's functions is to bind and inactivate RB1, a master regulator of the G1/S cell cycle checkpoint. In G1, RB1 binds E2F1-3, represses transcription of E2F target genes, and blocks entry into S phase. For cells to enter S, cyclin-dependent kinases must phosphorylate RB1 and disrupt its interaction with E2F1-3, activating E2F target gene transcription. In MCPyV-infected cells, LT binds RB1 and prevents it from interacting with E2F1-3. Thus, LT is thought to drive the host cell into S phase to initiate viral replication. Yet how LT/ST transcription is regulated is poorly understood.

We discovered E2F1-3 bind the MCPyV Non-Coding Control Region (NCCR) via a consensus E2 site situated close to the LT/ST transcriptional start site. Inhibiting E2F-NCCR binding downregulates LT/ST expression in both Merkel cell carcinoma cells and in 293A cells transfected with the circular MCPyV genome. Our findings suggest that LT/ST transcription occurs when MCPyV-infected cells enter S phase. To test this hypothesis, we have begun to examine E2F-NCCR binding in primary dermal fibroblasts, the putative MCPyV host cell. Following serum starvation, most fibroblasts are arrested in G0/G1 with undetectable E2F1-3 expression. After 24h serum stimulation, around half of fibroblasts are in S phase with robust E2F1-3 expression and RB1 phosphorylation. In DNA pulldowns, E2F1-3 bind the NCCR using serum-stimulated but not serum-starved fibroblasts, suggesting that LT/ST transcription occurs upon S phase entry.

A similar link between LT expression and S phase has been reported for BKPyV. We examined the NCCRs from a range of PyVs and identified E2 sites in the NCCRs of PyVs closely related to MCPyV. These E2 sites are bound by E2Fs and are required for robust NCCR activity in luciferase assays. Surprisingly, BKPyV's NCCR does not contain a consensus E2 site but binds E2F3 weakly. In conclusion, our findings challenge the existing paradigm that PyV infection stimulates S phase entry via LT expression and support the hypothesis that PyVs express LT/ST and replicate in response to S phase entry.

NF-κB reprograms MCPyV early transcription to suppress viral replication

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The Merkel cell polyomavirus (MCPyV) Early Region encodes Large and Small Tumour antigens (LT/ST), which are splice variants that drive viral replication. In addition to LT/ST, the Early Region expresses an Alternate LT Open reading frame (ALTO), which is encoded within the second exon of LT in the +2 reading frame. ALTO has been shown to inhibit viral replication in dermal fibroblasts and 293A cells. We previously showed that ALTO downregulates LT/ST transcription by activating NF-κB, which subsequently binds the MCPyV Non-Coding Control Region (NCCR). While LT and ALTO expression are mutually exclusive in MCPyV-infected fibroblasts, how ALTO expression is regulated is currently unknown.

While investigating the role of ALTO and NF-κB signaling on MCPyV replication in 293A cells, we observed that RELA overexpression, an NF-κB subunit, suppresses LT/ST expression and replication, while upregulating ALTO. Using 5'-RACE assays, we discovered that RELA induces expression of ALTO-specific transcripts, whose transcription initiates from within the Early Region. Using long read nanopore sequencing, we mapped the 3' end of the transcripts to the conventional polyadenylation signal used by LT and ST transcripts. To determine which MCPyV transcripts express ALTO protein, we transfected 293A cells with in vitro transcribed and 3'-polyadenylated LT, ST, and ALTO transcripts. While LT and ST transcripts contain the ALTO coding sequence, we only detected ALTO protein in cells transfected with ALTO transcripts. To determine whether NF-κB directly activates ALTO transcription, we performed DNA pulldowns using a probe spanning the ALTO transcriptional start sites within the Early Region. We found that NF-κB proteins bind much more strongly to this putative ALTO promoter than the NCCR. After cloning the putative ALTO promoter into a luciferase reporter plasmid, we detected significant upregulation of luciferase activity when the reporter is co-transfected with RELA plasmid. In summary, NF-κB appears to suppress MCPyV replication by binding to the MCPyV genome, inhibiting LT/ST transcription from the NCCR, and activating ALTO transcription from within the Early Region. Furthermore, our findings reveal an NF-κB/ALTO positive feedback loop that appears to have evolved to allow MCPyV to persist in response to anti-viral host responses by entering a latent state.

HPV16, but not HPV18, represses chemokine signalling in tonsil epithelia through targeted modulation of host cell chromatin

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HPV infection in the oropharynx is responsible for around 50% of all oropharyngeal cancer (OPSCC) cases worldwide. HPV16 accounts for >90% of HPV-OPSCC, whilst HPV18 is rarely found (<3%). The reason(s) for the disparity in HPV-type prevalence at this anatomical site are unknown, but one theory is HPV-type specific modulation of immune signalling dictates viral persistence and oncogenic potential.

Using a model of HPV replication in primary human tonsil keratinocytes (HTK), we show that HPV16 and 18 drive wholesale transcriptional reprogramming of infected keratinocytes but also observed distinct differences in the genes targeted by HPV16 and HPV18. Notably, HPV16 more strongly repressed innate immune signalling pathways than HPV18 including cytokine molecules (e.g. CCL2, CCL7 and CCL8) important for the activation and migration of immune cells into the infected epithelia, which was not rescued by type II IFN treatment of the cells. To understand the mechanisms driving transcriptional reprogramming by either HPV16 or HPV18, we studied global changes to chromatin topology using ATAC-Seq, which showed a strong correlation between chromatin accessibility and differential gene expression. Analysis of the CCL gene cluster at Chr17q12 (encoding CCL1, 2, 7, 8, 11 and 13) revealed a significant decrease in chromatin accessibility throughout the locus following HPV16, but not HPV18 establishment in multiple HTK donors.

De novo motif analysis of differentially accessible peaks using HOMER identified that HPV16, but not HPV18, significantly downregulated the accessibility of motifs best matched to signal transducer and activator of transcription (STAT), essential in regulating innate immune responses, and B-cell lymphoma 6 (Bcl6), a transcriptional repressor that functions to recruit corepressors (NCOR1/2) to induce epigenetic repression of target loci. We show that STAT signalling pathways appear to be intact in both HPV16 and HPV18 genome containing HTKs. However, Bcl6 protein levels are increased by HPV16, but not HPV18 replication suggesting that increased recruitment of Bcl6 to the CCL locus may drive HPV16-mediated epigenetic repression. Putting these results together, we hypothesise that HPV16-mediated stabilisation of Bcl6 results in enhanced recruitment to the CCL locus, driving epigenetic repression and chromatin compaction to prevent activation of cytokine production.

HPV18 mutes cGAS-STING signalling in the presence of increased cellular cGAMP levels

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While 90% of HPV infections are cleared by the immune system, persistent infection with Human papillomaviruses (HPVs) account for the majority of oropharyngeal and anogenital cancers worldwide. cGAS, a cytosolic double-stranded DNA sensor generates cGAMP upon binding to DNA, leading to downstream STING trafficking resulting in the activation of IRF3 and NFkB. This signalling pathway culminates in the expression of interferon stimulated genes and pro-inflammatory cytokines. In addition to this pathway, a conserved result of cGAMP mediated STING activation is the non-canonical induction of autophagy.

We previously demonstrated that HPV evades cGAS detection during initial infectious entry. Importantly, exogenous stimulation with excess cGAMP reduces the ability of HPV18 to establish long-term replication in primary cells. Nonetheless, established HPV(+) cell lines have increased cGAMP levels compared to parental control cells. These observations raise an important question, how do HPV(+) cells rewire cGAMP responsive cellular pathways?

HPV18 modulates cGAS/STING/IRF3 signalling during long-term infection. In our tissue culture-based model of persistent HPV infection, exogenous cGAS stimulation leads to robust phosphorylation of STING and IRF3 leading to downstream ISG expression. Importantly, in matched HPV18(+) cells this response is muted, suggesting that HPV18 modulates this pathway to support long term replication.

We further investigated the consequences of elevated cGAMP by examining how HPV18 gene expression influences induction of cGAMP-mediated induction of autophagy. In primary keratinocytes, STING activation induces LC3 lipidation, a hallmark of autophagy initiation, but does not activate the downstream master transcription factor, TFEB. Suggesting that this non-canonical autophagy pathway may not be fully active in keratinocytes. Nonetheless, cGAMP-treated HPV18(+) cells showed reduced LC3 lipidation compared with HPV18(−) cells. Because the regulatory mechanisms governing both IRF3 activation and LC3-associated autophagy require translocation of STING from the endoplasmic reticulum to the Golgi, we are investigating whether HPV18 alters STING trafficking to selectively dampen downstream effector responses.

MmuPV1 E6 and IKKα - There is More to E6R130A Than Meets the Eye

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Our recent work has established that MmuPV1 E6 is key driver for several phenotypes that are associated with the hallmarks of cancer and persistent papillomavirus infection. Prior work by the Munger lab has established that the MmuPV1 E6R130A mutant is incapable of promoting disease in vivo. They were able to show that this impairment was associated with a defect in binding to the transcription factor MAML1, which activates NOTCH signaling. We have determined that the E6R130A-expressing mouse keratinocytes is significantly impaired in promoting proliferation of mouse keratinocytes in vitro when E6 is overexpressed or during infection. To more comprehensively understand the impact of the E6R130A mutation on MmuPV1 E6 interactome, we performed co-immunoprecipitation on MmuPV1 E6-expressing cells followed by immunoblot (Co-IP/WB) for multiple MmuPV1 E6 binding partners that were identified in previous interactomic studies. Our Co-IP/WB analysis found that MmuPV1 E6R130A was defective for binding multiple cellular targets of MmuPV1 E6 including MAML1, IRF3, IKKα, IKKβ, and IKKγ. We were able to determine that MmuPV1 E6 reduced the steady state activity of NF-κB signaling and impaired NF-κB signaling following stimulation with TNFα. Similar results were observed in MmuPV1 infected mouse keratinocytes and in infected lesions in vivo. To mechanistically determine the impact of MmuPV1 E6 on NF-κB signaling through its interaction with the IKK complex, we performed immunoblot analysis on MmuPV1 E6-overexpressing and MmuPV1 infected mouse keratinocytes for IKK complex proteins. We found that MmuPV1 E6 does not change the protein abundance of IKK complex proteins. Recently literature has shown nuclear localization of IKKα impairs NF-κB signaling. We performed fractionation analysis to determine the impact of MmuPV1 E6 on IKKα localization. We found that MmuPV1 E6 increases nuclear IKKα compared to vector control and E6R130A-expressing cells in both overexpression and infection cell models. Importantly, we have determined that MmuPV1 E6 promotes transcriptional changes that are consistent with increased nuclear localization of IKKα as previously published. Collectively, our data suggests that a major role of MmuPV1 E6 is to impair NF-κB signaling by changing IKKα localization which contributes to transcriptional changes required for persistent infection.

Development of a two-colour fluorescence stability assay to identify novel regulators of HPV16 E7 stability

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Human papillomaviruses (HPV) contribute to approximately 5% of all human cancers worldwide, including almost all cervical cancer cases and a growing number of oral cancers. HPV E6 and E7 are the primary viral oncogenes, manipulating host cellular pathways to drive proliferation and survival. The expression of these proteins is essential for the survival of HPV+ cancers; however, their regulation at the protein level is poorly understood. Protein ubiquitination is an essential regulatory mechanism that can alter the stability, activity or localisation of protein substrates. Deubiquitinases (DUBs), enzymes which catalyse the removal of ubiquitin, have become an emerging target in cancer therapeutics. To investigate the role of deubiquitinases in HPV16 E7 stability, we developed a two-colour fluorescence stability assay using a bicistronic plasmid containing EGFP-tagged HPV16 E7 and mCherry separated by a P2A:T2A linker. In this system, GFP expression corresponds to 16E7 abundance, while mCherry serves as a normalisation control. To identify novel regulators of HPV16 E7 stability, we performed an siRNA screening assay by both flow cytometry and high-content microscopy using a DUB siRNA screening library. This identified several DUBs that may play a role in the stability of HPV16 E7, including USP33 and USP39. Subsequent validation confirmed the potential role of all of the significant hits from our screen, confirming the validity of our approach. Using HPV16+ CaSki cells, we depleted USP39 using shRNA and confirmed that 16E7 expression was reduced. Furthermore, USP39 depletion also resulted in reduced cell growth and colony forming potential. Our data demonstrate a novel methodology allowing for assessment of HPV16 E7 oncoprotein stability. Further studies will assess the direct role of these DUBs such as USP39 in the regulation of HPV16 E7 stability and their potential oncogenic functions in HPV-associated cancers.

Creation of a novel and inducible knock-in mouse skin model to analyze the functional role(s) of JC virus ORF4 protein, in keratinocytes, using HSV as a surrogate virus

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The human neurotropic virus, JC virus (JCV), causes a devastating and fatal disease of the central nervous system (CNS), progressive multifocal leukoencephalopathy (PML), by lytically infecting oligodendrocytes and astrocytes in a subset of immunocompromised individuals. The majority of the human population is infected with this virus during childhood, which resides primarily in the kidneys in a latent form. Every individual with autoimmune disease, including psoriasis, Crohn's disease, multiple sclerosis (MS), and rheumatoid arthritis patients, treated with immunosuppressive drugs and those with immunocompromised conditions, including AIDS patients, are at risk for reactivating JCV and developing PML. Reactivated virus carries more than 20% fatality rate, and even those who survive the disease are severely disabled due to brain damage caused by the viral infection and inflammation. Currently, there are no effective treatment options available for PML patients, nor are there animal models that manifest the infection and symptoms of PML. Thus, to develop such treatment options, it is fundamental to understand the molecular mechanisms underlying JCV's basic biology by critically analyzing the functional roles of its regulatory proteins. JCV encodes a small number of such proteins generated by the alternative splicing of the viral early and late transcripts.

We have recently discovered a novel JCV protein, ORF4 (173 aa), produced by alternative splicing of the viral late coding region. It was surprising to observe that ORF4 was found to be the only JCV protein that specifically targets the promyelocytic leukemia nuclear bodies (PML-NBs) in the nucleus and induces their reorganization. PML-NBs are known to play critical roles in intrinsic and innate immune responses against various viral infections, suggesting that the ORF4 protein, by targeting PML-NBs, inhibits their function and thereby significantly contributes to the viral life cycle. We initially characterized ORF4's functional roles during the infection cycle in vitro using mutagenesis and plan to do so in animal models in vivo as well. We have now created a novel tissue-specific knock-in mouse model for ORF4 to further analyze its PML-NB-targeting properties and its modulation of the antiviral immune system using HSV as a surrogate virus.

Targeting NSD2 to Modulate Tumor Immunogenicity in HNSCC According to HPV Status

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Head and Neck Squamous Cell Carcinoma (HNSCC) is the seventh most common cancer worldwide, with a rapidly increasing incidence projected to rise further in the coming decades. Notably, oropharyngeal cancers (OPC) have surged, driven by a 225% increase in Human Papillomavirus (HPV)-associated cases over the last twenty years. Although immunotherapies have improved clinical outcomes in HNSCC, only a minority of patients derive durable benefit, and both primary and acquired resistance remain significant challenges. In addition, treatment-related morbidity and healthcare costs continue to be substantial. Therefore, identifying predictive biomarkers and novel therapeutic targets to enhance treatment efficacy and overcome resistance is an urgent clinical priority.

We recently demonstrated that NSD2, a histone H3K36 methyltransferase, is upregulated in HNSCC compared to normal tissues, with higher expression in HPV-associated versus HPV-independent tumors (doi: 10.1186/s13046-025-03631-0). These findings position NSD2 as a promising therapeutic target and H3K36me2 as a potential biomarker for patient stratification. While NSD2 has been implicated as an oncogenic driver in multiple cancers and selective inhibitors are now emerging, its role in HNSCC-particularly across HPV subtypes-remains poorly defined.

Our RNA-sequencing analyses reveal that NSD2 knockdown (KD) regulates distinct gene programs depending on HPV status. In HPV-independent HNSCC cells, NSD2-KD specifically upregulates genes involved in immune responses, including antigen presentation, MHC class I pathways, interferon response and T-cell chemotaxis. Furthermore, preliminary data suggest that NSD2 targeting may induce a “viral mimicry” response.

Our findings suggest that NSD2 inhibition may enhance tumor immunogenicity in HNSCC, depending on HPV status, potentially converting immunologically “cold” tumors into “hot” ones and sensitizing them to immunotherapy. This work supports NSD2 as both a biomarker and a therapeutic target for combination strategies aimed at improving patient outcomes in HNSCC.

Detection of traces of EBNA2 in the Microenvironment of Pediatric Classical Hodgkin Lymphoma Reveals Its Immunomodulatory Role

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The detection of Epstein–Barr virus (EBV) traces using ultrasensitive techniques in B-cell lymphomas previously classified as EBV-negative by conventional methods suggests a broader role for the virus in lymphomagenesis. Its contribution in classical Hodgkin lymphoma (cHL) remains unexplored. This study aimed to characterize viral transcripts expression by sensitive methods.

A total of 48 pediatric cHL cases were analyzed. EBV status was determined using the conventional approach: EBER in situ hybridization (ISH). LMP1 and EBNA2 transcripts were assessed using highly sensitive dual ISH combined with CD30 immunohistochemistry (IHC) to distinguish tumor cells from cells of the tumor microenvironment (TME). IHC evaluated proteins expression for LMP1, CD4, CD8, CD56, CD68, CD163, PD-1, PD-L1 (TME and tumor cell), IL-10, FOXP3, Granzyme B (GrB), TGF-β, and T-bet. Event-free survival was analyzed using the Kaplan-Meier method.

Among the 32 EBERS+ cases, 32 expressed LMP1 transcripts in both tumor cells (LMP1t+) and the TME (LMP1m+), none expressed EBNA2 transcripts in tumor cells (EBNA2t-), and 23 expressed EBNA2 transcripts in the microenvironment (EBNA2m+). In the 16 EBERs-, 10 cases were classified LMP1t+, 11 cases LMP1m+ and 6 EBNA2m+. Remarkably, EBNA2 transcripts were exclusively observed in the TME. 5 cases did not express any of the analyzed transcripts.

PD-L1 expression in the TME was significantly higher in cases expressing EBNA2 transcripts compared to those without EBNA2 expression ($p=0.0373$, MW test). Similarly, GrB expression was significantly increased in EBNA2m+ cases ($p=0.0012$, MW test). The count of IL10+ cells in the groups defined by EBNA2 transcript expression showed a trend ($p=0.0768$, MW test). Only cases expressing traces of LMP1 in both tumor cells and the TME exhibited a predominant M1 profile (CD68+cells/CD163+ cells> 1.5) ($p=0.0339$, $p=0.0096$, Fisher´s exact test). No significant differences in event-free survival were observed according to viral transcripts expression ($p>0.05$).

EBNA2 expression in the TME, even in EBV- cases, might be associated with immune checkpoint regulation and cytotoxic responses, highlighting a previously underrecognized role of EBV and its viral traces in shaping the cHL microenvironment. Additionally, LMP1 expression in tumor cells and the TME could contribute to the M1-polarized profile previously described in pediatric EBV-associated cHL.

Immunogenetic Determinants of Viral Persistence in Breast Tumorigenesis: An In Silico HLA Binding Affinity Analysis

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Accumulating evidence implicates multiple oncogenic and oncomodulatory viruses in breast tumor development, including human herpesvirus 4 (HHV-4), human herpesvirus 5 (HHV-5), human papillomavirus (HPV), JC polyomavirus (JCV), human endogenous retrovirus K (HERV-K), bovine leukemia virus (BLV), and mouse mammary tumor virus (MMTV). However, the immunogenetic mechanisms governing host susceptibility to virus-associated breast tumors remain insufficiently understood. Given the central role of human leukocyte antigen (HLA) molecules in antigen presentation and viral clearance, we hypothesized that viral contribution to breast tumorigenesis is modulated by host HLA-dependent immune recognition efficiency. To test this hypothesis, we conducted a comprehensive in silico analysis of predicted binding affinities (PBAs) between viral proteins from the seven candidate viruses and 127 prevalent HLA alleles, including 69 Class I (HLA-I) and 58 Class II (HLA-II) molecules. These PBAs were systematically compared with established breast tumor-associated HLA (BC-HLA) immunogenetic profiles. Hierarchical clustering analysis revealed a distinct convergence pattern: for HLA-I, BLV, JCV, and MMTV clustered with tumor-associated HLA alleles, whereas for HLA-II, BLV, HERV-K, HPV, JCV, and MMTV demonstrated similar clustering behavior. Notably, across both HLA classes, viruses grouped within the tumor-associated HLA cluster exhibited significantly lower average PBAs compared to non-associated viruses. As reduced binding affinity is indicative of suboptimal antigen presentation, these findings suggest impaired immune recognition and delayed viral clearance. Such inefficiency may promote prolonged viral persistence, chronic immune dysregulation, and an increased likelihood of tumor initiation and progression. Collectively, our findings provide compelling immunogenetic evidence supporting a model in which inefficient HLA-mediated viral elimination contributes to breast tumor susceptibility. This study underscores the critical interplay between host HLA diversity and virus-driven tumorigenesis and highlights the potential of immunogenetic profiling in identifying individuals at elevated risk for virus-associated breast tumors.

Immune-mediated viral clearance and cellular mechanisms of immune memory in MmuPV1 infection

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Different inbred mouse strains exhibit divergent outcomes upon mouse papillomavirus (MmuPV1) infection. The FVB/N mouse strain is permissive for persistent MmuPV1 infections, and their H2q Major Histocompatibility Complex (MHC) haplotype is deficient in eliciting strong cytotoxic T lymphocyte (CTL) responses against several proteins encoded by this virus. In contrast, C57BL/6 mice are resistant to persistent MmuPV1 infections, and their H2b MHC supports robust CTL responses against the viral E6 protein. To test the hypothesis that these differences in susceptibility are mediated by MHC variation, we utilized congenic FVB.H2b mice carrying the H2b MHC allele from C57BL/6 strain. FVB.H2b and wild-type (WT) FVB mice (carrying the H2q allele) were infected at cutaneous sites (ears and tails) and monitored longitudinally for disease progression. While WT FVB mice developed persistent papillomas, FVB.H2b mice, while initially showing evidence of lesions arising, exhibited spontaneous lesion regression. This finding supports the hypothesis that MHC haplotype is a key determinant of viral clearance. We then performed T cell depletion in FVB.H2b mice to determine if this is a T cell-mediated response. Selective depletion of either CD4+ or CD8+ T cells alone was not sufficient to impair viral clearance; however, depletion of all T cells using anti-CD3 antibodies abrogated lesion regression, indicating that coordinated T cell responses are required for effective viral control in this congenic strain.

Despite the different primary infection outcomes, both FVB.H2b and WT FVB were resistant to rechallenge at different sites, suggesting that protective immunity is established regardless of MHC haplotype. This dissociation highlights distinct mechanisms governing effector and memory responses.

Together, these findings identify MHC variation as a critical regulator of papillomavirus clearance. Consistent with this concept, specific MHC alleles in humans are associated with increased risk of HPV infections, while others appear to have more protective effects. The establishment of protective memory across genotypes further suggests that MHC-independent pathways contribute to long-term immunity. Future studies will define the mechanisms underlying these distinct axes of antiviral responses.

Disruption of Host Innate Immune Response by HAdV-C5 via Human Ubiquitin E3 Ligase UBR4

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Human Ubiquitin Protein Ligase E3 Component N-recognin 4 (UBR4) is best known for its role in protein degradation via the ubiquitin proteasome. However, our lab amongst others, have identified UBR4 as a cellular hub protein. Specifically, our lab is investigating how human Adenovirus C5 (HAdV-C5) utilizes UBR4 during infections. HAdV expresses Early Region 1A and 1B (E1A and E1B) proteins that are required for a productive infection and deregulate host cellular interaction networks via targeting of hub proteins. Through these interactions, E1A and E1B proteins disrupt various aspects of the human cell. We have identified UBR4 as a novel interaction partner of E1A and E1B proteins and aim to elucidate the consequences of these interactions. Recently, we have discovered that under conditions of UBR4 knockdown, levels of interferon-stimulated genes (ISGs) are altered in infected cells. This difference is seen with interferon treatment during various stages of infection suggesting that UBR4 may function in the regulation of ISG expression. Moreover, when UBR4 is knocked down, histone H3 occupancy increases at ISG promoters in uninfected cells but decreases in infected cells. Consequently, we found that viral growth and viral genome copies decrease when UBR4 is knocked down in the cell. Our results identify UBR4 as a previously unknown player in chromatin organization and transcriptional regulation of ISGs in response to HAdV infection. Moreover, our studies provide insight in how HAdV disrupts UBR4 during viral infection in addition to unravelling the role of this protein in the cell.

The Balance Between Immune Surveillance and Viral Immune Evasion Drives the Persistence of Distinct HPV Lifecycle States

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Cervical HPV infections are considered immune controlled when HPV DNA or RNA is detected in tissue without visible disease. Shortly after infection, prior to immune control, lesions are typically productive. Persistent productive infections, particularly those involving multiple HPV types, are associated with a poor immune response.

We examined a large collection of samples from women with varying degrees of immune suppression due to HIV infection. Poor immune control was associated with three broad HPV lifecycle patterns: (1) productive LSIL, (2) non-productive LSIL, and (3) HSIL of increasing severity. These states frequently coexisted within individuals and were associated with disease recurrence after treatment. Improved immune responses appeared to suppress productive LSIL, leaving non-productive LSIL and HSIL. With repeated intervention and immune stimulation, disease becomes more spatially restricted, often presenting as small HSIL foci with high viral gene expression localised to cervical crypt entrances.

To characterise the molecular pathways driving immune control of the late stages of the viral lifecycle, we assessed immune cell infiltration alongside markers of CD8+ T cell function in situ, including IL-2, TNF-α, IFN-γ, and the transcription factor IRF-1, a key mediator of interferon-driven antiviral responses. Interestingly, stromal papillae served as conduits for immune infiltration, facilitating CD8 T cell access to the superficial cervical epithelium. In these layers, we also observed a mutually exclusive distribution between CD8 T-cell localization and E4 protein expression. These observations indicate that late stages of the viral lifecycle may occur in regions with limited CD8+ T cell access after epithelial differentiation. Distinct spatial patterns of IRF-1 expression were also observed, with strong nuclear signal in superficial epithelial layers and weaker expression in basal, p16-positive areas. This distribution suggests a spatially restricted immune response, reflecting a balance between immune surveillance and viral immune evasion. HPV-mediated disruption of IRF-1 signalling, potentially via E6 and E7, may contribute to reduced signalling in the basal layer.

Together, these findings support a model in which progressive immune failure enables viral persistence within epithelial niches. High viral gene expression and immune evasion mechanisms may limit immune infiltration and clearance. These data highlight a hierarchy of immune dysfunction and suggest that immune modulation may be most effective at the LSIL stage, with viral immune evasion proteins representing promising therapeutic targets.

Elucidation of a B cell specific inflammatory pathway that resembles the response to EBV infection

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Dysregulated inflammatory signalling is implicated in numerous diseases including viral infection and many cancers. Consequently, altering the inflammatory response is a promising target for emerging treatments. However, inflammatory pathways are not completely understood, and their therapeutic utility is consequently limited.

Work from our lab has revealed an inflammatory signalling pathway in B lymphocytes causing cell death. We have found misexpression of multiple classes of interspersed repeat sequences under pharmaceutical inhibition of EZH2 by GSK343 in resting murine B cell splenocytes. Furthermore, EZH2-inhibition induced inflammation fails to occur in a mouse model lacking functional cytosolic pattern recognition receptors (PRRs). This indicates B cell reliance on PRR recognition of repeat RNA element structures for inflammatory signaling. Notably, gene expression patterns induced by EZH2 inhibition resembled host cells infected by Epstein-Barr Virus. This represents a unique form of viral mimicry that we aim to decipher.

RNA-seq analysis revealed upregulation of pro-inflammatory factors related to monocyte and T cell activation in GSK343-treated B cells, but a notable absence of interferons. This suggests a novel inflammatory mechanism. Additionally, an IKKb conditional knockout mouse model has shown that secreted cytokines like CCL5 and CCL2 that are induced by EZH2 inhibition and blocked by PRR knockouts are dependent on NFkB. While this suggests a role for NFkB, other cytokines of interest such as CCL3 display no such dependence. A cytokine array experiment confirmed upregulation of many of the same cytokines and chemokines in response to GSK343 as our RNA-seq experiment and additionally identified IL-15, IL-16, and TNF as dependent on PRRs and GSK343 treatment for secretion. In summary, we found that misexpression of interspersed repeat elements, induced by dysregulation of heterochromatin via inhibition of EZH2, triggers PRRs and immune-mediated cell killing. We are employing CRISPR knockouts in genes of interest prior to transplantation into mice to assess their requirement in this pathway under EZH2 inhibition. Finally, this CRISPR approach will enable a screen of inflammatory genes to more thoroughly characterize this pathway.

Elucidation of this signalling mechanism will inform potential use of already approved EZH2 inhibitors for novel inflammatory targets in B cell malignancies and other diseases.

Modulation of Intrinsic Immunity by Merkel Cell Polyomavirus in Skin Organoids

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Merkel cell polyomavirus (MCPyV) is a human tumour virus responsible for most cases of Merkel cell carcinoma, an aggressive skin cancer predominantly in elderly and immunosuppressed individuals. In tumors, the viral genome is monoclonally integrated into the host genome ensuring expression of viral oncoproteins small T antigen (sT) and truncated large T antigen (tLT), while expression of the viral tumor suppressor ALTO (alternate open reading frame for LT) is suppressed. As with many chronic human viruses, physiologically relevant infection models are limited.

We recently established a productive MCPyV infection model using induced pluripotent stem cell-derived skin organoids (SkOs), enabling the investigation of viral immunomodulation in a context that closely mimics natural infection. Previous studies identified sT and ALTO as key regulators of host immune responses with sT suppressing type I interferon signalling and counteracting ALTO-mediated immune activation.

Building on these findings, we sought to validate and extend observations from overexpression systems using the SkO infection model to better understand MCPyV immune evasion and persistence. Therefore, SkOs were infected with MCPyV mutants harbouring sT alterations that impair innate immune interference or ALTO mutations that abolish NF-κB activation. Long-term infection experiments (up to 90 d) were conducted, including partial treatment with a JAK/STAT inhibitor previously shown to enhance MCPyV infection.

Comprehensive analysis included qPCR, whole-mount immunofluorescence, immunohistochemistry, DNA- and RNA-scope, and transcriptomic profiling, complemented by luciferase assays and immunoprecipitation in 2D infection models.

Long-term SkO infections revealed that mutations in the PP4C/NEMO binding region of sT restrict MCPyV infection and progeny production while still permitting viral genome persistence. In contrast, mutations in the ALTO SH2 domain that impair phosphorylation and thus NF-κB activation function result in enhanced MCPyV replication.

MCPyV persistence and replication rely on a finely balanced regulation of host signaling pathways. The interplay between sT-mediated suppression of interferon signaling and ALTO-driven activation of NF-κB is critical for maintaining viral persistence and progeny production.

EBV inhibits antiviral immunity through the generation of MDSCs in systemic CAEBV disease and EBV-associated HLH

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Systemic CAEBV disease is characterised by persistent, severe IM-like symptoms for >6 months, including fever and lymphadenopathy with hepatosplenomegaly, liver dysfunction, persistent high EBV DNA load of at least 10,000 IU/mL, and expanded populations of EBV infected T cells and/or NK cells. CAEBV disease is either indolent, persisting for years but always developing into aggressive T/NK malignancies, or it is highly aggressive often presenting as EBV-associated HLH, a clinical emergency requiring rapid diagnosis and immediate appropriate treatment. CAEBV disease is not associated with a genetic immune defect, yet large expansions of EBV-infected T/NK cells are evident despite the presence of apparently functional antiviral CD8+ cytotoxic T cells (CTLs).

This impaired viral clearance is far from understood, but one potential mechanism is the large expansion of polymorphonuclear myeloid derived suppressor cells (PMN-MDSC) observed in the blood of every patient, driving the generation of an immune suppressed microenvironment. However, the drivers of PMN-MDSC generation, mechanism of immune suppression and functional outcomes in CAEBV disease remain unknown.

Here we show EBV-infected T- and NK cells release high concentrations of cytokines and chemokines that function to drive the PMN-MDSC phenotype from neutrophils and prolong their survival. These PMN-MDSCs inhibit the growth of CD4+ and CD8+ T cells and NK cells by secretion of Arginase-1, and inhibit the function of antiviral CTLs by nitration of the TCRb and CD8 resulting in blockade of antigen-specific activation of the CTLs. Importantly, we show the EBV-infected T- and NK cells actually grow in an arginine-depleted microenvironment, by upregulating genes involved in glycolysis and gluconeogenesis, and these metabolic changes are driven by the EBV-encoded latent membrane 1 (LMP1).

Collectively, these data reveal how EBV can induce PMN-MDSC to generate an immune-suppressive microenvironment enabling viral persistence in T/NK cells, as well as explaining the progressive immune suppression observed in CAEBV disease over time and why CAEBV disease is refractory to immunotherapy, including checkpoint inhibition. Indeed these mechanisms can be interpreted as a novel immune evasion tactic by the virus.

Breaking the balance: Human papillomavirus E7 interrupts IL-18 inflammatory signaling in head and neck cancers

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The incidence of human papillomavirus-positive (HPV+) head and neck squamous cell carcinoma (HNSCC) is increasing at epidemic rates. HPV+ HNSCCs retain expression of the E6 and E7 viral oncoproteins, which are critical for tumour growth, survival, and avoidance of the immune system. To aid in viral immune evasion, E7 can disrupt the methylation of several host gene promoters that interfere with HPV infection. One potential target of E7 is IL-18, a pro-inflammatory cytokine with a critically important role in epithelial barrier function. IL-18 activity is negatively regulated by its antagonist, IL-18 binding protein (IL-18BP). Our analysis of RNA-seq data from The Cancer Genome Atlas program showed increased IL18BP and reduced IL18 mRNA levels in HPV+ HNSCC compared to HPV- HNSCC and normal adjacent tissues. Examination of a single-cell RNA-seq dataset shows this reduction in IL18 mRNA specifically in epithelial clusters of the oral mucosa. These findings suggest a concerted antagonism of the IL-18 pathway in HPV+ HNSCC patients by breaking the balance of IL-18 inflammatory signaling and its negative regulation by IL-18BP. From these observations, we hypothesize that expression of the viral E7 oncoprotein in HPV+ HNSCC suppresses the IL-18 pathway, assisting in evasion of anticancer immune responses.

Within The Cancer Genome Atlas cohort, we identified higher levels of methylation at CpG sites in the IL18 promoter in HPV+ HNSCC patients, indicating a possible mechanism for transcriptional repression of IL-18 in HPV+ HNSCC. Transient transfection of E7 in HPV- HNSCC cell lines demonstrated that E7 expression alone can repress IL18 transcription and secreted IL-18 protein levels. With our extensive panel of HPV16 E7 mutants, we identified that conserved region 3 (CR3) of HPV16 E7 is crucial for IL-18 pathway repression and highlighted specific surface-exposed amino acid residues within CR3 that appear necessary for this repression. Although highly relevant for the persistence of HPV infection, IL-18 suppression is also likely critical for suppression of anti-tumour immunity in HNSCC; thereby preventing immune-mediated detection and clearance of virally-transformed cells. The identification and understanding of IL-18 suppression by HPV provides the opportunity to advise future therapies designed to restore IL-18 function in HPV+ HNSCC.

Characterization of the immune microenvironment at the sites of cervical HIV/ HPV co-infection

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Women living with HIV (WLWH) have an approximately threefold higher risk of human papillomavirus (HPV) infection compared with HIV-negative women and experience higher rates of cervical lesions with accelerated progression to severe cervical disease, even under antiretroviral therapy (ART) with preserved peripheral CD4 counts. Although localization of HIV-1 in the cervix of WLWH has been reported already, the specific cervical cell types harboring HIV and the mechanisms by which local HIV infection promotes immune evasion remain poorly understood.

We utilized an extensive collection of cervical biopsy specimens from HPV-positive patients with or without HIV-1 infection who were treated for high-grade intraepithelial lesions (HSILs) in South Africa, Kenya, and Poland and Barcelona. Local HIV-1 was detected by in situ RNAscope hybridization and immunofluorescence of HIV structural protein p24. Additional staining for cell surface immune markers, including CD4, CD8, CD20, PD-1, and CD68, was performed to map immune cell subtypes and characterize local immune microstructures.

High-resolution imaging identified active HIV-1 RNA transcription and viral particle production compartmentalized within secondary lymphoid follicle-like structures adjacent to cervical crypt entrances, a common site of local immune evasion. HIV-1 signals were predominantly concentrated within CD4+ regions, implicating cervical follicles as active viral reservoirs. Quantitative image analysis demonstrated notable variances in local CD4+ and CD8+ cell densities between HIV-positive and HIV-negative cohorts. Furthermore, a modest correlation was observed between local CD4+ cell density, disease extent indicated by p16 expression, and peripheral CD4+ T-cell counts.

Taken together, our study suggest local HIV-1 infection may alter immune cell distribution and recruitment at cervix, contributing to immune evasion and disease progression. The limited correlation between peripheral lymphocyte counts and local immune responses indicates that peripheral lymphocyte monitoring inadequately reflects localized cervical immune dynamics, highlighting the need for immune microenvironment assessments to inform future therapeutic and preventative strategies.

Assessing HPV type specific immune interactions influencing persistence in tonsil epithelia

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High-risk HPV infection within the oropharynx is responsible for around half of all oropharyngeal squamous cell carcinomas (OPSCC) worldwide, particularly in the palatine tonsils and base of tongue. The vast majority of HPV+OPSCC are caused by HPV16 whilst other oncogenic types, including HPV18, are rarely found. The tumour microenvironment (TME) of HPV+ OPSCC is characterised by an increased immune cell infiltration compared to HPV- OPSCC, which also correlates with better prognosis. We hypothesized that HPV subtype-specific immune cell modulation may be contributing factor in the differential contribution of high-risk types to oropharyngeal carcinogenesis in immune-rich tonsil epithelia.

To understand how HPV replication in tonsil epithelia manipulates innate immune signalling and keratinocyte-immune cell interactions, we established HPV16 and HPV18 replication in primary tonsil keratinocytes (HTK) harvested from tonsillectomies. RNA sequencing revealed differential steady-state expression of cytokines (CCL2 and CCL5) in HPV16-HTK compared to HPV18-HTK and HTK control. Stimulation with interferons did not rescue this phenotype. To determine whether HPV16 and HPV18 differentially manipulate the activation and migration of specific immune cell subtypes we established Transwell migration assays with cryopreserved donor-matched tonsil mononuclear cell (MNCs). While HPV-negative HTKs induced only low levels of MNC migration in the absence of IFNg stimulation, HPV16 replication in the same donor increased MNC cell migration and this was even further increased with HPV18 replication. Stimulation of the cells with IFNg increased migration in all experimental conditions. Migration after IFNg stimulation was again lower in HTK compared to HPV16-HTK, suggesting that HPV16 replication results in increased immune cell infiltration. Notably, HPV18 replication significantly increased MNC migration following IFNg stimulation in comparison to HPV16, suggesting that HPV16 and HPV18 differentially alter host keratinocyte innate immune signalling.

These results suggest that both HPV16 and HPV18 replication in tonsil epithelia results in increased immune cell activation and migration towards the infected keratinocyte, as observed in HPV+ OPSCC. However, the dramatically increased migration of MNCs towards HPV18-HTK compared to HPV16-HTK may significantly impact the ability of HPV18 to persist in immune-rich tonsil epithelia rendering this oncogenic HPV type less likely to drive carcinogenesis at this body site.

Viral genome state and host immunity in HPV-driven cervical cancer: insights from a longitudinal cohort in India

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Persistent infection with high-risk human papillomaviruses (HPV) is the main cause of cervical cancer (CC). The disease progresses from an asymptomatic infection to cervical intraepithelial neoplasia (CIN) and to invasive cancer, often over decades. A key molecular event in this process is the integration of the HPV genome into the host genome and the dysregulated expression of oncogenes E6 and E7. Although researchers have mapped integration events and distinguished them from episomal viral persistence, the factors controlling this transition, especially the role of the host immune system, remain poorly understood.

CC is the second deadliest cancer in Indian women, making up almost one-fifth of cases worldwide, with more than 123,907 new cases and about 77,348 deaths annually. Even though effective vaccines exist, coverage is limited, and screening is inadequate. This situation provides a valuable opportunity to study how HPV infection develops and progresses over time in well-defined groups of people.

In this study, we follow a group of high-risk HPV-positive women in India for over two years. The group includes women with asymptomatic infection, CIN, and invasive CC. Further, we use a multi-omics approach to combine viral genomics and immune profiling, by using long-read whole genome sequencing to determine episomal or integrated states of the HPV genome. We also use RNA sequencing for transcriptomic profiles of host's PBMCs to understand its association with viral persistence and disease progression.

This study is part of a larger consortium that examines how the immune system responds to HPV, including B-cell and T-cell responses, as well as systemic and mucosal antibody responses. By linking the state of the viral genome with immune markers, we aim to determine the relationship between immune patterns and HPV integration and disease progression. We are also expanding our analysis to include tumour-associated lymphocytes to better understand the local immune environment at different stages of disease.

Overall, this systems-level approach provides a way to connect the viral genome state with the host's immunological landscape. Our results may help reveal immune correlates of HPV persistence and clearance, guiding new immune-based strategies for preventing and treating cervical cancer in high-risk regions.

HPV E6/E7 promotes TBX3 oncogenic activity through CDK5-mediated phosphorylation

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Cervical cancer is a leading cause of cancer-related deaths in women worldwide and is driven by persistent infection with high-risk human papillomavirus (HPV) strains. While the HPV oncoproteins E6 and E7 establish and maintain the malignant phenotype, their oncogenic functions depend on cooperation with host proteins. We have shown that the T-box transcription factor 3 (TBX3) is overexpressed in HPV-positive cervical cancer patient tissues and cell lines where it interacts and co-operates with E6/E7 to promote cell proliferation and migration. We hypothesised that E6/E7 may also regulate the cofactors that TBX3 interact with to promote these oncogenic phenotypes. To test this, affinity purification followed by mass spectrometry was used to analyse the TBX3-associated proteome in the presence and absence of E6/E7. Curiously, E6/E7 were found to reduce the binding of TBX3 to protein phosphatase 2A (PP2A) and Kaplan-Meier analyses demonstrated that high PP2A levels correlate with improved cervical cancer patient survival. Furthermore, using knockdown and overexpression cell culture models we show that PP2A inhibits TBX3 stability and cervical cancer cell proliferation and migration. This data show for the first time that PP2A has tumour suppressor activity in cervical cancer. Importantly, PP2A dephosphorylated TBX3 within its activation domain at serine 438 (S438) and using site-directed mutagenesis we show that phosphorylation at S438 is essential for TBX3's oncogenic activity. We therefore used in silico kinase prediction analysis to identify the kinase involved and the only candidate identified was cyclin-dependent kinase 5 (CDK5). Using an in vitro kinase assay, we confirm that CDK5 phosphorylates TBX3 within its activation domain. Furthermore, CDK5 knockdown decreases TBX3 protein levels without affecting its mRNA expression and significantly inhibits cervical cancer cell proliferation and migration. Together, these findings identify a novel E6/E7-regulated PP2A/CDK5 phospho-regulatory axis that controls TBX3 phosphorylation status and oncogenic function in cervical cancer. Importantly, E6/E7 shifts the dynamic balance toward sustained TBX3 phosphorylation by CDK5 promoting cervical cancer progression.

Understanding oncogene-induced replication stress in tumorigenesis using HPV disease model

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Genomic instability consisting of point mutations, chromosomal structural variations and DNA copy number alterations (CNA) is a hallmark of cancer. Whether genomic instability is a cause or consequence of cancer remains unknown. One proposed mechanism of cancer initiation is oncogene-induced replication stress (Oi-RS). We used oncogenic human papillomavirus type 18 infected human foreskin keratinocytes (HPV18+ HFK) passaged both short (low passage) and long (high passage) term as a longitudinal mechanistic model to study Oi-RS. Persistence of HPV18 genomes in HFKs induces genomic instability via constitutive inactivation of tumour suppressor genes p53 and Rb by viral proteins E6 and E7 respectively. Hence our model excellently recapitulates the complexity of cancer initiation while remaining physiological and disease relevant.

Using DNA fibre assay, we have observed that HPV18+ HFKs displayed accelerated fork stalling and fork progression with oscillating fork speeds from low to high passage cells. Interestingly, the oscillation in fork speeds correlated with viral E6 protein levels in HPV18+ HFKs. RNA sequencing analysis showed an increase in E2F and Myc targets in high passage compared to low passage HPV18+ HFKs. HPV-induced fork acceleration is found to be more dependent on the repriming DNA polymerase PRIMPOL than on the translesion synthesis polymerase REV1. Moreover, low p53 levels correlated with PRIMPOL-dependent high fork speeds and high p53 levels with low fork speeds in HPV18+ HFKs. In addition, overexpression of p53 in HPV18+ HFKs showed a reduction in fork speed. DNA damage response foci such as γH2AX, 53BP1 and RAD51 were increased in HPV18+ HFKs in immunofluorescence. Bulk and single cell genome sequencing revealed that aneuploidies and CNAs emerge over time in HPV18+ HFKs with some emergent CNAs like HPV-positive cancer genomes. Western blot analysis showed increased Vimentin levels and Phalloidin immunofluorescence detected finer and shorter F-actin structures in HPV18+ HFKs. High passage HPV18+ HFKs formed tumours in chick embryo xenograft model.

In summary, we have showed that HPV-induced Oi-RS and chromosomal instability promote clonal selection and display cancer-like rates of genomic instability in high passage HPV18+ HFKs leading to transformation.

Host genetic landscape of HPV E6/E7-driven immortalization and transformation

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High-risk human papillomaviruses (HPVs), such as HPV16 and HPV18, are responsible for ~5% of human cancer cases, particularly as a driver of cervical and oropharyngeal cancers. Vaccination could prevent most high-risk HPV infections; however, vaccine uptake rates remain low in many parts of the world, and HPV vaccination cannot treat precancerous lesions or cancers. Additionally, there are currently no mechanism-based therapies for HPV-associated precancerous lesions or cancers, presenting a public health need.

The essential drivers of HPV-mediated carcinogenesis are the HPV E6 and E7 oncoproteins, which are necessary and sufficient to immortalize human epithelial cells. While several host genes targeted by the HPV16 E6 and E7 proteins have been identified that contribute to immortalization, there is a current lack of functional genomics data exploring the full host genome. The goal of this project is to perform a genome-wide study to generate these functional genomics data and identify cellular genes that promote or suppress the immortalization and transformation of epithelial cells driven by HPV16 E6 and E7 oncogenes.

Here we used the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology that inhibits (CRISPRi) or activates (CRISPRa) one gene per cell, in primary human foreskin keratinocytes (HFKs) from two healthy donors. Genome-wide screening of both CRISPRi and CRISPRa guide RNA libraries in HPV16 E6- and E7-coexpressing HFKs was performed to identify genes that enhance or inhibit the viability/growth of these cells. Furthermore, we used an anoikis-resistance assay to identify genes that impact the survival of the HPV16 E6 and E7 co-expressing keratinocytes in the absence of surface adhesion. Hits from these screens are currently being validated and assessed as the potential targets of small molecule inhibitors against early-stage HPV-positive cancers. Our long-term goal is to develop novel small molecule therapeutics for HPV-positive cancers by integrating the screening results with other orthogonal omics studies under the AI-Enabled Cures Frontier program in Gates Foundation Accelerator.

mEEP, a novel syngeneic model of HPV+ oropharyngeal carcinoma

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Human papillomavirus (HPV)-associated oropharyngeal cancer (OPC) represents a growing public health burden. Although most patients achieve durable remission with standard chemoradiotherapy, treatment-related morbidities significantly compromise quality of life. Current models are insufficient to guide de-escalation strategies for favorable-risk patients or to inform curative approaches for those with recurrence-prone disease. The efficacy of immune checkpoint blockade (ICB) in OPC remains limited, and targeted therapies are emerging as promising combination partners — yet efforts to advance these agents clinically are hampered by a critical shortage of immunocompetent murine models for HPV-positive OPC.

HPV^+ OPCs frequently harbor APOBEC-associated oncogenic mutations, most notably PIK3CA hotspot mutations. To model how such alterations shape early malignant evolution in the context of HPV oncogene expression, we generated HPV16 E6/E7–immortalized murine tongue epithelial cells expressing PIK3CAE545K (mEEP) — a recurrent APOBEC3-associated driver mutation in HPV^+ OPC. Although non-transformed in vitro, mEEP cells acquire malignant properties following in vivo passage and can be propagated as stable tumor-adapted derivatives. mEEP-derived tumors recapitulate key features of human HPV^+ OPC, including poorly differentiated squamous cell carcinoma morphology, transcriptomic similarity to human tumor profiles, and enrichment of WNT-driven stem cell transcriptional signatures. We are currently characterizing the mEEP tumor microenvironment and leveraging this model to dissect mechanisms of immune evasion and therapeutic response. mEEP represents a tractable, genetically defined syngeneic platform to accelerate preclinical investigation of immunotherapy combinations in HPV^+ OPC.

HPV16 E7 Reprograms Glutamine Metabolism to Promote Metabolic Plasticity in HPV-Associated Cancers

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Human papillomavirus (HPV) oncogenes, similar to other oncogenic viruses, are implicated in reprogramming host cellular metabolism to support malignant transformation and tumor progression, however, the specific metabolic pathways regulated by HPV remain incompletely defined. Analysis of The Cancer Genome Atlas (TCGA) datasets revealed upregulation of glutamine synthetase (GS; encoded by GLUL) in cervical cancers compared with normal cervical tissue. GLUL expression was also elevated in HPV-positive relative to HPV-negative HNSCC tumors, suggesting that HPV oncogenes promote metabolic adaptations enabling glutamine synthesis. Since glutamine metabolism plays a role in cancer cell growth and survival, we investigated whether HPV oncogenes regulate glutamine utilization.

To determine whether HPV16 E6 and E7 oncogenes drive this phenotype, we ectopically expressed each individually or in combination in HPV-negative cell lines. Tumor sphere, flow cytometry, and clonogenic assays showed that E7 expression alone was sufficient to promote partial glutamine independence in HPV-negative cervical cancer cells, whereas E6 had no comparable effect. Co-expression of E6 and E7 did not enhance this phenotype beyond E7 alone. Consistent results were observed in HPV-negative head and neck squamous cell carcinoma cells, where E7 was the primary driver of enhanced survival under glutamine-limiting conditions. These findings identify HPV16 E7 as the principal driver of partial glutamine independence.

To define how HPV16 E7 regulates glutamine metabolism, we examined glutamine utilization under varying nutrient conditions. Under glutamine deprivation, E7 reduced dependence on extracellular glutamine and induced GS expression, enabling de novo glutamine synthesis and promoting survival under nutrient stress. This phenotype was reversed by the GS inhibitor methionine sulfoximine (MSO), indicating functional dependence on GS activity.

Under glutamine-replete conditions, HPV16 E7 increased expression of glutamine transporters SLC1A5 (ASCT2) and SLC7A5 (LAT1), while repressing GLS and GLUL, suggesting a shift toward glutamine exchange rather than catabolism. Metabolic assays confirmed increased extracellular glutamine and reduced glutamate secretion. E7 enhanced phosphorylation of the mTORC1 downstream target S6 following amino acid refeeding, indicating increased nutrient signaling.

Together, these findings suggest that HPV16 E7 coordinates glutamine uptake and synthesis to promote metabolic plasticity, enabling tumor growth across fluctuating nutrient environments and identifying glutamine metabolism as a potential therapeutic vulnerability.

Lipid reprogramming of stratified squamous epithelium by the high-risk HPV E6 and E6/E7 oncoproteins

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Genome integration of high-risk human papillomavirus (HPV) and subsequent expression of the viral E6/E7 oncogenes is a major cause of cervical cancer. Emerging evidence suggests that HPV reprograms the host metabolism to support viral persistence and cellular transformation. However, global lipidomic reprogramming induced by HPV oncogenes remains poorly understood, particularly at early stages of HPV-induced transformation. We sought to define the regulation of lipid metabolism in stratified squamous epithelia of transgenic mice expressing the HPV16 E6 oncogene alone or in combination with E7.

Untargeted lipidomics was used to identify novel lipid biomarkers in the skin and female reproductive tract (FRT) of HPV16 E6 and E6/E7 transgenic mice compared to wild-type (WT) mice. To investigate enzymatic dysregulation of lipids by HPV oncogene expression, we employed Lipid Network Explorer (LINEX2), which analyzes lipidomics data through lipid enrichment analysis. We also used the Global Natural Product Social Molecular Networking (GNPS) platform to enhance lipid identification, exploring molecular networking to improve feature annotation. Our lipidomic analysis produced several new observations. First, E6 expression caused a consistent alteration of glycerophospholipids, with particularly significant substrate-product shifts in the phosphatidylcholine (PC) to lysophosphatidylcholine (LPC) pathway in the skin. Second, E6/E7 expression dysregulated glucosylceramide (GlcCer) biosynthesis. Third, both E6/E7 expressing skin and FRT tissues exhibited a redox imbalance and increased levels of oxidized lipids, including oxylipins and several oxidized PCs. These findings suggest that HPV oncoproteins drive lipid reprogramming, potentially contributing to early HPV-related tumorigenesis.

These findings provide new insights into HPV-induced lipid reprogramming and establish a framework for future studies examining the functional and clinical relevance of lipid alterations in HPV-associated cancers.

APOBEC mutagenesis in cancers associated with DNA tumor viruses

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Human papillomaviruses (HPV) and polyomaviruses (PyV) are strongly associated with cancers of the cervix, head/neck, bladder, and other tumor types. Another characteristic of these types of cancer is high levels of APOBEC3 signature mutations, which are conservatively defined as C-to-T and C-to-G single base substitution mutations in TCA and TCT trinucleotide motifs. Ironically, the APOBEC3 family of single-stranded DNA cytosine deaminases functions to prevent viral infections and, apparently, is not able to overtly block either HPV or PyV infection. Moreover, HPV and PyV utilize viral oncoproteins and perhaps additional mechanisms to trigger APOBEC3 upregulation at the transcriptional level, which still fails to restrict virus infection but explains, at least in part, the higher levels of APOBEC3 signature mutations evident in virus-associated tumors. Interestingly, both HPV and PyV are depleted of APOBEC3-preferred TCA and TCT trinucleotide motifs, implying that APOBEC3 enzymes may contribute to virus diversification over an evolutionary time scale. These observations combine to imply that HPV and PyV employ an as-yet-uncharacterized APOBEC3 counteraction mechanism that is robust but not completely resistant to APOBEC3 deamination activity. This talk will discuss this APOBEC/DNA tumor virus paradox and include recent insights from cellular and murine models.

Non-CpG and CpG methylation in Merkel cell carcinoma

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DNA methylation is a major epigenetic mechanism regulating gene expression occurring predominantly at CpG motifs and associated with gene repression. It is also observed at non-CpG motifs primarily in stem cells and neurons, with variable effects on gene expression depending on the nucleotide context. Aberrant DNA methylation is a common feature of cancer and has been correlated with tumor development. Merkel cell carcinoma (MCC), an aggressive neuroendocrine skin tumor, can be driven by Merkel cell polyomavirus (MCPyV) (MCCP) or UV exposure (MCCN). Through whole-genome bisulfite sequencing of nine patient-derived xenograft cell lines, we identified aberrant CpG methylation in MCC, which clustered MCC samples away from other tumor types and distinguished MCCN from MCCP as previously reported. We also identified a distinctive non-CpG methylation pattern shared between MCCP and MCCN and not observed in other cancers. The genes associated with these methyl cytosines are significantly enriched for neurological pathways. These methylated cytosines are predominantly located at CAG motifs in introns and are associated with higher gene expression, which is similar to the methylation patterns and effects within stem cells. Clustering by genes with these non-CpG marks also discretely separates MCC from other cancer cell types.

Compared to other cancers, RNA-seq analysis revealed higher expression of methylation machinery in MCC, including DNMT1 and DNMT3A. BCL11B, a transcription factor potentially involved in non-CpG methylation, is also highly expressed in MCC. Knockdown of DNMT1, DNMT3A, and BCL11B resulted in a decrease in non-CpG methylation by dot blot. Further om MCCP, non-CpG methylation appears to be partially driven by the MCPyV large T antigen, as knockdown of MCPyV large T antigen in the CVG cell line, an MCPyV-positive cell line, significantly reduced expression of DNMT1 and BCL11B.

In conclusion, both MCCN and MCCP harbor a distinct stem cell-like non-CpG methylation profile. This non-CpG methylation appears to promote the neuroendocrine features of MCC through enhanced expression of genes involved in neurological pathways, and is driven by DNMT1, DNMT3A, and BCL11B.

NEDD8-dependent Proteasomal Degradation of p53 by the Beta-HPV49 E6 Oncoprotein is Independent from E6AP, MAML1 and MDM2

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The oncogenic functions of beta HPV are incompletely understood. We recently showed that beta3-HPV49 gains immortalization competence upon inactivation of the viral E8^E2 repressor, which enables high level expression of the E6 and E7 oncogenes. Immortalization by hr-HPV types depends on E7 binding to the retinoblastoma protein, promoting cell cycle progression and subsequent replicative stress, which stabilizes the tumor suppressor p53. Therefore, E6 binds to p53 and induces its degradation via the ubiquitin-ligase E6AP.
All beta-HPV E6 bind to MAML1, a co-activator of the Notch pathway, while their involvement in the p53 pathway is diverse. Some previous studies have suggested that beta3-E6 binds to E6AP and p53 to induce its degradation, similar to hr-HPV, whereas others have reported downregulation of p53 target genes without direct binding of E6 to p53 or E6AP. So far, no model has emerged that robustly explains these conflicting findings.

A sensitive reporter assay reveals that HPV49 E6 induces p53 degradation only when untagged or tagged at the C-terminus with a single HA epitope. Consistent with this, co-immunoprecipitation confirms that C-terminally 1xHA-tagged HPV49 E6 interacts with MAML1, p53, but not with E6AP. Co-expression of HPV49 E6 and E7 immortalizes normal human keratinocytes (NHK). Knockdown of E6 in these cells stabilizes p53 protein, but not p53 mRNA, and activates p53 target genes. However, neither knockdown of E6AP nor MAML1 in these cells rescues the destabilization of p53 by HPV49 E6.
Proteasome inhibition with MG132 in HPV49 E6/E7-cells increases p53 to the same levels achieved by E6 knockdown, indicating a proteasome-dependent process. In contrast, treatment with Nutlin-3a, an MDM2 inhibitor, the primary E3 ubiquitin ligase controlling p53, does not restore p53 stability, excluding MDM2 involvement.
Treatment with the neddylation-inhibitor MLN4924 rescues p53 levels in HPV49 E6/E7 cells, but not in NHK, hinting at a NEDD8-dependent E3-ligase. Surprisingly, knockdown of cullin 1-9 and RBX1/2, the major NEDD8-dependent E3-ubiquitin ligase complexes, does not rescue p53 in HPV49 E6/E7-cells.

Together, these findings suggest that HPV49 E6 promotes proteasome-mediated degradation of p53 independent from E6AP, MDM2 and MAML1, but in a NEDD8-dependent manner, suggesting a novel mechanism.

Biochemical Activity of HPV16 E6 and E7 Variants Does Not Correlate with Cancer Association

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Human papillomavirus type 16 (HPV16) is the most prevalent high-risk HPV type and accounts for more than 50% of cervical carcinomas and oropharyngeal cancers. Despite its clinical importance, the interplay of viral and host factors that determine whether an HPV16 infection progresses to cancer remains incompletely understood. A major limitation for such studies has been the reliance of most molecular studies on the original HPV16 clone. Since its identification in 1983 multiple HPV16 lineages and sublineages with distinct geographic distributions have been described, and epidemiologic data suggest that different HPV15 variants may confer varying levels of cancer risk.

In the largest analysis to date, thousands of unique HPV16 genomes were identified in a cohort of 5,570 HPV16-positive cervical specimens collected from women with high-grade premalignant lesions, cervical cancer, or no detectable cervical abnormalities (Mirabello et al. Cell, 2017; PMID: 28886384). Notably, some of these variants contained nonsynonymous mutations in the viral E6 and E7 oncogenes.

To determine whether E6 or E7 variant alleles associated with high-grade lesions or cancer exhibit greater oncogenic potential than those found predominantly in asymptomatic infections, we analyzed eleven E7 and eleven E6 variant alleles identified in both clinical contexts. We evaluated protein stability, interactions with established host targets, and, for E6 variants, effects on p53 steady-state levels. Several E7 variants, including some identified in high-grade lesions, showed impaired binding to UBR4 and/or PTPN14, suggesting partial loss of function. Similarly, multiple E6 variants detected in cervical lesions exhibited reduced stability or diminished interactions with p53, UBE3A, or PDZ domain–containing proteins.

Together, our findings suggest that HPV16 E6 and E7 variants present in cervical lesions can encode proteins with partial or reduced activity, arguing against a simple model in which increased intrinsic oncogene activity alone drives cancer risk. Instead, our results support a more complex framework in which viral genetic variation and host genetic background together may influence HPV16 carcinogenic potential.

YAP1/HMGCS1 signaling axis regulates Clamp Loader Rad17-dependent lipid droplet formation in cervical cancer

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Inactivation of the RB1 and PTPN14 cooperatively enables the carcinogenic activity of the human papillomavirus E7 oncoprotein

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High- and low-risk human papillomavirus (HPV) E7 proteins bind to several of the same cellular proteins but have different biological activities. E7 proteins from both high- and low-risk HPV genotypes bind directly to at least two tumor suppressors, RB1 and PTPN14. We previously characterized mutations in high-risk HPV E7 proteins that selectively disrupt the ability of E7 to bind either RB1 or PTPN14. We found that RB1 inactivation and PTPN14 degradation are separable activities of high-risk HPV E7 proteins and that neither mutant alone was sufficient to extend the lifespan of primary keratinocytes. We then used the mutants to establish a genetic complementation system, finding that the two mutants expressed together could, like wild-type high-risk HPV E7, extend keratinocyte lifespan.

Here we sought to determine whether and how different effects on RB1 and/or PTPN14 contribute to the different biological activity of high- versus low-risk HPV E7 proteins. High-risk HPV E7 proteins target both RB1 and PTPN14 for degradation whereas low-risk HPV E7 proteins reduce the steady-state levels of PTPN14, but not RB1. We found that in proliferative keratinocytes, high-risk HPV E7 proteins could induce the expression of E2F target genes such as CCNE2 and MCM2, but low-risk HPV E7 proteins could not. Both high- and low-risk E7 reduced PTPN14 protein levels and reduced expression of differentiation genes. We extended the complementation approach and found that experimental depletion of either RB1 or PTPN14 could cooperate with low-risk HPV6 E7 to extend the lifespan of primary keratinocytes. This result prompted the observation that PTPN14 depletion and RB1 inactivation by HPV E7 acted synergistically to induce certain cell cycle regulatory genes.

Our findings advance the model that inactivation of at least two tumor suppressors is required for the carcinogenic activity of high-risk HPV E7. Although RB1 and PTPN14 regulate distinct signaling pathways, their combined inactivation may also contribute to the biological activity of HPV E7.

HPV E6/E7 promote the interaction of TBX3 with its oncogenic partners, Hsc70 and nucleolin, in cervical cancer

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Cervical cancer remains the fourth most common cancer among women worldwide, with over 85% of cases and deaths occurring in low- and middle-income countries (LMICs). This is despite availability of effective prophylactic vaccines against the human papillomavirus (HPV), the main aetiological agent of cervical cancer. Furthermore, in LMICs, most women have poor access to vaccination and screening programs which results in patients presenting with advanced stage disease for which current treatments are ineffective. We therefore need to improve our understanding of the molecular mechanisms by which the HPV oncoproteins E6 and E7 cooperate with host proteins to drive cervical cancer progression. This will reveal important virus-host protein networks that can be exploited for targeted therapies. In this regard, we have previously shown that the T-box transcription factor 3 (TBX3) is overexpressed in HPV-positive cervical cancer and it promotes cervical cancer cell proliferation and migration through its interaction and cooperation with E6/E7. Given that cofactors determine the functional specificity of T-box transcription factors, we investigated whether E6/E7 enhance TBX3-driven oncogenicity by promoting its interaction with host proteins. To test this, affinity purification/mass spectrometry was used to analyse the TBX3-associated proteome in the presence and absence of E6 or E7. We identified nucleolin and heat shock cognate 70 (Hsc70) as TBX3 cofactors whose binding to TBX3 was enhanced in the presence of E6 and E7. Hsc70 was shown to stabilise TBX3, nucleolin, E6 and E7, while TBX3 and nucleolin were mutually required to promote cervical cancer cell proliferation and migration. Importantly, as nucleolin is considered a druggable target with several nucleolin-targeting drugs currently in clinical trial, we assessed the therapeutic potential of the nucleolin-targeting aptamer AS1411 against TBX3/nucleolin dependent cervical cancer. Treatment with AS1411 was shown to displace TBX3 and nucleolin from the nucleus to the cytoplasm and significantly inhibit cervical cancer cell proliferation and migration, functionally disrupting this oncogenic protein network. Together, these findings identify TBX3/nucleolin/Hsc70 as a novel host oncogenic axis exploited by E6/E7 to promote cervical cancer progression and highlight a promising host-directed therapeutic aptamer that may be used as a cheaper and more effective treatment in resource-constrained settings.

Toll-like Receptor 3 (TLR3) and E7-induced lnc-FANCI-2 in Cervical Cancer

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High-risk (HR)-E6 and -E7 play crucial roles in cervical cancer by degrading p53 and pRB, respectively, which promotes cell immortalization and growth. However, E6 and E7 alone, without activated RAS, are insufficient to transform cells. Notably, mice with p53 deficiencies develop normally, and disruption of Rb1 does not lead to retinoblastoma. This suggests that additional oncogenic pathways, such as chemical carcinogens, hormonal stimulation, and noncoding RNAs, must cooperate with the loss of p53 and pRb in HR-HPV-driven tumorigenesis. Research has shown that HR-E6 and HR-E7 induce aberrant expression of both oncogenic and tumor-suppressive noncoding RNAs (including miRNAs and lncRNAs), which are involved in various biological functions. Specifically, HR-E7 interacts with the host transcription factor YY1 to transactivate the expression of lnc-FANCI-2 (https://doi.org/10.1073/pnas.2014195118). Using CRISPR-Cas9 technology, the lnc-FANCI-2 promoter or YY1 binding sites (BS) within the promoter were knocked out in HPV16-positive CaSki cells. Single cell clones lacking lnc-FANCI-2 expression were analyzed for cell growth, colony formation, soluble cell receptor changes, and genome-wide RNA-seq. Compared to wild-type (WT) cells, both lnc-FANCI-2 promoter KO and YY1-BS KO cells exhibited reduced cell proliferation and colony formation, altered soluble cell receptor profiles, increased expression of KRAS signaling genes, and decreased expression of genes responsive to interferon (IFN) pathway (https://doi.org/10.7554/eLife.102681.3). To investigate the reduced IFN response in lnc-FANCI-2 KO cells, RNA-seq data were analyzed and genome-wide expression of IFN-related genes was compared. TLR3 expression, which is responsible for recognizing double-stranded RNAs in endosomes and activating phosphorylation of IRF3 to regulate IFN response genes, was significantly lower in lnc-FANCI-2 KO cells than in WT cells. Western blot analysis showed that poly I:C stimulation strongly induced IRF3 phosphorylation and IRF1 production in WT cells, but only minimally in lnc-FANCI-2 KO cells. These results indicate that lnc-FANCI-2 facilitates the IFN response in HPV16-positive cells, likely through TLR3. HPV infection modulates the expression of lncRNAs, including lnc-FANCI-2, via the viral oncoprotein E7. lnc-FANCI-2 acts as a restriction factor for RAS signaling but a facilitator of IFN responses. Consequently, cervical cancer patients with high expression levels of lnc-FANCI-2 have a better prognosis and improved survival outcomes.

Investigating the Trafficking Mechanisms of Merkel Cell Polyomavirus (MCPyV)

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Polyomaviruses (PyVs) are small DNA viruses that cause debilitating diseases in humans, including the often-fatal Merkel cell carcinoma by Merkel cell polyomavirus (MCPyV). To cause infection, these non-enveloped viruses must transport through the crowded host cellular environment to reach the nucleus where transcription and replication of the viral genome leads to lytic infection or cellular transformation. During entry, the prototypic simian virus 40 (SV40) is endocytosed and trafficked to the endoplasmic reticulum (ER) where it penetrates the ER membrane to reach the cytosol. Once in the cytosol, the virus is disassembled and transported to the nucleus through the nuclear pore complex (NPC). While much of our knowledge of the human PyV life cycle has come from the study of SV40, key differences have been observed with MCPyV infection. One prominent distinction lies in its use of mitotic nuclear breakdown (NEBD) to enter the nucleus rather than through the NPC like other PyVs. Another difference that we have observed is in the apparent mechanism of MCPyV intracellular trafficking. While SV40 has been characterized to form distinct foci within the ER membrane that facilitate its escape into the cytosol, our preliminary data show that MCPyV does not form ER foci. Further, we learned from cellular fractionation assays that MCPyV does not reach the cytosol during its infectious life cycle but instead remains within a detergent insoluble vesicle while trafficking within cells. This poses interesting questions of whether MCPyV ever reaches the ER or cytosol during its infectious life cycle. Interestingly, these data support the hypothesis that MCPyV's use of NEBD for nuclear entry is determined by its alternate trafficking mechanism in cells. We aim to characterize the overall trafficking mechanism of MCPyV, examine the identity of these detergent insoluble vesicles and determine what host factors the virus may be interacting with during the entry pathway.

Unravelling the Role of CFTR in BK polyomavirus Infection

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Kidney transplantation is the most effective treatment for patients with end stage renal disease. Following transplantation, the risk of organ rejection is managed by immunosuppressants. However, these drugs also increase the likelihood of re-activation of BK polyomavirus (BKPyV). Of the ~80,000 kidney transplants occurring yearly, 30-40% of these patients will experience clinically-relevant viraemia (BKPyV in the blood) and approximately 10% of cases develop extensive kidney damage known as BKPyV-Associated Nephropathy, resulting in a 5-6 times higher chance of long-term kidney transplant graft loss.

Current anti-viral strategies against BKPyV have had limited success in reducing viral load and nephropathy in patients. None are recommended in the 2024 British Transplant Society Guideline on BKPyV. Reducing immunosuppression is standard care, allowing the immune system to clear the infection, but can take months, and risks rejection and premature graft failure. Consequently, for renal transplant patients, BKPyV re-activation remains a substantial problem associated with significant morbidity and mortality.

Our previous work investigating viral entry in primary renal proximal tubule epithelial (RPTE) cells, identified Glibenclamide as a potent inhibitor of infection. From this we utilised other ion channel inhibitors to determine that cystic fibrosis transmembrane regulator (CFTR) channel activity is essential during early stages (~2hr post-infection) of BKPyV entry. We have since identified that CFTR function is also critical for JCPyV and SV40 infection, in SVGA and RPTE cells respectively, but not required for BKPyV or SV40 entry into vero cells. The suggests there are cell-dependant differences in how polyomaviruses infect cells. We are now unravelling the mechanistic detail of CFTR's involvement within polyomavirus infections by investigating; 1) the localisation of CFTR during virus trafficking through the endosomal system, and 2) the functional role it is having during early infection. These characterisations will further reveal CFTR's potential as a therapeutic target to prevent or treat BK virus kidney transplant damage.

A Forward Genetics Platform for Papillomavirus Research

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Human papillomavirus (HPV) research has been constrained by the absence of a forward genetic system that directly links viral genotype to phenotype. To address this limitation, we developed a genetic selection platform in which plasmids separately encoding HPV L1 and L2 are packaged into HPV capsids, generating mixed virus stocks in which each encapsidated plasmid encodes the specific mutant capsid protein present in that particle. This concordance between genotype and phenotype enables recovery of mutant plasmids following phenotypic selection.

As proof of principle, we performed a reconstruction experiment using HPV16 L1. Cells were co-transfected with a plasmid expressing wild-type L1 in 100-fold excess over an antibody-resistant mutant L1 plasmid, and virus stocks were prepared. Following neutralization with an antibody that blocks infection by wild-type, DNA recovered from infected cells was ~2500-fold enriched for the mutant, demonstrating efficient selection of rare capsid variants.

We applied this system to select antibody escape mutants from a library of random L1 mutants generated by error-prone PCR targeting a segment of L1 including the FG loop, a major surface-exposed region recognized by many neutralizing antibodies. Next-generation sequencing identified mutations at sites that were previously identified as binding the antibodies as well as at novel positions. The neutralization phenotype inferred by sequencing was confirmed by testing individual recovered mutants. Importantly, this screen revealed amino acid preferences and cooperative mutational interactions—pairs of substitutions that individually fail to confer resistance but together enable escape from neutralization. These findings would not be accessible by conventional structural approaches.

This platform enables studies that were previously not feasible, including: defining mechanisms of viral entry and inhibition through isolation of escape mutants; selecting L1 variants that form capsids with improved properties such as assembly, stability, or immunogenicity with applications in virus-like particle vaccine development; characterizing the immune response to HPV infection or vaccination; selecting and characterizing conditional viral mutants such as temperature-sensitive or inhibitor-resistant variants; and generating reagents and information for therapeutic development. This genetic system provides a versatile new framework to support both basic HPV research and the development of improved vaccines and antiviral strategies.

The human papillomavirus L2 protein in the capsid is an ensemble of related structures poised to exit the virus particle

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The human papillomavirus (HPV) capsid contains 360 molecules of the L1 protein, which assembles into pentamers that form the icosahedral capsid shell, and up to 72 molecules of the L2 protein. The C-terminus of the L2 protein must emerge from the capsid, insert into the endosome membrane, and protrude into the cytoplasm to bind to cellular proteins that mediate trafficking of the virus to the nucleus during infection. Computational analysis predicts that much of L2 is disordered, but the stoichiometry, structure and location of L2 in the capsid remain elusive. Here, we use cryoEM single particle analysis of HPV16 pseudovirus capsids with and without the L2 protein combined with AlphaFold3 predictions and molecular dynamics simulations to develop a structural model of L2 within the capsid. Our results reveal that a single molecule of L2 associates with the inner surface of L1 pentamers. The relatively rigid structure of the pentamer forces the long intrinsically disordered segments of L2 to adopt multiple, related, compact conformations in the capsid, whereas the structured elements of L2 remain folded but adopt varied positions in space largely beneath the pentamer. A prominent feature of L2 associated with the pentamer is a finger-like projection extending into the central pore of the pentamer. Remarkably, AlphaFold3 predicts that many different segments of the disordered domains of L2 can form fingers. Furthermore, in some models the C-terminus of L2 extends through the pentamer pore to the exterior surface of the capsid. Consistent with these models, we show by immunogold staining and transmission electron microscopy that the C-terminus of multiple L2 molecules is exposed on the surface of each capsid, presumably positioned to insert into the endosomal membrane. These findings provide a structural basis for understanding how L2 is organized in the capsid poised to initiate its role during virus entry.

Functional elements of the Human Papillomavirus L2 protein act sequentially from its C-terminus during virus entry

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Successful infection by Human Papillomavirus (HPV) requires trafficking of the non-enveloped virus particle from the endosome, through the Golgi, and eventually into the host cell nucleus. This process relies on the minor capsid protein L2, which is predicted to contain N-terminal alpha-helices, a central structured region (CSR), and two relatively long disordered regions (>100 amino acids): the N-terminal disordered region (NDR) and the C-terminal disordered region (CDR), which flank the CSR. The 473-amino-acid HPV16 L2 protein protrudes through the vesicular membrane to engage cytoplasmic trafficking factors such as retromer and COPI. Membrane protrusion is mediated by a cell-penetrating peptide at the extreme C terminus of L2. While the entry mechanisms up to Golgi stacks are well studied, post-Golgi trafficking mechanisms remain largely elusive.

Here, we identify the L2 NDR as the essential coordinator of late entry stages. Although both the NDR and CDR exhibit biochemical features of intrinsically disordered protein regions, systematic mutagenesis reveals that most of the NDR functions through sequence-dependent mechanisms, unlike the CDR, which acts as a length-dependent segment in the endosome. Imaging studies demonstrate that sequences in the NDR are dispensable for HPV trafficking to the Golgi stack but critical for subsequent nuclear targeting. Specifically, a C-terminal segment within the NDR is critical for the progression of the incoming virus from Nesprin2-associated perinuclear compartments. Sequences in the N-terminal portion of the NDR are not required for this step but act later during trafficking to support nuclear entry.

These findings define the L2 NDR as a sequence-specific disordered domain that governs late trafficking steps of HPV entry. By integrating our findings with established roles for the CDR and CSR in the endosome and Golgi, respectively, we propose a model that HPV progressively deploys L2 from its C-terminus toward N-terminus to coordinate stepwise entry processes during trafficking to the nucleus. This mechanism provides a conceptual framework for how a single viral protein manages sequential protein-protein interactions occurring during the multi-step journey into the nucleus. It also provides a new template for understanding viral trafficking and identifying potential high-precision targets for antiviral intervention.

Genetic complementation studies of Human PapillomaVirus type 16 minor capsid protein L2 mutations that prevent infection

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We performed a genetic complementation study to better understand the functions during infection of the human papillomavirus type 16 (HPV16) minor capsid protein, L2. HPV16's non-enveloped capsid is formed by 360 copies of the L1 major capsid protein. The capsid also includes between 12-72 molecules of L2, whose location and function are poorly understood.

Initially, we compared head-to-head how L2 mutations, both novel and previously described, impact particle assembly, L2 incorporation, DNA encapsidation and viral infectivity (of 293TT cells, HeLa cells and the vaginal tract of mice) using a pseudovirion (PsV) system. HPV16 PsV incorporating a luciferase reporter plasmid instead of the viral genome were generated in 293TT cells and validated for particle assembly by density gradient centrifugation, and inclusion of L2 by Western blot. The efficiency with which luciferase reporter plasmid was encapsidated by each mutant was measured by qPCR. Multiple L2 mutants across several domains severely reduced infectivity without compromising virion assembly.

A panel of potent L2 mutants was selected to further investigate functional relationships between L2 domains. Genetic complementation assays were performed to assess functional rescue of infection. Trans-complementation experiments, in which preparations of each different mutant PsV were mixed 1:1 prior to addition to cells, failed to restore infectivity. Likewise, when preparations of each mutant PsV were mixed 1:1, 3:1 or 10:1 with wild type PsV prior to addition to cells, no dominant negative effects were seen. These results suggest that L2 functions cannot be complemented or disrupted between separate virions.

In contrast, cis-complementation, achieved by co-packaging two different L2 mutants at a 1:1 ratio within the same PsV particle, resulted in partial restoration of infectivity for specific mutant pairs. These findings indicate that L2 molecules can function cooperatively within a single virion to perform some functions. Other mutants showed no cis-complementation, suggesting a single L2 molecule may perform certain critical functions during infection.

Overall, this study demonstrates that some, but not all, L2-dependent functions are cooperative within individual viral particles, although not between particles. Genetic complementation provides a functional framework for understanding L2 domain interactions during HPV16 infection.

Interplay Between Promyelocytic Leukemia Nuclear Bodies and Phospholipases C Facilitates Early Events in the HPV16 Lifecycle

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Promyelocytic leukemia nuclear bodies (PML NBs) play a critical role in intrinsic immunity and their interactions with viral infections are complex, as viruses can both evade and exploit PML NB functions. It is well established that incoming HPV virions are associated with PML NBs, however, the exact mechanism through which HPV targets PML NBs to facilitate establishment of infection remains unclear. We have recently demonstrated that HPV virions are delivered into the nucleus inside a membrane-bound transport vesicle during mitosis and associate with PML protein prior to the release of the viral genome from the vesicle. The viral genome release is mediated by phospholipases C (PLC) and the depletion of PLC β4, δ4 and γ1 delays egress of HPV genome from the vesicle. Herein, we demonstrate that the PML II isoform is selectively recruited to incoming HPV harbouring transport vesicles. Interestingly, all PML isoforms associate with the overexpressed L2 protein in HeLa cells to a similar extent. Since PML II has been known to associate with lipid droplets in the nucleus, we conclude that recruitment of PML II is mediated via its interaction with lipids of the HPV harbouring membrane bound vesicles rather than with the L2 protein. We observed that the association of PML NB with the HPV16 viral genome is transient and lost over time. We also found that phospholipase C β4 and γ1, localized in close proximity to PMLII, associated with the transport vesicle. This suggests a role for PMLII in recruiting certain PLCs to facilitate vesicle resolution and release of the viral genome. Overall, our data suggests that the interplay between PMLII isoform and phospholipases C plays a critical role in early events of the HPV lifecycle. These insights reveal promising therapeutic targets and highlight the molecular complexity of virus-host interactions.

Sp140L is a Novel Restriction Factor of Herpesvirus Infection

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Epstein-Barr virus (EBV) is an oncogenic gammaherpesvirus that can transform B cells into lymphoblastoid cell lines in vitro. To establish latent infection, EBV must evade (or use) the intrinsic cellular immune processes. PML nuclear bodies (PML-NBs) are key components of this intrinsic response, repressing transcription of herpesviruses that lack appropriate countermeasures. The speckled protein (SP) Sp100 is a core component of PML-NBs that contributes to virus repression. The SP family also includes Sp110, Sp140 and (only in primates) Sp140L. Viral countermeasures to Sp100 include EBV protein EBNA-LP that binds and inhibits Sp100 and Herpesvirus Saimiri (HVS) ORF3 which degrades Sp100.

We previously showed that EBNA-LP-knockout EBV (LPKO) is unable to transform naïve B cells and exhibits reduced transcription of its genes. In single cell RNA-seq data, LPKO-infected B cells exhibited reduced viral gene expression and increased expression of interferon stimulating genes associated with Speckled protein expression. CRISPR knockout of either Sp100 or Sp140L (but not other PML-NB components) rescued outgrowth of LPKO-infected naïve B cells, and similarly, rescued transgene expression from ORF3-knockout HVS in human fibroblasts. By directing CRISPR mutations to specific exons, we found that truncations in either the SAND or PHD/bromo domain of Sp140L, or the SAND but not PHD/bromo domain of Sp100 – also rescued these phenotypes. Conversely, ongoing experiments suggest ICP0-mutant Herpes simplex virus is susceptible to repression by Sp100 but not by Sp140L.

This suggests that, in human cells, Sp140L is an essential cofactor of Sp100 for the restriction of oncogenic herpesviruses, and likely some other DNA viruses. We hypothesize they work together to sense and repress transcription of nuclear viral DNA and prevent transformation of naïve B cells by EBV.

Mutant mania! Generating E1A HAdV mutants to investigate the role of E1A-Protein Kinase A interactions during HAdV infection

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Early in human adenovirus (HAdV) infection, the viral hub protein E1A interacts with numerous cellular proteins to rewire the host environment and make it suitable for HAdV replication. Previous work from the Mymryk lab has identified protein kinase A (PKA) as a key target of E1A during infection. PKA is essential for cAMP signalling pathways regulating cellular transcription, which can be spatially modulated within the cell through A-kinase anchoring proteins (AKAPs). We have demonstrated that the N-terminus of E1A serves as a mimic of a cellular AKAP, allowing E1A to relocate PKA to the nucleus to promote viral gene transcription, meanwhile sequestering PKA from its normal functions. This interaction between E1A and PKA is essential for productive HAdV replication, however, it is unknown what the effect of PKA sequestration is on the numerous essential host PKA-dependant mechanisms.

N-terminal E1A-protein interactions are difficult to investigate individually due to the overlapping nature of different protein binding motifs in the region. To bypass this challenge, we have generated a library of N-terminal E1A mutant HAdVs investigate how cellular PKA signalling is impacted during infection. The AdenoBuilder platform was used to generate charge-switch point mutations to residues in E1A that are predicted to interact with PKA. Viral genomes were Gibson assembled and electroporated into HEK293 cells to generate virus. Mutant viruses were found to express lower levels of E1A and reduced viral genome replication compared to wild-type HAdV, using western blotting and qPCR respectively, in HAdV infected A549 cells. Each mutant E1As ability to interact with PKA in the nucleus of HAdV infected A549 cells has been determined and quantified using a proximity ligation assay and fluorescent microscopy.

The impact of each point mutation on other N-terminal E1A-host protein interactions will be characterized using co-ip mass spectrometry. Following characterization of the mutant HAdVs, they will be used in combination to characterize changes in cellular gene expression during HAdV infection caused by the interaction between E1A and PKA using RNA-seq. Taken together, these investigations will allow for a greater understanding of how E1A alters the cellular environment to make it suitable for productive HAdV infection.

The development of a kidney organoid model of JC Virus infection

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JC virus (JCV) is a ubiquitous human polyomavirus that infects the majority of healthy individuals. The transmissible form of the virus, termed archetype JCV, leads to life-long asymptomatic infection of the kidneys and is characterized by a highly conserved genome across individuals. In contrast, in patients with severely weakened cellular immunity, JCV can reactivate and stochastically undergo genomic rearrangements through which it gains the ability to infect glial cells of the central nervous system, including oligodendrocytes and astrocytes. Lytic infection of oligodendrocytes results in the rare and often fatal demyelinating disease progressive multifocal leukoencephalopathy (PML). Currently there are no treatments for PML nor in vivo models that accurately recapitulate the pathological processes of PML.

3D organoids are cell culture models derived from human pluripotent stem cells or adult stem cells and can mimic tissue morphology, cellular heterogeneity, and function of human organs. To better understand the viral-host interactions that allow archetype JCV to establish a benign infection in the kidneys and genomically rearranged variants of JCV to cause a destructive infection in the brain, we aim to use organoid models of the brain and kidney. Although JCV-infection in brain organoids have been reported previously, JCV infection of kidney organoids has not. We describe successful JCV infection and replication within human kidney organoids using different viral variants through detection of increasing production of viral genome and late viral protein over 21 days. The most prominently infected epithelia exhibit AQP3-positivity, reflecting collecting duct-like differentiation. We hypothesize this model could further be used to improve preclinical screening for drug candidates for PML and to validate this application, we are testing various viral replication inhibitors in this system. This research illustrates how organoids are being used to change preclinical modeling of rare diseases and advance our understandings of diseases which have been historically difficult to study.

Unraveling Merkel Cell Polyomavirus Infection Dynamics and Persistence in Human Hair-Bearing Skin Organoids Using Multi-omics

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Merkel cell polyomavirus (MCPyV) is a human tumor virus responsible for the majority of Merkel cell carcinoma (MCC) cases, a rare, highly aggressive skin. Virus-positive MCCs are characterized by integration of the viral genome into the host genome, a low mutational burden, and strict dependence on the expression of viral oncoproteins. Understanding the mechanisms by which the highly prevalent virus leads in rare cases to MCC and how a limited set of viral oncoproteins drive tumorigenesis is of broad relevance beyond MCC research.

In the absence of reliable in vitro and in vivo models that faithfully represent the early stages of the viral life cycle, many aspects of MCPyV biology remain poorly understood. Here, we utilize iPSC-derived hair-bearing skin organoids (SkO), a recently established model (PMC7593871) to develop an in vitro infection model for progressive MCPyV infection. This platform enables the investigation of the events preceding MCPyV integration and cancer development.

The integration of bulk, single-cell, and spatial transcriptomic analyses revealed a complex interplay between MCPyV infection and the host immune response. Combining short- and long-read sequencing enabled precise characterization of the viral transcriptome at single-cell resolution. Furthermore, the integration of DNA/RNAscope, scRNA-seq, and spatial transcriptomics, identified distinct fibroblast subpopulations that support MCPyV production.

This is the first MCPyV in vitro infection model that supports progressive viral infection. We provide the first evidence that MCPyV can persist across multiple skin cell types, characterized by restricted expression of early viral genes, while productive infection is confined to a distinct fibroblast subpopulation. Collectively, these findings highlight the skin organoid system as a physiologically relevant platform for studying MCPyV infection and pathogenesis, and underscores its potential for future investigations into viral persistence, oncogenic viral transformation and tumorigenesis.

Cellular origin of cervical reserve cells: primary target for HPV infection and the onset of cervical neoplasia and dysplasia

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Metaplastic changes at the uterine cervix are a normal physiological occurrence, however they increase the risk for the emergence of neoplasia which may lead to cancer. The cellular origin of the cervical remodelling is enigmatic. Chumduri et al (2021) postulated that the cervical columnar and squamous epithelia arise from two distinct lineage-specific stem cell types, however this result was disputed by Lohmussar et al, (2021). Our data suggests the columnar cells can transdifferentiate to a reserve cell which can then proliferate and differentiate to form a squamous epithelium. We used in situ staining of human cervical tissue (HPV negative) and analysed single-cell transcriptomic profiling data published from healthy human cervix. Functional studies of cervical organoid models confirmed that endocervical columnar cells can undergo remodelling to form precursor cells which can be promoted to outgrow as ectocervical squamous cells. This concept of mucosal transition zone homeostasis fits well with other recent observations on mucosal stem identity at the anal transition zone as well as iPSC identity (Li et al, 2018) and provides a basis for future investigations into transition zone homeostasis in other tissues with high clinical relevance.

Exploring the functional significance of the interaction between the HPV capsid protein L2 and the oncogenic transcription Factors TBX2 and TBX3 in the HPV life Cycle

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Cervical cancer is the leading cause of cancer-related deaths in South African females and 99% of cases result from infection with high-risk Human Papillomavirus (HPV) strains, particularly HPV-16. The HPV minor capsid protein L2 assists with establishing this infection by interacting with host cellular proteins to deliver the viral genome into the host nucleus. Identifying and characterizing these host proteins may lead to targets for prophylactic and/or therapeutic HPV treatments. Of interest to this study are preliminary data showing that L2 interacts with the homologous oncogenic transcription factors TBX2 and TBX3. While we have previously shown that TBX3 interacts with the HPV E6/E7 oncoproteins to promote cervical cancer, the impact of the interaction between L2 and TBX2/TBX3 on HPV infection is unknown, and this is explored in this study.

To confirm the interaction previously reported between HPV L2 and TBX2/TBX3, co-immunoprecipitation and immunocytochemistry were performed in the CaSki and HeLa cervical cancer cells transfected with L2. To create a tool to study the impact of TBX2 and TBX3 on HPV infection, HPV-luciferase pseudoviruses were produced following standard protocols. HaCaT cells (host for HPV infection) in which TBX2 or TBX3 were depleted by siRNAs or treated with a TBX2/TBX3-targeting anthelmintic drug, niclosamide, were infected with pseudoviruses. Infectivity was measured by luciferase assays and confocal microscopy.

The in vivo interaction between L2 and TBX2 as well as with TBX3 was validated, pointing towards a function of this interaction during HPV infection. Pseudoviruses were successfully produced and used to show that TBX2 and TBX3 promote HPV infectivity. Importantly, the TBX2/TBX3-targeting drug, niclosamide, inhibited HPV infection.

This study identifies TBX2 and TBX3 as novel targets for prophylactic and therapeutic treatment of HPV infection. It further demonstrates that niclosamide is a promising TBX2/TBX3 targeting drug that can be repurposed to inhibit HPV infection.

Investigating the interplay between SV40 chromosome architecture and activity

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The genome of polyomavirus Simian Vacuolating Virus 40 (SV40) is organized into eukaryotic-like chromatin throughout its lifecycle. Following contact with a host cell and trafficking to the nucleus, partially disassembled SV40 virions must complete early transcription and synthesis of early viral proteins, replicate their genome, transcribe late viral proteins, and encapsidate newly replicated genomes for packaging into new virions. Successful completion of the infectious cycle requires equitable distribution of newly formed viral chromosomes to each of these functional activities. While the mechanism for licensing genetically identical chromosomes for each purpose is not well-defined, it appears that distinct structural features are consistently attributable to chromosomes of each functional group. Utilizing sequential Chromatin Immunoprecipitation (ChIP), we have quantified the distribution of chromosomal licensing to replication, transcription, and encapsidation within infected cells. Additionally, we have utilized sequential ChIP experiments to map nucleosome positioning and patterns of histone modifications along the genomes of chromosomes belonging to each functional group. These experiments have revealed that a dominant majority of viral chromosomes seem to be inactive, and are not participating in replication, transcription, or encapsidation at a given time. A minor percentage of chromosomes seem to be functionally active and display distinct patterns of nucleosome positioning and histone modification depending on their functional activity. Activating histone modifications acetyl-H3 and acetyl-H4 and repressive H3K9me1, H3K9me3, and H3K27me3 are present in distinct patterns for each functional group. In the regulatory region of SV40 chromosomes, several nucleosomes were found to contain both activating modification acetyl-H4 and repressive modification H3K9me1 at the same site. This suggests that viral chromosomes contain bivalent nucleosomes, possibly involved in mechanisms of viral latency. The presence of distinct histone modification patterns, including bivalent nucleosomes within the regulatory region, supports a model in which chromatin-based regulation contributes to functional licensing of viral genomes and progression of the viral infection cycle.

Host chromatin surveillance reshapes hepatitis B viral gene expression and interplay with endogenous retroelements

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The Human Silencing Hub (HUSH) complex is best known for silencing retroviral elements and has been implicated as an essential restriction factor for HIV, however, its role in the hepatitis B virus (HBV) life cycle is not known. HBV persists as a circular episomal DNA genome, but aberrant replication can generate double-stranded linear DNA that can integrate into the host chromatin. These viral integrants are replication-defective, yet remain a ubiquitous source of immune tolerising viral surface antigens. The host factors that regulate transcription from episomal and integrated HBV templates remain incompletely defined, and we hypothesise a multifactorial role for HUSH in the regulation of HBV transcription.

Here, we used shRNA-mediated depletion of the HUSH components TASOR and MPP8 in HBV-infected hepatoma cells, followed by targeted RT-qPCR and long-read transcript profiling. Importantly, this infection model supports the full HBV replicative life cycle. In contrast to a simple repressor model, MPP8 depletion reduced the abundance of HBV RNA across 9 days of infection, whereas TASOR depletion had little-to-no effect on HBV transcriptional outputs. MPP8 loss was associated with a reorchestration of viral promoter activity and subsequent transcription start site usage, which may influence the balance of viral protein expression required to establish chronic infection. MPP8 depletion was accompanied by an increase in spliced HBV transcripts, whilst maintaining canonical transcript architecture; suggesting selective rewiring of viral transcriptional output rather than a global disruption of RNA structure. Despite a well-defined role for HUSH in the regulation of integrated HIV genomes, depletion of MPP8 and TASOR in hepatoma cells harbouring transcriptionally active HBV integrants we observed modest and context-dependent responses to HUSH depletion.

As HUSH is classically linked to transposon silencing, we examined host transposable element expression in the same infection system. Interestingly, we found that HBV infection attenuated the magnitude of the HUSH-linked LINE-1 response, consistent with infection-associated blunting of canonical host transposon control in this model. Together, these data suggest that host chromatin surveillance reshapes HBV transcription during active viral replication. Additionally, we demonstrate that HBV infection can perturb host retroelement landscape, with implications in host cell RNA sensing and interferon signalling.

Cloning and Analysis of an Epstein-Barr virus Strain from an LCL Established with virus from Kenyan Breast Milk

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The majority of in vitro studies of EBV biology have relied on a small number of EBV strains derived from cancer cell lines, or mutated by prolonged and unnatural culture strategies. It is therefore important that our current understandings of EBV biology are tested using strains that are more representative of normal circulating EBV. There is therefore a need for EBV genomes that are representative of circulating strains from different geographic regions.

We have developed a new protocol for cloning EBV genomes. This uses cas9 to cut the EBV genome between BVRF1 and BVLF1, as pioneered by Teru Kanda, but integrating the BAC such that the C-termini of these ORFs are recoded to avoid repetitive DNA flanking the BAC. Then five days post transfection, we extract total genomic DNA from the cells, and then enrich the EBV DNA using a biotinylated EBNA1 DNA-binding domain (containing a Y518F mutation to avoid covalent attachment of EBNA1 to DNA). Enriched DNA is transformed into bacteria to recover the BAC-cloned EBV genome.

This strategy can reproducibly clone Jijoye EBV from its parental cell line. To enable cloning of EBV from LCLs, we co-transformed the BAC capture vector with a cas9-RNP, that enabled cloning of an intact EBV genome from an LCL (BM209-2) established by infecting B cells with breast milk from a Kenyan individual (Daud et al 2014). Both Jijoye and BM209 BACs were used to generate 293 virus producer cell lines. These both generated low levels of encapsidated EBV genomes, that were increased approximately 200-fold when a Cre-expression vector (to excise the BAC element) was included alongside the virus-induction, suggesting that BAC-cloned EBVs may commonly exceed the efficient packaging limit of the virus. EBV transformation by BM209-2 is inefficient, and the resulting LCLs comprised fewer IgD+ cells and more plasmablasts present during outgrowth than for B95-8 transformation. Preliminary experiments suggest that LCLs from which the BAC had been excised had a different balance of latency proteins, implying that the presence of the BAC may have unexpected effects on latency gene expression.

NFX1-123 is required for keratinocyte growth, differentiation, and HPV 16 infection maintenance

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Persistent high-risk human papillomavirus (HR HPV) infections cause nearly 5% of cancers worldwide, including cervical and oropharyngeal cancers. HR HPVs sustain long-term infections in stratified epithelial tissues by maintaining copies of viral genomes in basal keratinocytes and utilizing cellular differentiation processes in the upper layers to coordinate key steps in the viral life cycle. We have demonstrated previously that the endogenous host protein NFX1-123 bound directly to the HR HPV type 16 oncoprotein E6 (16E6) and that this partnership increased keratinocyte proliferation and differentiation, two processes which are both required for persistent HPV 16 infections. As there is a range of endogenous expression of NFX1-123 in normal basal keratinocytes, we aimed to investigate the role of NFX1-123 abundance on the initiation and persistence of HPV 16 infections.

In this study, we utilized keratinocytes with overexpressed, endogenous, or reduced levels of NFX1-123 and infected these cells with an HPV 16 quasivirus (Qsv) to interrogate changes to keratinocyte growth and viral DNA copy numbers with NFX1-123 modulation. Keratinocytes with reduced NFX1-123 had blunted differentiation and stratification in raft cultures, indicating that NFX1-123 expression is required for typical keratinocyte growth and differentiation. This blunting of stratified epithelial architecture with NFX1-123 knockout correlated with decreased HPV 16 Qsv DNA copies, while overexpression of NFX1-123 supported increased viral copy numbers. Similarly, when we reduced NFX1-123 in W12 cells, a cervical cell line harboring episomal (W12E) or integrated (W12I) HPV 16 genomes, we saw a decrease in raft culture growth, differentiation and thickness. We also observed fewer viral genome copies in W12E cells with knocked out NFX1-123 compared to W12E cells with endogenous NFX1-123, in both differentiating and undifferentiated keratinocytes. These findings indicate that NFX1-123 expression is required for keratinocyte growth and differentiation and highlight the importance of NFX1-123 on the initiation and maintenance of persistent HPV 16 infections that may progress to cancer.

Understanding Immune-Mediated Resolution of HPV-Related Disease and the Control of Subclinical Infections

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Human papillomavirus (HPV) infection is the primary cause of cervical precancerous lesions, which can potentially progress to cervical cancer. The pathogenesis of HPV involves a dynamic interplay between viral gene expression and the host immune response. As the mechanisms underlying the immune system's control of HPV infection remain elusive, this study aims to explore the molecular mechanisms by which immune cells regulate HPV-related disease and subclinical infection.

Clinical cervical samples from over 300 patients with and without HPV-related disease were analysed, alongside the corresponding diagnosis and treatment data. Spatial biology approaches, including multiplex immunofluorescence and RNAscope, were applied to assess viral biomarkers, immune cell phenotypes, and cytokine signatures, enabling mapping of viral activity in relation to local immune responses.

Among 30 patients with a prior colposcopic biopsy-confirmed disease who lacked high-grade squamous intraepithelial lesion (HSIL) at follow-up after loop electrosurgical excision procedure (LEEP), LEEP specimens from 6 patients exhibited features of disease resolution, characterised by increased T cell infiltration, accompanied by reduced expression of p16/MCM, a surrogate biomarker of high-risk HPV E6/E7 deregulation. These findings are parallel to immune-mediated regression observed in the rabbit oral papillomavirus (ROPV) models. Notably, regression was not associated with complete viral clearance. Following disease resolution, HPV infection appeared to persist in the epithelial basal reservoir during subclinical infection. To support the presence of persistent subclinical infection, using hysterectomy specimens from 97 patients without cervical abnormality, we found low-level viral RNA expression in 17 patients with normal histology alongside the presence of tissue-resident memory T (Trm) cells. These findings suggest Trm-driven, cytokine-mediated immune surveillance in the control of subclinical HPV infection.

This study establishes a spatially defined 'pseudotime' linking disease resolution to subclinical infection. We propose that immune-mediated control of HPV infection is achieved through suppression of viral gene expression rather than complete viral clearance. These insights will implement new knowledge into the prognosis and effective treatment of HPV-associated diseases.

Stochastic Modeling of Epithelial Homeostasis in persistent HPV16 and HPV11 infection

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Human papillomaviruses (HPVs) are DNA viruses associated with lesions ranging from benign to cancerous. Low-risk HPV11-E6 shows behaviour consistent with benign lesions, whereas high-risk HPV16-E6 confers a proliferative and competitive advantage to keratinocytes within the basal layer. Under physiological conditions, epithelial homeostasis is maintained through a stochastic balance between progenitor cell self-renewal and differentiation, which is disrupted by persistent viral protein expression.

We performed a quantitative analysis of HPV-E6 expressing keratinocytes to investigate homeostatic regulation, focusing on the role of the E6 protein. Experiments were carried out using NIKS keratinocytes in confluent monolayers, in mosaic and single-clone assays, tracking colony growth and clone size distributions over time. Co-staining for Keratin 10 and Ki-67 identified differentiated and proliferative states.
Colony size distributions diverged in an E6 genotype-dependent manner. HPV11-E6 expressing cells overlapped with controls, whereas HPV16-E6 expressing cells showed a marked shift towards larger clones. Using a stochastic progenitor model fitted to clone size distributions, we captured clone-level heterogeneity and showed that the HPV-induced shift in epithelial homeostasis emerged from altered cell fate decisions. A deterministic mean-field approximation captured average system behaviour in mosaic competition assays.
HPV E6 did not simply increase proliferation but shifted epithelial homeostasis by raising the cell density at which differentiation was triggered, consistent with an increased steady-state fraction of proliferative cells. Integration of scRNA-seq data with pseudotime trajectory inference revealed distinct cell-state organisations between low- and high-risk HPV types, including transitioning subpopulations, and suggested alternative routes towards terminal differentiation. These observations are informing a revised biological-mathematical model in which low- and high-risk HPVs might follow distinct cell fate choices.
Finally, we are developing a preliminary spatio-temporal modelling framework to investigate emergent collective properties, linking altered cell fate decisions to changes in tissue organisation.

In conclusion, HPV infection alters epithelial homeostasis in a genotype-dependent manner. The integration of quantitative experiments and modelling shows that HPV E6 confers a competitive advantage by redefining progenitor fate balance, providing a framework to understand how HPV-driven shifts in epithelial homeostasis underpin viral persistence and highlight homeostatic control as a potential target.

Mitochondrial ROS levels are enhanced by the adenovirus E4orf4 protein and play a role in viral replication and virion stability

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The adenoviral E4orf4 protein is a small 14 kDa protein expressed early during viral infection. E4orf4 uses several mechanisms to fine-tune viral and cellular signaling. These include adjusting early viral gene expression by various means, inhibition of the DNA damage response, and induction of caspase-independent programmed cell death when expressed alone. Recently, our work has shown that E4orf4 can localize inside the mitochondria, where it interacts with electron transport chain (ETC) proteins, decreasing mitochondrial respiration while increasing generation of reactive oxygen species (ROS). A deeper dive into this phenomenon suggests that it occurs through close interactions between E4orf4 and ETC complexes 3 and 4. Whether the generation of higher ROS levels contributes to adenoviral infection, and what may be the underlying mechanisms of such a potential contribution, is yet to be fully understood. Our recent studies of the consequences of anti-oxidant treatments for viral replication revealed concentration-dependent variable effects. Furthermore, anti-oxidant treatments changed not only the efficiency of viral infection but also affected virion stability and resilience to heat shock, suggesting that alterations in viral replication stemmed, at least in part, from ROS-induced changes in virion stability. Interestingly, in addition to E4orf4, several capsid proteins also associated with the mitochondria and were found in four distinct large assemblies. This is a surprising finding given that, according to our knowledge to date, adenoviral capsid assembly occurs predominantly in the nucleus. This further raises the question whether ROS-induced alterations in some mitochondria-associated capsid proteins contribute to capsid assembly in the nucleus. Our results will be presented and brought forth for discussion.

Merkel cell polyomavirus small T antigen regulates the progression of the viral life cycle by promoting the maturation of late transcripts

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The small T (ST) protein of Merkel cell polyomavirus (MCV) is essential for viral DNA replication and gene transcription. MCVΔST, which lacks the viral ST gene, is unable to sustain genomic replication, and the expression of early and late viral genes is reduced compared to wild-type MCV. Consequently, expression of the major capsid protein VP1 is lost, and progression from the early to late stages of the viral life cycle is prevented.

We identified that a significant proportion of viral transcripts in MCV-replicating 293 cells are unspliced and remain in the nucleus, rather than being exported to the cytoplasm. In particular, the majority of MCV late transcripts read through the polyadenylation signals, causing wraparound transcription, and only a limited number underwent splicing and polyadenylation. This suggests that RNA maturation is a rate-limiting step in producing viral capsid proteins. Remarkably, late gene splicing, which is necessary for generating VP1 mRNA, is largely absent in MCVΔST, although unspliced late transcripts are detectable. Expression of the exogenous ST protein in MCVΔST rescued late gene polyadenylation and VP1 splicing, indicating that ST is required for the maturation of viral late transcripts. Furthermore, a replication-defective, single-nucleotide mutation in the MCV origin and aphidicolin treatment abolished VP1 splicing, suggesting a critical role of viral DNA replication in late gene splicing. Poly-A-enriched and total RNA sequencing analyses also revealed that the relative abundance of cytoplasmic polyadenylated late mRNA compared to total late RNA in the nucleus was significantly lower in MCVΔsT than in wild-type MCV. Late gene polyadenylation, VP1 mRNA splicing and leader-to-leader splicing, resulting from the wraparound transcription, were barely detectable in MCVΔST. Instead, immature late (poly-A read-through) transcripts were exported to the cytoplasm.

Taken together, our study suggests that MCV ST promotes late gene expression and maturation, along with promoting viral DNA replication, emphasizing the multifactorial functions of ST that are essential for the progression of the MCV life cycle.

Investigating the spatial localisation of the HPV16 late protein, L1 and HPV16 virions in organotypic raft cultures

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Completion of the HPV lifecycle relies upon the production and correct localisation of the viral capsid proteins within the host keratinocytes. Viral late proteins, including the major capsid protein L1, are produced within the differentiated cells of the epithelium. Following translation in the cytoplasm, L1 is transported to the nucleus for virion formation. Previous studies using ectopic expression of L1, or L1 together with the minor capsid protein L2, have given conflicting data on capsid protein localisation while studies of L1 expression in raft tissue models, or in patient tissues, have provided information at low resolution. Therefore, localisation of L1 within the nucleus, and how this enables virion assembly in natural models of infection is poorly understood.

We developed a novel technique to study cells within the top layer of W12E organotypic rafts (W12E cells clone 20865 containing episomal genomes) for analysis of L1 localisation. This allowed the upper, most differentiated layer of cells at the top of the rafts to be harvested, antibody stained and imaged to high resolution.

The subcellular localisation of L1 within these fully differentiated keratinocytes was examined. Almost all cells stained positive for L1. Three L1 staining phenotypes were observed. Phenotype A, no nuclear L1 staining. Phenotype B, diffuse L1 nuclear staining, which was absent from the nucleoli and areas of dense nuclear material. Phenotype C, tightly condensed foci of L1 staining. Z-stack analysis by confocal microscopy demonstrated that each phenotype was consistent throughout the cell. All phenotypes showed speckled L1 staining at the nuclear periphery. Furthermore, quantitative analysis of cells with each phenotype at 10, 14 and 16 days of raft culture suggested that over time there was a progression of phenotypes A to B to C. Finally, high-resolution confocal microscopy suggested that the intranuclear foci of L1 were composed of multiple structured particles and transmission electron microscopy of cells from the upper layers of W12 raft cultures revealed clusters of virus-like particles. We propose that these clusters may represent the foci of L1 staining observed by confocal microscopy, suggesting that papillomaviruses assemble in subnuclear domains.

One virus, two pathways – Lytic and nonlytic cell egress

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Transmission from infected to noninfected cells is essential for virus maintenance in organisms and populations. Lytic-infected cells efficiently propagate infection by releasing hundreds to thousands of progeny virions together with subviral particles, unpacked genomes, and cell constituents, whereas nonlytic cells are less effective and maintain an intact plasma membrane. We hypothesized that lytic and nonlytic egress pathways of human adenovirus (AdV) are switch-controlled, e.g., by activators or repressors. AdVs are contagious agents and account for ~2-15% of the respiratory tract infections worldwide, depending on age and country {Radin, 2014, 10.1371/journal.pone.0114871; Arnold, 2021, doi.org/10.1016/j.mpmed.2021.09.013}. Human-to-human transmission occurs through respiratory droplets, interpersonal contact or contaminated surfaces, the latter causing eye and gastro-intestinal disease. AdVs infect epithelial cells and immune cells. The former give rise to high levels of progeny, cell lysis and viremia, the latter low amounts of progeny without cell death {Garnett, 2009, 10.1128/JVI.02392-08; Sequeira, 2025, 10.1128/jvi.01825-24}. Although previous work showed that lytic or nonlytic pathways are affected by the cell state such as cell cycle {Georgi, 2020, 10.1128/AAC.01002-20; Andriasyan, 2021, 10.1016/j.isci.2021.102543; Petkidis, 2024, 10.1128/msphere.00454-24; Suomalainen, 2020, 10.1242/jcs.252544; Snaider, 2022, 10.1128/jvi.00442-22}, the underlying cell biological mechanisms of AdV egress have remained unknown. Previously, full cycle infection assays in arrayed, medium-throughput fluorescence microscopy formats scored AdV focus formation and identified comet-shaped and round plaques, representing lytic and non-lytic infections, respectively {Yakimovich, 2015, 10.1371/journal.pone.0138760}. The FDA-approved HIV protease inhibitor nelfinavir was found to block lytic AdV egress, without affecting replication or progeny formation in the cell nucleus {Georgi, 2020, 10.1128/AAC.01002-20; Gantt, 2011, 10.1128/AAC.01295-10}. Fluorescence microscopy and correlative light and electron-microscopy in live and fixed cells now show that both the nonlytic and the lytic pathways require the rupture of the nuclear envelope and the release of virions and subviral entities to the cytoplasm, followed by the detachment of plasma membrane blebs, or plasma membrane rupture, respectively. Analysis of LC-MS/MS proteomics data, membrane trafficking, drug sensitivity and viral mutants has identified the secretory pathway as a switch between lytic and non-lytic virus egress. The results have implications for counteracting AdV in immunosuppressed individuals, oncolytic viral therapy, and modulating the inflammatory anti-viral host response.

Deregulation of high-risk HPV in cervical precancer centres around the crypt entrance epithelial niche

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Human papillomaviruses (HPVs) cause cancers at vulnerable epithelial sites such as the cervical transformation zone (TZ), a hormonally responsive region adjacent to the squamocolumnar junction (SCJ). High-risk HPV (hrHPV)-driven neoplasia at the TZ is believed to arise from infection of reserve cells, a stem cell-like population implicated in metaplasia.

Using transcript-specific HPV16 probes, we spatially mapped infected basal and reserve cells at the TZ and quantified promoter activity and mRNA splicing at single-cell resolution.
In productive infections (typically located at the ectocervix), viral gene expression in the basal layer was strikingly heterogeneous and regulated primarily at the p97 promoter. Individual basal cells were found to transcribe up to 250 times more copies than neighbouring cells within the same lesion, while others remained below the detection threshold. In productive low-grade squamous intraepithelial lesions (LSIL), a coordinated upregulation of E6, E7, and E2 was observed in mid-epithelial layers, a pattern absent from non-productive HSIL.
Within the cervical TZ, several distinctions emerged. Relative p97 activity in the basal layer was more than tenfold higher than in productive ectocervical lesions, mirrored by a 14-fold increase in E6/E7 expression. Reserve cells supported hrHPV gene expression variably, depending on their position within the epithelium. Differences in transcription factor expression between crypt entrances and surface epithelium point to a distinct transcriptional niche that promotes deregulated E6 and E7 expression. We additionally resolved the spliced E8^E2 repressor transcript at single-cell resolution, offering new insight into how its expression varies between productive and abortive lesions and across TZ microenvironments, with implications for local restraint of E6/E7. Abortive HPV16 infections were frequently confined to crypt entrances, suggesting possible type-specific regulatory mechanisms.

These findings highlight the transcriptional complexity of hrHPV infection at the TZ and underscore the role of microenvironmental cues in viral gene regulation and pathogenesis.

HPV18 regulates alternative splicing of E6/E7 through PRMT1-dependent m6A methylation of viral mRNA

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Human papillomavirus (HPV) infections rely on controlled and balanced expression of the viral oncogenes E6 and E7 during infection, establishment and for long-term persistence of the viral genome. E6 and E7 mRNAs are produced through alternative splicing of polycistronic pre-mRNAs, generating diverse splice isoforms with distinct coding potential. HPV genomes likely evolved non-consensus splice sites to tune, rather than maximize, transcript processing. Therefore, splice site selection is regulated by interactions between viral cis-acting regulatory RNA elements and cellular trans-acting factors, though it is unclear how HPV mediates these interactions.

To address this question, we previously identified protein arginine N-methyltransferase 1 (PRMT1) as a host factor upregulated by HPV18 infection in primary human cells. PRMT1 is the main human enzyme responsible for the asymmetric arginine dimethylation of proteins. During HPV18 infection, PRMT1 inhibition increases intronic m6A levels on viral mRNA, which correlates with a highly dysregulated viral splicing pattern. To dissect the role of viral mRNA m6A methylation on viral splicing in response to reduced PRMT1 activity, we developed a dual luciferase minigene system. Using this approach, we identified methylated DRACH motifs within the 3' exon and intronic regions of E6 important for splicing regulation, suggesting a role PRMT1 and m6A in viral oncogene splicing. We are currently testing the impact of these mutations on HPV18 genome establishment.

Mechanistically, PRMT1 has been shown to methylate RNA binding motif protein 15 (RBM15) leading to degradation. RBM15 is a regulatory subunit of the m6A writer complex, that targets m6A preferentially to introns. Importantly, RBM15 localizes to HPV18 replication foci and its recruitment to viral mRNA is regulated by PRMT1. An RBM15 mutant unable to be methylated by PRMT1 further implicates RBM15 in the viral lifecycle. Taken together, our findings provide evidence that oncogenic HPV types regulate alternative splicing of E6 and E7 through PRMT1-dependent m6A methylation, with PRMT1 modulating cellular RBM15 levels to direct m6A deposition on viral transcripts, thereby facilitating long-term persistent infections.

The role of N6-methyladenosine in regulating the expression of HPV capsid transcripts

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HPV infects the basal keratinocytes of stratified epithelia and viral early genes are expressed in these proliferating cells, while late genes encoding the capsid proteins are only expressed once the infected keratinocytes differentiate. How viral late gene expression is restricted to differentiated cells is an area of ongoing investigation. We are specifically interested in how differentiation-dependent cellular changes regulate the processing of late viral transcripts. We previously demonstrated that the splicing of HPV18 early transcripts is regulated by the RNA modification N6-Methyladenosine (m6A). m6A modifications recruit “reader” proteins which regulate diverse aspects of mRNA processing, including splicing and transcript stability.

We used orthogonal approaches to demonstrate that differentiation of primary keratinocytes changes the number and distribution of m6A modifications on late viral transcripts. Using Nanopore Direct RNA sequencing, we identified DRACH consensus motifs in viral transcripts that are differentially m6A modified in organotypic raft-differentiated cultures compared to undifferentiated cultures. Notably, the E4 splice enhancer that promotes inclusion of the E4 ORF in late viral transcripts contains a DRACH site which is m6A modified less frequently upon cellular differentiation. To test the hypothesis that differentiation dependent changes in m6A modification of specific DRACH sites in viral transcripts regulate late viral transcript processing, we developed an HPV16 based minigene. This plasmid-based construct expresses viral genes under the control of an HCMV promoter rather than the differentiation-dependent HPV16 late (p670) promoter. Importantly, expression of minigene-derived late viral transcripts is increased upon cellular differentiation, suggesting that the steady state levels of spliced, late viral transcripts are not solely controlled though promoter activity. Indeed, inhibition of METTL3, the enzyme responsible for m6A methylation, further increased the levels of E1^E4 and E4^L1 containing transcripts following differentiation in semi-solid medium. To dissect the contribution of individual m6A sites to differentiation-dependent late transcript processing, we are mutating DRACH motifs that show differential methylation upon cellular differentiation within the HPV16 minigene. This work will further our understanding of the requirements for the productive HPV lifecycle, knowledge which could be applied in efforts to limit viral transmission or stimulate a capsid targeted immune response during infection.

The mammalian DREAM complex regulates gene expression and DNA damage repair to impact longevity

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RB-family proteins are multifunctional adaptors that link cell-cycle regulation with transcriptional control. Viral oncoproteins such as HPV E7, adenovirus E1A, and SV40 large T antigen target RB-family complexes to promote entry into S phase and facilitate viral replication, largely through disruption of RB–E2F–mediated transcriptional control. In contrast, the RB paralogues p107 and p130 have less well-defined roles in cell-cycle entry, raising questions about their functional significance in physiology and viral pathogenesis. To address this, we investigated the roles of p107 and p130 in vivo.

We engineered mice to express a point mutation in p107 and a deletion of p130, thereby disrupting assembly of the DREAM complex, in which p107 and p130 participate but RB does not. Using ubiquitous conditional deletion of p130 in young adult mice, we assessed the physiological consequences of DREAM loss throughout life.

Disruption of DREAM assembly resulted in shorter lifespan, but these mice showed little signs of aging prior to their demise. Moribund DREAM assembly mutants displayed systemic amyloidosis with deposits in the heart, liver, spleen, and kidneys, but not in brain or bone marrow late in life. Laser-capture microdissection followed by mass spectrometry identified apolipoproteins as the predominant components of these deposits, with apolipoprotein A-IV being most abundant. Mechanistically, we found that DREAM directly regulates chromatin structure at Apoa4 and its loss leads to sustained higher expression and eventually amyloid deposition. In addition, based on roles for DREAM in longevity, we also investigated DREAM loss in mutation accumulation across the lifespan of these mice. We used single molecule duplex sequencing to assess and quantitate mutations. This revealed that DREAM loss decreased single-base substitution type changes and small deletions, implying superior DNA damage repair in its absence. Together, these findings identify DREAM as a playing a key role in transcriptional control with multiple impacts on organismal longevity with both benefits and drawbacks to its loss of function.

PPARα-activated transcriptome of HPV16(+) oropharyngeal cells

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Human papillomavirus (HPV)-associated oropharyngeal squamous cell carcinomas (OPSCCs) are the most common HPV-associated cancer in the United States, with over 20,000 cases per year. HPV(+) oropharyngeal cancers are biologically and clinically distant from the HPV(+) ano-genital cancers (e.g., cervical cancers). Nearly all HPV(+) OPSCCs are caused by HPV16, most arise in the tonsils, and they are five times more common in men. There is currently no established pre-cancer screening or early treatment for HPV(+) OPSCCs.

In the natural course of HPV infection, a productive viral lifecycle yields infectious viral particles. Importantly, it is a non-productive outcome for this virus to transform host epithelial cells. It is thought that HPV delays but still requires normal epithelial differentiation to complete its lifecycle. However, the interplay between tonsillar differentiation and HPV16's lifecycle is poorly understood. Clinical data suggest that tonsil crypts are preferential sites of transformation, while the tonsil surfaces are rarely origin sites of HPV16(+) OPSCCs. Our research aims to decipher interactions between HPV16 and the distinct tonsillar differentiation programs in the surface and crypt.

From our prior scRNA-seq data, we identified transcription factors with increased predicted activity in HPV16(+) differentiated tonsil surface cells, including peroxisome proliferator-activated receptor alpha (PPARα). Inversely, in proliferating cells, viral expression was negatively correlated with predicted PPARα activity. We found that PPARα activation via fenofibrate reduces HPV16(+) tonsil cell growth, compared to their parental HPV-negative controls, and reduces viral oncogene expression. To further investigate this phenotype, we performed bulk RNA-seq on fenofibrate treated vs vehicle-only (DMSO) treated control cells and identified 522 up-regulated and 455 down-regulated genes in PPARα-activated HPV16(+) tonsil cells. These data suggest that HPV upregulates PPARα activity to promote cellular differentiation in the presence of SP1 transcription factor activity. In parallel, a reduction in redox homeostasis genes results in increased expression of ROS related genes highlighting metabolic reprogramming of HPV16(+) cells.

Overall, these findings help address the molecular basis of HPV16's productivity versus transformation in tonsils and highlight a therapeutic opportunity for targeting HPV(+) OPSCCs. PPARα agonist fenofibrate has been proposed as a repurposed drug for HPV(+) cancers and is currently entering clinical trials.

Degradation of Mettl3 and Mettl14 by high-risk E6 stabilizes RNA:DNA hybrid formation and alters the epitranscriptome of HPV positive cells

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RNA:DNA hybrids or R-loops serve as significant sources of genomic instability and regulators of cellular gene expression during HPV pathogenesis. Our studies and others have shown that R-loop levels are elevated through numerous mechanisms involving high-risk E6 as well as APOBEC3B, and these regulate viral replication and gene expression. The increases in R-loops were found to be due to their increased stabilization caused by E6 mediated reductions in the levels of N(6)-methyladenosine (m6A) modified RNAs in these structures. It was, however, unknown whether this repression of m6A was specific to R-loops or a global phenomenon.

Our studies identified that primary keratinocytes stably maintaining high-risk HPV episomes have significantly lower total levels of m6A modified RNAs, including mRNAs. Importantly, altering the pools of m6A modified mRNAs through inhibition of the m6A demethylases impaired viral genomic maintenance and gene expression. High-risk HPV E6 induced decreases in m6A through E6AP-mediated degradation of Mettl3 and Mettl14, while low-risk HPV E6 did not. To determine whether specific cellular or viral transcripts were altered in their m6A modification status, we performed m6A-RNA immunoprecipitation sequencing (m6A-RIP seq) on RNA from primary keratinocytes and HPV positive cells. After filtering out reads mapping to tRNAs and rRNAs, more than 30,000 m6A-mRNA sites were found to be differentially modified between HPV positive and normal cells. Over 25,000 sites contained reduced levels of m6A modifications in HPV positive cells, with an average reduction of ~2.5-fold across all cellular mRNAs tested, while about 5,000 sites were increased. Many of these changes occurred on transcripts of pathways critical for HPV pathogenesis, including reduced modification of genes involved in the defense response to viruses, positive regulation of IL2 production, and response to type 1 interferon, while genes involved in DNA replication, transcription, and the cell cycle were increased. Moreover, mapping m6A modification sites to HPV transcripts identified significant modification around the E6/E6*, E7, E1, and E2 ORFs. These data indicate that m6A modification plays a significant role in regulating R-loop formation and controlling cellular and viral gene expression in HPV positive cells.

How does alternative splicing shape HPV E6/E7 evolution, or the other way around?

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To be spliced or not to be is a defining feature of high-risk (HR) type human papillomaviruses (HPVs), enabling regulation of the viral oncogene expression, E6 and E7. Notably, only the 26 HR-HPV types among hundreds contain splice sites within the E6 coding region. In this context, intron retention in the E6/E7 pre-mRNA produces E6 mRNA, whereas alternative splicing generates E6*I/E7 mRNA. A central question is how alternative splicing has impacted the evolution of HPV E6 and E7.

We re-evaluated splice sites in the 26 HR-HPV E6*I mRNA, classifying splice donors as being of the SD226-, SD154- or SD184-type and splice acceptors as being of the SA409- or SA509-type. Both SA409 and SA509 are highly conserved across HR-HPVs, raising the question of why only one is used at a time. This suggests the presence of regulatory mechanisms enforcing mutually exclusive activation. Supporting this, ancestral tracing showed these splice sites already existed in early alpha-genus HPVs, including both high- and low-risk types. Furthermore, swapping SA409 and SA509 sequences did not redirect splicing to the substituted sites, indicating that splicing site sequences alone do not determine usage. Instead, additional cis-regulatory elements and/or trans-acting factors likely control splice site selection.

Using reconstructed ancestral E6 sequences, we observed the emergence and transition of splice site usage in vitro at key evolutionary nodes. Substituting 13 dispersed nucleotides from the SA409 type-ancestor into and SA509-type background induced SA409 usage while abolishing SA509. These nucleotides likely form critical regulatory elements governing splice acceptor selection. We propose that changes in RNA secondary structure or protein interactions underlie this shift. Such adaptations, uniquely acquired by carcinogenic HPVs during evolution, may be essential for proper E6/E7 expression and contribute to oncogenic potential.

Mechanisms of HPV16 oncogenicity regulation through splicing by TRAP150

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High-risk HPV E6 and E7 are two oncogenes directly associated with the immortalization of cells infected by HPV and the development of cancer, inhibiting p53 and pRB respectively. The expression of E6 and E6*I/E7 mRNAs is regulated by the alternative splicing of the same polycistronic pre-mRNA.

Previously, we have identified Enhancer one (Enh-1), a splicing-enhancer which induces splicing between SD226 and SA409, increasing E6*I/E7 mRNA expression. In this context, we also identified TRAP150, a DDR-related protein associated with alternative splicing, as a Enh-1-interacting protein. Notably, TRAP150 overexpression induced E6E7-pre-mRNA splicing, increasing E7 expression (Jönsson et al., 2023). Furthermore, the disruption of Enh-1 in a HPV16 subgenomic plasmid or episomal DNA failed to immortalize primary human keratinocytes, underscoring the critical role of the Enh-1-TRAP150 axis in regulating HPV oncogene expression.

To understand the mechanism behind TRAP150 regulation of E7 expression, we created multiple truncated versions of this protein. Out of these, those which included the N-terminal of TRAP150 increased E7 protein expression to the same degree as the full-length protein, the most efficient and smallest of which being N190, which showed a comparable or superior effect on E7 expression. This 190aa protein, a BCLAF-1 homolog (BHD) with two RS-rich regions, from the N-terminal of TRAP150, is nearly one fifth of the size of the full-size TRAP150's 955aa. The development of mutants lacking these RS-domains revealed the role these play in E7mRNA and protein expression. Additionally, five substitution mutants were developed, substituting specific amino-acid residues, from the BHD, homologous to BCLAF-1 to assess the potential role of HD in HPV16 mRNA splicing. Taken together, these findings indicate that the N-terminal portion of TRAP150 alone is sufficient to induce splicing, raising a model in which TRAP150 functions as an adaptor protein in the regulation of alternative splicing. Ongoing studies aim to identify TRAP150-interacting proteins involved in this process.

PTPN14 is a transcriptional target of specific isoforms of p63

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Human papillomavirus (HPV) E7 proteins target the tumor suppressor PTPN14 for degradation. PTPN14 is expressed specifically in basal epithelial cells and PTPN14 degradation activates the YAP1 oncoprotein, a driver of cell stemness and self-renewal. Our data support that by inactivating PTPN14, HPV E7 promotes the retention of cells in the in the basal layer of a stratified epithelium. To better understand the role of PTPN14 in epithelial biology and in HPV-infected cells, this project aims to determine how and why PTPN14 expression is restricted to basal epithelial cells.

We hypothesize that PTPN14 expression is regulated by one or more isoforms of p63. p63 transcription factors are master organizers of epithelial tissues and specific isoforms of p63 promote either proliferation or commitment to differentiation. We analyzed published single-cell RNA-seq data from human interfollicular epithelium to determine that PTPN14 and TP63 are expressed in the same subset of basal epithelial cells. In addition, there is a predicted p63/p53 binding site in intron 3 of PTPN14.

Here we report several observations indicating that certain p63 isoforms regulate the expression of PTPN14. We found that the binding site in intron 3 of human PTPN14 is highly conserved across primate species and is preferentially bound by p63, not p53. Using ChIP-qPCR, we observed enrichment for H3K27ac at the p63 binding site, indicating that this binding site acts as a super enhancer. We experimentally induced the expression of six p63 isoforms in keratinocytes, finding that two of them (TAp63β and TAp63γ) drive an increase in PTPN14 RNA levels. We are currently testing whether the p63 isoforms that promote PTPN14 expression control H3K27 acetylation at the p63 binding site and using CRISPR/Cas9 editing to develop cells that lack the p63 binding site in PTPN14. Our long-term goal is to determine whether PTPN14 acts as an effector of differentiation-promoting isoforms of p63 by inhibiting the cell stemness effector YAP1.

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

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Tumor viruses, including Merkel Cell Polyomavirus (MCPyV) and Human Papillomavirus (HPV), cause at least 15% of human cancers worldwide. MCPyV is causally associated with Merkel Cell Carcinoma (MCC), a rare yet aggressive neuroendocrine skin cancer with a high mortality rate. Approximately 80% of MCCs contain integrated regions of the MCPyV genome that facilitate continued expression of multifunctional viral oncoproteins called tumor (T) antigens. Similarly, HPV is the etiological agent of ~5% of cancers worldwide, including a high proportion of anal and oropharyngeal cancers. High-risk HPVs produce viral oncoproteins E6 and E7, expression of which becomes upregulated upon viral integration into the host genome. Both MCPyV and HPV oncoproteins inactivate host tumor suppressor pathways and drive carcinogenesis while preferentially recruiting DNA Damage Response (DDR) proteins to viral genomes, exacerbating host replication stress. In this study, we are testing the hypothesis that viral oncoproteins expressed by MCPyV and high-risk HPVs alter host replisomes to increase replication stress and potentiate their oncogenic potential, as the majority of cancers have dysregulated or mutated replication stress factors.

To identify mechanisms of virus-induced replisome manipulation, we used a quantitative unbiased screening method called Isolation of Proteins On Nascent DNA (iPOND), combined with stable isotope labeling by amino acids in cell culture (SILAC) and mass spectrometry to characterize proteins differentially recruited to actively replicating DNA in cells expressing these viral oncoproteins. We identified the HELLS chromatin remodeling complex as a putative target for MCPyV manipulation in epithelial cells expressing MCC-derived T antigens (tLT+ST), evidenced by significant enrichment of constituent proteins HELLS and CDCA7 in host replisomes. We also observe increased expression in 2D and 3D culture models comparable to the HELLS/CDCA7 upregulation seen in MCC cell lines. Our preliminary data suggests that T antigen-driven, rapid DNA synthesis is dependent on HELLS expression. Ongoing experiments will characterize interactions between iPOND-derived targets and viral oncoproteins through co-immunoprecipitation. Future experiments will determine how these interactions impact tumor development and malignant progression in preclinical mouse models. These studies will help identify tumorigenic processes that could serve as targets for novel cancer therapies.

KAT5 (TIP60) Is Required for ST- MYCL- Driven Chromatin Activation and Oncogenic Transcription in Merkel Cell Carcinoma

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Merkel cell polyomavirus (MCPyV)-positive Merkel cell carcinoma (MCC) depends on Small T antigen (ST)-driven transcriptional programs mediated by MYCL and EP400/TIP60 chromatin machinery. However, the functional role of TIP60 complex activity in sustaining these programs remains incompletely defined.

Here, we demonstrate that KAT5 (also known as TIP60) is required for ST-MYCL- driven chromatin activation and oncogenic transcription in MCPyV-positive MCC. Building on prior identification of ST-MYCL-TIP60 target genes, we used acute degron-mediated depletion to define the direct consequences of TIP60 complex disruption.

dTAG-mediated degradation of KAT5 occurs within 30 minutes and results in rapid loss of histone acetylation, followed by a marked anti-proliferative effect. Integration of RNA-seq, TMT proteomics, ChIP-seq, and ATAC-seq reveals coordinated downregulation of MYC target genes and cell-cycle pathways at both transcriptional and protein levels. Binding and Expression Target Analysis (BETA) identifies KAT5-dependent genes as enriched for MYC-driven transcriptional programs, consistent with a direct role in ST-MYCL-driven transcription.

Chromatin accessibility profiling demonstrates reduced accessibility at regions enriched for neuroendocrine transcription factor motifs, including NEUROD1, indicating that KAT5 is required to maintain the neuroendocrine transcriptional state. Acute EP400 degradation also impairs proliferation, although less strongly than KAT5 loss, suggesting that KAT5 functions as a central effector of TIP60 complex activity.

Together, these findings establish KAT5 as a critical chromatin effector that sustains ST-MYCL driven transcriptional programs and maintains the neuroendocrine state in MCPyV-positive MCC. These results identify TIP60 complex activity as a central dependency and potential therapeutic vulnerability in MCPyV-positive MCC.

Dissecting the role of genomic instability in human papillomavirus (HPV)-driven cancer initiation

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Human papillomavirus (HPV) infection is the causal factor in the vast majority of cervical and a growing proportion of oropharyngeal cancers. Beyond dysregulation of viral oncoprotein expression, the key determinants of transformation remain incompletely understood. To decipher the molecular and genomic events that occur along the HPV-driven tumourigenesis pathway, we and collaborators have developed a tractable in vitro model of HPV disease progression. HPV18-containing primary keratinocytes undergo long-term continuous propagation in culture, whereby they progressively acquire a plethora of cancer-like phenotypes and ultimately undergo transformation. This system recapitulates well the natural processes of carcinogenesis, including oncogene activation and genomic instability, allowing us to elucidate the mechanisms of HPV-driven disease.

Long-read Nanopore sequencing of cultures at multiple time points after HPV18 establishment revealed an accumulation of clonal DNA copy number alterations (CNAs) over time. Significantly, several of these CNAs can be observed in panels of early squamous cancer lesions and HPV18+ cancer patient samples. We have also employed single-cell whole genome sequencing to identify and track the evolution of rarer, sub-clonal genomic alterations. These approaches were combined with fixed cell immunofluorescence, which revealed that keratinocytes commonly display mitotic defects and chromosome mis-segregation following HPV establishment, the rates of which reach levels similar to those observed in established cancer cell lines. A large proportion of chromosome segregation errors were acentric chromosome fragments and chromatin bridges originating from DNA damage and/or replication stress, indicating this is a major source of genomic instability in HPV+ cells. Finally, our Nanopore sequencing data also provides exciting evidence of promiscuous, low-level HPV genome integration occurring far earlier than anticipated, followed by stringent selection to a single clonal integration site during prolonged culturing.

This study enhances our understanding of the temporal order of events from HPV establishment to cancer initiation. This will ultimately support strategies for the early detection and prevention of HPV+ cancers, for example in allowing us to develop novel biomarkers to predict cancer risk in HPV-infected individuals.

HPV16 E6 Sustains a DLL4–Notch1 Proliferative Reserve Cell Identity Linked to Cervical Cancer Prognosis

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High-risk human papillomavirus (HPV) infection is an important driver of high-grade intraepithelial neoplasia and cervical cancer. For reasons that remain unclear, disease develops most frequently in specialised epithelial niches, such as at the cervical transformation zone. Because disruption of Notch1 signalling, either by mutation or deregulated HPV E6 expression, can contribute to neoplastic progression in stratified squamous epithelia, this study examined whether Notch1 and its Delta-like ligands (DLL) help explain site-specific susceptibility to HPV-driven disease.

First, DLL1 and DLL4 expression was mapped in the various cell populations present in normal cervical biopsy specimens using immunofluorescence. Second, in vitro keratinocyte 2D monolayer models, growth assays, and organotypic raft cultures were used to assess the role of DLL-Notch signalling in uninfected epithelial cells and HPV16-associated neoplasia. An RNA sequencing-based signature was then applied to 279 HPV-positive cervical carcinomas from The Cancer Genome Atlas to infer tumour cell of origin and evaluate associations with clinical outcome. Finally, the prognostic impact of DLL4 expression was investigated in three independent cervical cancer cohorts.

The study identified three molecular subtypes of cervical carcinoma, with reserve cell-derived tumours being the most frequent and associated with a relatively favourable prognosis. Reserve cells were characterised as DLL1-negative/DLL4-positive — a proliferative phenotype that is observed transiently during squamous metaplasia and wound healing, but is maintained by HPV16 E6 in raft models of low-grade and, more prominently, in high-grade neoplasia. Elevated DLL4 expression was associated with an increased risk of cervical cancer recurrence and cancer-related death. Taken together, DLL4-Notch1 signalling marks a proliferative state that is transiently present during physiological processes but intrinsic to cervical reserve cells, making them strongly resemble neoplastic tissue even before HPV infection has occurred.

HPV Status Defines MBD2-Dependent Regulation of Cellular Plasticity in Head and Neck Cancer

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DNA methylation is a central epigenetic mechanism regulating gene expression and is frequently dysregulated in cancer. Methyl-CpG Binding Domain Protein 2 (MBD2), a key reader of DNA methylation, modulates transcription through recruitment of chromatin remodeling complexes such as NuRD1. While traditionally considered a transcriptional repressor, emerging evidence suggests that MBD2 functions are highly context-dependent cancer2–6 Here, we investigate the role of MBD2 in head and neck squamous cell carcinoma (HNSCC), with a focus on differences driven by human papillomavirus (HPV) status.

HPV-positive and HPV-negative HNSCC represent distinct biological entities with divergent clinical outcomes; however, the epigenetic mechanisms underlying these differences remain poorly defined. Using chemical inhibition and siRNA-mediated knockdown, we demonstrate that MBD2 exhibits striking HPV-dependent functions. In HPV-negative HNSCC models, MBD2 inhibition significantly reduced cell viability, clonogenicity, and epithelial-to-mesenchymal plasticity (EMP), a key driver of metastasis and therapeutic resistance. Transcriptomic analysis revealed downregulation of PI3K, MAPK, and TGF-β signaling pathways, and chromatin studies identified direct MBD2 binding at epithelial regulators including E-cadherin.

In contrast, HPV-positive cells were largely insensitive to MBD2 inhibition, showing minimal effects on viability and transcriptional programs. Instead, MBD2 was enriched at promoters of TGF-β pathway components, suggesting rewiring of epigenetic regulation in the presence of viral oncogenes. Functional assays confirmed a context-specific role for TGF-β signaling in mediating plasticity phenotypes.

Collectively, these findings identify MBD2 as a critical regulator of cellular plasticity in HPV-negative HNSCC and highlight its potential as a therapeutic target in this clinically aggressive subtype. More broadly, they demonstrate how HPV status shapes epigenetic dependencies in cancer.

Investigating Anti-Retroviral Therapy-Induced Stemness and Epithelial Mesenchymal Transition Shifts That May Enhance Susceptibility to HPV16 Driven Oropharyngeal Carcinogenesis

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Anti retroviral therapy (ART) has been associated with increased opportunistic high risk Human Papillomavirus 16 (HPV16) infections among HIV positive individuals, contributing to a higher incidence of HPV related cancers such as oropharyngeal squamous cell carcinoma (OPSCC). Although ART has significantly extended the lifespan of HIV positive patients, this increased longevity has coincided with a rise in non AIDS defining cancers (NADCs), including HPV positive OPSCC, at rates exceeding those of the general population. Epidemiological studies following HIV positive cohorts post ART initiation consistently report this upward trend. While extended life expectancy is a major contributing factor, it does not fully account for these cancer patterns. Most patients presenting with advanced stage OPSCC lack a documented history of pre malignant lesions, resulting in a scarcity of early stage clinical samples and limiting our ability to define biomarkers or identify therapeutic targets. Using the organotypic raft culture system, our lab has shown that ART treatment of primary gingiva epithelial tissue induces substantial transcriptional reprogramming, characterized by increased proliferation-associated pathways and decreased differentiation-associated pathways, changes consistent with a shift toward stem like and epithelial mesenchymal transition (EMT) permissive states. These findings suggest a potential mechanistic link between ART exposure and enhanced susceptibility to HPV mediated carcinogenic progression. Based on this, we hypothesize that ART acts as a cofactor that promotes HPV16 positive oral epithelial transition toward a more stem like and EMT permissive phenotype. To test this, we used multi color flow cytometry to quantify markers associated with stemness and EMT in continuously passaged HPV16 transfected human tonsil keratinocytes treated with Tenofovir, a nucleotide reverse transcriptase inhibitor (NRTI). We expect ART treatment to produce variable but consistent shifts in marker expression, revealing emerging patterns of (1) increased stem like characteristics, (2) EMT like loosening of epithelial structure, and (3) basal like expansion.

Integrative structural analysis of the interaction between the onco-protein E7 of human papilloma virus 16 and the clathrin adaptor protein 2

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Clathrin-mediated endocytosis (CME) is the primary endocytic pathway in eukaryotes and plays an essential role in cellular homeostasis and membrane recycling. Due to its central importance, many viruses have evolved strategies to hijack CME as a means of promoting viral survival and proliferation. High-risk human papilloma viruses (HPV) are the primary cause of cervical cancer and are associated to anogenital and oropharyngeal cancers.

The interplay between the onco-protein E7 of the high-risk HPV type 16 and the clathrin adaptor 2 (AP2), a key mediator of clathrin polymerization on the cytosolic membrane, has already been described and associated to the transformative potential of HPV-infected cells; nonetheless, the molecular details of this interaction are still unclear.

Here, we propose a mechanistic model of the binding between AP2 and HPV 16 E7. We characterized this event in vitro, with an integrative structural analysis, using mass spectrometry, molecular dynamics, biophysical techniques and single particle cryogenic electron microscopy. This led us to resolve the structure of the complex at 4 Ångstrom of resolution that shows how the interaction is orchestrated by the formation of two binding sites. Additionally, our results suggest that E7 alone is able to induce a large conformational change in AP2, sequestering it from the cytosolic reservoir. An event that would in turn impair the formation of new endocytic pits and consequently reduce the recycling of membrane receptors. The implications of these findings extend to drug discovery and rational design of therapeutics targeting HPV and HPV-related cancers.

SIRT1 inhibition disrupts replication-checkpoint control and reveals a p53-p21 vulnerability in HPV-driven cancers

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High-risk human papillomavirus (HPV)-driven cancers rely on host pathways that sustain proliferation despite viral oncogene-mediated suppression of tumour suppressor checkpoints. Among these, the NAD+-dependent deacetylase SIRT1 is consistently upregulated and functionally linked to HPV oncogenesis, yet how it supports proliferation and how its inhibition is translated into specific cell-fate outcomes remain unclear. Here, using isogenic normal oral keratinocytes (NOK) and HPV16 E6/E7-expressing counterparts, we identify SIRT1 as a regulator of replication–checkpoint coupling in HPV-driven cells.

Proteomic and functional analyses show that SIRT1 associates with components of the DNA replication machinery and sustains replication output. Pharmacological SIRT1 inhibition reduces MCM complex abundance and globally slows replication fork progression without overt replication stress or DNA damage in our model. Despite similar effects on replication dynamics, SIRT1 inhibition triggers distinct outcomes in the two cellular contexts, with NOK engaging a reversible G1/S checkpoint, whereas HPV16-positive cells show p53-p21 activation accompanied by accumulation in G1 and reduced S-phase entry, together with repression of mitotic regulators and, upon cell-cycle progression, a sustained G2/M arrest culminating in a senescence-like state. Mechanistically, p21 contributes to mitotic blockade through Cyclin B1 suppression, linking p53 reactivation to loss of mitotic competence. Importantly, SIRT1 inhibition enhances radiosensitivity both in vitro and in vivo, resulting in more durable tumour control in HPV-driven models.

Together, these findings identify SIRT1 as a non-oncogene dependency in HPV-associated cancers and uncover a p53-p21-dependent mitotic vulnerability that can be therapeutically exploited.

Diagnostic Utility of HPV-16/18 E6 mRNA Fold Change Measured by In-House qPCR in Cervical Cancer Triage for Differentiation of CIN3 and Cervical Cancer from Lower-Grade Lesions

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Cervical cancer is a significant public health issue in low- and middle-income countries due to limited screening and triage options. While HPV DNA testing is sensitive, it cannot differentiate transient from transforming infections. This study developed and validated an in-house semi-quantitative real-time PCR (qPCR) assay for HPV-16/18 E6/E7 mRNA to enhance risk stratification.

A SYBR Green–based qPCR assay was optimized using genotype-specific primers for HPV-16/18 E6/E7 and the RPS9 housekeeping gene. Primer concentration, cycling conditions, melt-curve specificity, and Ct cutoffs were systematically determined. Ct thresholds were established using 40 HPV DNA–positive clinical samples (25 E6/E7 mRNA–positive and 15 negative), supported by ROC analysis and Youden's index. Comparative analytical validation against a commercial assay assessed sensitivity and specificity.

Clinical evaluation was performed on 70 HPV DNA–positive cervical swab samples with histopathologically confirmed diagnoses ranging from chronic cervicitis to invasive carcinoma. E6 mRNA positivity increased significantly with disease severity ($p<0.001$) and showed a strong inverse correlation with HPV DNA load ($R^2 = -0.89$, $p<0.001$). ROC analysis identified E6 fold change (E6 FC) as the most accurate marker for distinguishing CIN3+ lesions from lower-grade disease (AUC = 0.9329). An optimal cutoff of E6 FC ≥ 0.648 yielded 95.24% sensitivity and 86.36% specificity. The in-house assay demonstrated robust analytical performance (85% sensitivity, 100% specificity). These findings highlight E6 fold change as a reliable molecular triage marker and support the use of this effective in-house qPCR assay to strengthen cervical cancer screening and triage strategies.

Macaca mulatta Papillomavirus Type 1 and Simian Immunodeficiency Virus Co-Infections in Rhesus Macaques to Model Persistent Oral Human Papillomavirus and Human Immunodeficiency Virus Co-Infection and Oropharyngeal Cancer Induction

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HPV-driven oropharyngeal squamous cell carcinoma (OPSCC) is rising notably in high-income countries with marked male predominance. HIV-induced immunosuppression increases HPV acquisition, persistence, and risk of cancer. People living with HIV tend to have roughly 2- to 4-fold higher oral HPV prevalence and ~1.5- to 3-fold higher OPSCC risk compared with immunocompetent adults. The mechanisms driving persistent oral oncogenic HPV infection, precancer development, and HIV-HPV co-infection remain poorly understood, and no animal model, thus far, recapitulates this biology.

Our prior work found that Macaca mulatta papillomavirus type 1 (MmPV1), phylogenetic-ally related to oncogenic HPV16, is a robust surrogate for HPV-associated disease in female rhesus macaques (RMs). In MmPV1 seronegative and anogenital viral DNA negative animals, cervical inoculation with $\geq 10^9$ MmPV1 viral genome equivalents (VGE) resulted in persistent infections with neoplasia developing in those with highest viral loads.

We hypothesize that oral MmPV1 inoculation in SIV-immunosuppressed male RMs will produce persistent oropharyngeal infections progressing to precancerous lesions that model HPV-driven disease in humans. Three male RMs found to be MmPV1 capsid IgG- and oral viral DNA-negative were infected with 100 TCID50 of SIVmac239 for 12 weeks to deplete CD4+ T cells. Each of three oral sites (palatine tonsil, tongue base, soft palate) were inoculated with MmPV1 at ~10^{11} VGE using a GREER Pick® to puncture the mucosa and deliver the inoculum. Longitudinal sampling is ongoing and confirms sustained SIV loads, low CD4+ cell counts, and increased oral MmPV1 DNA levels by 1 to 2-months post inoculation in all three RMs. Oral brush tonsil sampling from one RM revealed dysplastic cells at 3-months post MmPV1 inoculation; MmPV1 RNA in situ hybridization (ISH) is pending. Plasma and saliva collected every two months will be analyzed for cytokines and chemokines using Meso Scale Discovery immunoassays, with immune mediator levels correlated with MmPV1 persistence and pathology. Terminal histopathology, p16 staining, MmPV1 RNA-ISH will assess precancerous progression in oral tissues. This study aims to establish the first physiologically relevant non-human primate model of oropharyngeal papillomavirus infection and tumorigenesis under SIV-induced immunosuppression, providing a platform to elucidate viral persistence, carcinogenic mechanisms, and to develop therapeutic strategies for HPV-associated OPSCC.

MicroRNA-Mediated Regulation of Matrix Metalloproteinase Inhibitors in HPV-Transformed Cells

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The extracellular matrix (ECM) provides a physical and biochemical scaffold for cells within the tumor tissue. Far from playing a passive role in cancer progression, it is responsible for modulating a variety of processes, including cell proliferation, migration, and metastasis, among others. During the carcinogenic process, a disruption in the fine balance of its components, characterized by increased levels and activity of enzymes known as matrix metalloproteinases, is commonly observed. This scenario may result, at least in part, from the downregulation of the biological inhibitors of these proteases, namely RECK and members of the TIMP family. We have reported this pattern in precursor lesions, cervical cancer samples, and their derived cell lines. Interestingly, the expression of E6/E7 from Human Papillomavirus 16 (HPV16) is negatively correlated with RECK/TIMP-2 in primary human keratinocytes, suggesting the effect of HPV oncoproteins in this downregulation. However, the underlying molecular mechanisms remain unclear. In the present study, we evaluated the role of microRNAs in the negative regulation of RECK/TIMP-2 in HPV-transformed cells. These genes are poorly mutated in cervical cancers (approximately 1%), supporting the idea of post-transcriptional modulation of their expression.

The microRNAs miR-21-5p and miR-429 were predicted to regulate RECK and TIMP-2 expression, when using the TargetScan, microT-CDS and miRDB computational tools. Transcriptomic data available in the Gene Expression Omnibus (GEO) repository indicate higher expression of these microRNAs in cervical cancer samples, as well as in precursor lesions. miR-21-5p and miR-429 were inhibited using antisense oligonucleotides in SiHa (HPV16+), SW756 and HeLa (both HPV18+) cells. In agreement with the in silico analyses, qPCR and Western blot assays revealed increased levels of RECK and TIMP-2 in all three cell lines upon microRNA inhibition. In parallel with the increase of these inhibitors, zymography assays revealed a reduced proteolytic activity of MMP-9, a target of RECK and TIMP-2. Furthermore, the loss of function of these molecules led to a decreased rate of cell migration and viability. Collectively, these data support a role for miR-21-5p and miR-429 in the negative regulation of RECK and TIMP-2, contributing to the malignant phenotype of cervical cancer cells.

Estrogen Receptor α (Esr1) Mediates Estrogen's Ability to Promote Papillomavirus-induced Cutaneous Disease in Male and Female Mice

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Persistent papillomavirus infection is a key driver of neoplastic progression in both mucosal and cutaneous epithelia. Although estrogen is a well established cofactor in HPV associated cervical carcinogenesis, its role in cutaneous papillomavirus disease has remained poorly understood. Using a murine papillomavirus type 1 (MmuPV1) infection model in immunocompetent FVB/N mice, we investigated whether estrogen influences viral pathogenesis in infected cutaneous epithelia. Estrogen treatment significantly increased wart incidence and growth independent of sex and was associated with pronounced epithelial hyperplasia, dysplasia, koilocytosis, and features of invasive squamous cell carcinoma. Systemically, estrogen reduces circulating leukocytes, while locally suppressing adaptive immune infiltration and enriching neutrophils within lesions, indicating broad immunosuppression coupled with selective innate enhancement. MmuPV1 E1^E4 and L1 RNA and protein levels were elevated in warts of estrogen-treated WT mice, indicative of enhanced productive infection. Importantly, Esr1 germline knockout mice exhibited significantly reduced wart incidence and size in both males and females, lower MmuPV1 E1^E4 and L1 transcript levels, coupled with the absence of estrogen induced depletion of circulating immune cells, demonstrating that ERα signaling mediates estrogen-driven epithelial proliferation, immune modulation, disease progression and productive viral infection. Collectively, these novel findings indicate that estrogen is a potent cofactor in cutaneous papillomavirus disease, acting through ERα-dependent pathways to drive virally induced neoplastic diseases, including cancer.

Single-cell RNA sequencing reveals potential tumor-associated macrophage-mediated immunosuppression in equine papillomavirus type 2-associated squamous cell carcinoma

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Introduction: Equine papillomavirus type 2 (EcPV-2)-associated genital disease in horses recapitulates the full spectrum of HPV-driven carcinogenesis, from benign precancerous lesions to invasive squamous cell carcinoma (SCC). This positions the horse as a naturally occurring large-animal model. Despite the clinical importance, underlying molecular pathomechanisms remain poorly understood.

Objective: We aimed to analyze cellular and molecular dynamics during EcPV-2-associated disease progression using single-cell RNA sequencing.

Materials and Methods: We used 10XGenomics single-cell RNA sequencing on fresh tissue samples from 7 naturally infected male individuals. Differential gene expression analysis was performed on SCC samples. CellChat, an in silico tool, was used to visualize predicted cell-cell communication networks.

Results: We successfully annotated cell types, including immune cells, endothelial cells, fibroblasts, and epithelial cells, thereby providing a comprehensive tissue atlas. Differential gene expression analysis revealed enriched expression of SPP1, GPNMB, LGALS3, and FABP5 in macrophage and monocyte-derived macrophage clusters, known markers of tumor-associated macrophages (TAMs) associated with an immunosuppressive microenvironment. Using CellChat, we identified both macrophages and monocyte-derived macrophages as dominant signal senders in SCC. Furthermore, we observed elevated SPP1/CD44 interactions, a pathway linked to tumor progression.

Conclusion: This is the first single-cell characterization of the EcPV-2 tumor microenvironment. Our data suggest that SCC progression is driven by immune suppression, chronic inflammation, and the establishment of a tumor-supportive microenvironment, with TAMs acting as central mediators predominantly through SPP1–CD44 signaling. These findings provide a basis for further investigation of TAM-mediated immune evasion in papillomavirus-driven carcinogenesis.

Beyond the Infected Cell: Reframing HPV Pathogenesis Through the Microenvironment

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Human papillomavirus (HPV)-driven carcinogenesis has historically been studied through an epithelial-centric lens. Foundational studies have emphasized viral oncogene function, epithelial transformation, and host signalling within infected keratinocytes. Growing evidence suggests that viral persistence, episomal maintenance, malignant progression, and therapeutic responses are also profoundly shaped by the surrounding microenvironment. We previously demonstrated that fibroblasts are active regulators of HPV+ epithelial cell biology. Using co-culture systems, we found that fibroblasts support HPV episome maintenance in both keratinocyte and cancer models, whereas fibroblast withdrawal increased viral integration events, as measured by the exonuclease assay. RNA sequencing further identified transcriptional reprogramming associated with fibroblast withdrawal, including pathways linked to proliferation, differentiation, histone regulation, and DNA damage/repair responses. Consistent with these findings, fibroblast withdrawal increased genomic instability, as demonstrated by elevated DNA damage in comet assays and aberrant replication fork dynamics measured by DNA fiber analysis.

Clinically, HPV+ epithelia represent only one component of a multifaceted local microenvironment. While epithelial-focused models are highly informative, they provide only a partial understanding of viral persistence and disease progression. Beyond fibroblasts, the broader stromal-immune architecture likely plays additional roles in HPV+ disease. Local fibroblasts can influence recruitment, polarization, and function of macrophages, T cells, and other infiltrating immune populations through cytokine gradients, extracellular matrix remodelling, and metabolic crosstalk in an HPV-dependent manner. Our investigations highlight IL6/STAT3 signalling as a key regulatory pathway. ELISA analyses demonstrate elevated IL-6 levels in ex vivo slice cultures of primary HPV+ oropharyngeal tumors, while transcriptional analyses of keratinocyte models implicate E6 as a dominant driver of IL6 dysregulation. Overall highlighting viral genome context, epithelial differentiation, and stromal interactions as regulators of this pathway. We propose that HPV pathogenesis should be viewed as a multicellular ecosystem in which viral oncogenes function within epithelial cells that continuously interact with stromal and immune compartments. Integrating these interactions is essential for understanding disease heterogeneity and identifying new biomarkers and therapeutic vulnerabilities. Future progress in HPV+ biology will require models that better recapitulate the complexity of viral-host interactions.

HPV Infection at the Cervical Transformation Zone: Reserve Cell Biology, and the Risk of HSIL Recurrence

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Human papillomaviruses establish a reservoir of infection in epithelial basal cells, with viral synthesis and release dependent on cell differentiation. At the cervical TZ, reserve cells serve as stem-like progenitors, undergo squamous metaplasia, and are the principal targets of hrHPV infection. Although LLETZ removes most high-grade squamous intraepithelial lesions (HSIL), post-treatment recurrence remains disproportionately high among women living with HIV (WLHIV). We therefore examined the spatial distribution and stem-like characteristics of reserve cells, deregulation of hrHPV E6/E7, and the local immune microenvironment at the TZ.

Using multiplex immunofluorescence, large-scale digital imaging, and RNAscope, we analysed LEEP biopsies from WLHIV (n=20) and women without HIV (n=13), all of whom underwent at least two surgical excisions, for recurrent or persistent HSIL. We characterised disease extent, crypt and excision depths, hrHPV E6/E7 expression, and immune cell distribution across serial tissue sections. p16/MCM and E4 distinguished abortive from productive infection, while p63, K17, and K5 identified reserve cell populations at crypt entrances and across the TZ.

WLHIV showed a significantly higher proportion of HSIL across the TZ. After repeated excision, HSIL became increasingly confined to crypt entrances, where the expanding K5/K17/p63 reserve cell population acts as a persistent stem-like reservoir. RNAscope revealed that E6/E7 deregulation was prominent at this site, independent of the surface epithelium at the TZ. However, crypts harbouring histologically normal reserve cells were consistently localised at depths exceeding those of neoplastic crypts, suggesting that reserve cell positioning within crypts' microenvironment is regulated and that standard excision may fail to eliminate this reservoir.

Analysis of the stromal immune microenvironment revealed tertiary lymphoid follicles adjacent to HSIL-involved crypt entrances. However, T cells were absent from infected crypts with high E6/E7 mRNA and instead localised to the surface epithelium with lower E6/E7 levels. T cell density was significantly lower in WLHIV, indicating localised depletion independent of systemic immune reconstitution on ART. These observations support E6/E7-driven immune evasion at crypt entrances, linking reserve cell persistence and immune failure to the anatomical basis of HSIL recurrence. These findings have implications for redefining treatment margins and therapeutic strategies in high-risk populations, particularly WLHIV.

Spatial analysis of the chronic hepatitis B and D liver reveals new aspects of viral pathogenesis and cancer

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Hepatitis B and D viruses are a major health problem with >20 million infected people worldwide at risk of developing cirrhosis and liver cancer. HDV coinfection associates with an accelerated course of liver disease, however, the molecular pathways leading to cirrhosis and hepatocellular carcinoma (HCC) are not well defined. Our limited knowledge of the mechanism(s) underlying hepatitis B and D pathogenesis and lack of in vitro or small animal models that develop disease are major roadblocks in the development of new curative treatments.

Spatial transcriptomics provides unprecedented insights into patterns of gene expression, making it a valuable tool for studying tissue architecture and disease aetiology. The latest advances in tissue transcriptomics allow single-cell resolution and integrating probes to quantify both viral and host gene expression offers unique insights into the behaviour of individual infected hepatocytes in the liver. Our recent studies of HDV-HBV co-infected and HBV mono-infected cases show a robust upregulation of interferon, inflammatory, epithelial-mesenchymal transition and pro-oncogenic signalling pathways in hepatitis B/D co-infected and HBV-integrant bearing hepatocytes.

Extending our transcriptomic analysis from virus infected cells to adjacent hepatocytes and non-parenchymal cells allowed the identification of viral niches that were enriched for Natural Killer, fibroblasts, T cells and Kupffer cells. Importantly, we noted differences in immune cell recruitment to HDAg, HDAg/HBsAg and HBs-expressing hepatocytes. These data support a model where virus infected cells create spatially segregated communities with mesenchymal/ immune cell populations and we identify a key dependency for liver zonation. Integrating the transcriptomic data with multiparametric immune markers and tissue pathology uncovers the key cell types associating with inflammatory damage. These findings highlight the power of spatial molecular virology combined with pathology to uncover the cellular and molecular basis of viral-associated liver disease.

Single-Cell Analysis of HPV-Dependent and HPV-Independent Epithelial Transformation Pathways in HNSCC

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Persistent infection with high-risk human papillomaviruses (HPVs) accounts for approximately 5% of all human cancers and a substantial subset of head and neck squamous cell carcinomas (HNSCC). Despite the availability of prophylactic vaccines, the incidence of HPV-positive (HPV+) HNSCC continues to rise, reshaping the etiologic landscape of HNSCC. Current treatment paradigms were largely developed for HPV-negative (HPV-) disease, even though HPV+ tumors typically exhibit distinct anatomic origins, molecular drivers and improved therapeutic response. Further elucidation of HPV-associated carcinogenic pathways is needed to inform prevention strategies and refine treatment, including potential de-escalation approaches for HPV+ HNSCC.

Single-cell RNA sequencing enables high-resolution characterization of cellular and molecular reprogramming during malignant transformation. We performed a joint analysis of published single-cell transcriptomic datasets from laboratory-generated normal and HPV-infected stratified squamous epithelium (SE) as well as clinical HPV+ and HPV-HNSCC specimens, to identify cell populations and pathways driving HPV-dependent and HPV-independent transformation. Preliminary pseudotime analyses implicate HPV-enriched superficial (HIDDEN) cells and differentiated cycling SE cells as likely contributors to HPV+ HNSCC development, whereas basal SE cells predominate in the HPV- context. Ongoing transcriptome analyses of these populations seek to define dysregulated pathways along distinct cancer evolutionary trajectories, with the goal of informing novel preventive and therapeutic interventions for HPV+ (and HPV-) disease.

HPV16 genomic status stratifies risk of disease recurrence in oropharyngeal squamous cell carcinoma

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Purpose: A subset of patients with human papillomavirus–positive oropharyngeal squamous cell carcinoma develops disease recurrence despite generally favorable outcomes. Reliable biomarkers for risk stratification remain lacking. We investigated whether comprehensive viral genomic profiling refines prognostic classification.

Experimental Design: Tumor samples from 100 patients with p16-positive and human papillomavirus DNA–positive oropharyngeal squamous cell carcinoma enrolled in two independent cohorts were analyzed using targeted viral capture sequencing. Viral genotype, sublineage, single-nucleotide variation, viral–host integration sites, viral structural variants, clonality, and viral copy number were characterized. Associations with cumulative incidence of progression, progression-free survival and overall survival were assessed at 60 months.

Results: Human papillomavirus 16 was detected in 94 tumors, of which 83 belonged to the HPV16_A1 sublineage. Within this sublineage, 20 tumors harbored strictly episomal viral genomes, whereas 63 exhibited integration and/or viral structural variants. No progression events occurred in the strictly episomal HPV16_A1 subgroup, while 12 occurred among tumors HPV16_A1 with genomic alterations. At 60 months, no progression events were observed in strictly episomal HPV16_A1 tumors, compared with a 23.1% cumulative incidence of progression in HPV16_A1 tumors with genomic alterations. Previously reported high-risk single-nucleotide variants and a novel upstream regulatory region variant were associated with worse progression-free survival. Integration sites were enriched in open chromatin and promoter regions and clustered within viral E2 and E4 genes.

Conclusions: Within human papillomavirus 16 A1 tumors, viral genomic architecture defines clinically distinct subgroups. Strictly episomal (WT) genomes identify a low-risk population, whereas integration and structural variants characterize higher-risk disease.

FAM151B-DT: A Dual-Function LncRNA Linking HPV Status to Prognosis in Head and Neck Cancer

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Head and neck cancers comprise biologically distinct diseases in which HPV status reshapes tumour behaviour, patient prognosis, and therapeutic response. Defining the biological basis of this divergence is essential to explain contrasting clinical behaviour and to guide the development of more precise therapeutic strategies.

Long non-coding RNAs (lncRNAs) are key regulators of cellular behaviour and can be manipulated in cancer. Exploring lncRNA molecular signatures in patients may help unravel biological pathways contributing to different clinical outcomes, providing biomarkers for predicting tumour aggression and facilitating development of targeted therapeutics.

Whole transcriptome sequencing was performed on HPV16-positive oropharyngeal and HPV-negative oral cavity tumours with matched proximal healthy tissue. Differential expression analysis revealed increased expression of the uncharacterised lncRNA FAM151B-DT in HPV-positive tumours. Analysis of The Cancer Genome Atlas (TCGA) HNSCC dataset showed that FAM151B-DT expression correlates with improved patient survival. This expression profile is reflected across HPV-positive and HPV-negative HNSCC cell lines, specifically involving the FAM151B-DT transcript isoforms -201 and -202. Expression and subcellular localisation of these transcripts are associated with HPV oncoproteins, with FAM151B-DT showing predominantly cytoplasmic localisation in HPV-containing cells.

Knockdown of FAM151B-DT causes HPV-positive HNSCC cells to adopt an aggressive amoeboid mode of migration, becoming more invasive with a rounded morphology capable of producing bleb-like protrusions. This phenotype is supported by increased expression of ROCK1 and ROCK2, with ROCK inhibition reducing invasion. Amoeboid migration is accompanied by increased resistance to chemotherapeutic agents, including docetaxel, in collagen-suspended knockdown cells.

Conversely, overexpression of FAM151B-DT increases cell proliferation. We have evidence that FAM151B-DT contains a novel open reading frame capable of being translated into a stable micropeptide. Mutation of the start codon significantly decreases cellular proliferation compared to the wild-type transcript, suggesting that both the lncRNA and the micropeptide contribute to proliferative capacity. We hypothesise that increased cell growth sensitises cells to chemotherapeutic agents, correlating with improved patient prognosis.

Together, these findings provide new insight into the role of the lncRNA FAM151B-DT in regulating cell proliferation and invasion, consistent with a ‘Grow or Go’-like trade-off in HPV-driven cancer.

Cervical cell lift enables tissue-sparing surface mapping, biological grading, and longitudinal tracking of HPV-associated cervical lesions

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Introduction: Persistent high-risk human papillomavirus (HPV) infection in the cervical transformation zone causes cervical cancer. Although most sexually active individuals acquire high-risk HPV, only a small proportion develop precancerous lesions or cancer. Cervical screening must therefore identify, grade, and treat lesions with malignant potential, rather than simply detect HPV infection. Current screening and triage stratify risk at patient or sample level, but do not map lesion location, extent, or biological grade across the cervical surface.

Methods: To enable lesion-level detection and grading, we developed Cervical Cell Lift (CCL), a tissue-sparing surface sampling method that captures epithelial cells while preserving their native spatial organisation. By retaining positional information, CCL converts the sampled cervical surface into an analysable cellular map rather than a pooled cytology specimen. CCL integrates cytomorphology with biomarker staining to generate lesion maps that identify, localise, and grade high-grade HPV-associated disease. Here, nuclear morphology and haematoxylin-based staining were combined with immunostaining markers, including MCM to identify precancerous epithelial change and E4 to define productive viral infection, providing both high-grade lesion detection and biological context. This approach enables marker-driven spatial grading while providing a flexible platform for integrating alternative biomarker panels with morphology-based analysis.

Results: Using CCL, we generated spatially resolved maps of HPV-associated epithelial disease across the cervical surface. High-grade precancerous lesions were detected as discrete regions with abnormal nuclear morphology and strong MCM positivity, enabling lesion localisation and biological grading while preserving positional information. E4 staining identified productive viral infection spatially distinct from, or adjacent to, high-grade regions, highlighting biological heterogeneity within HPV-associated lesions. The combination of morphology, MCM, and E4 allowed regions dominated by high-grade precancerous change to be distinguished from areas showing productive viral activity. Matched histology showed close correspondence between CCL-defined high-grade regions and the anatomical location and pathological grade of underlying lesions.

Conclusions: CCL therefore shifts HPV-associated disease assessment from determining whether abnormal cells are present to mapping where high-grade lesions are located and how they are biologically graded. Because CCL preserves positional information through tissue-sparing surface sampling, it enables repeated assessment of the same cervical region over time, supporting longitudinal tracking of lesion persistence, progression, regression, and treatment response. CCL provides a platform for studying the natural history and spatial biology of HPV-associated cervical precancer, including where access to colposcopy, pathology, or advanced immunostaining is limited.

A highly pathogenic HPV16 sub-lineage exhibits sustained oncogene expression and genome amplification

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High-risk human papillomavirus type 16 (HPV16) accounts for approximately 60% of HPV-related cervical cancers globally and exhibits significant genetic diversity. The most prevalent lineage A (European) comprises four sub-lineages (A1–A4), with A3 and A4 occurring predominantly in Asian populations. Lineage D is the second most common in the Americas and is strongly associated with increased viral persistence and an elevated cervical cancer risk, particularly sub-lineage D2 (African-American) and D3 (Asian- American). Despite well- described sub-lineage–disease associations, sub-lineage–specific viral and host determinants that control the HPV life cycle and pathological consequences of infection remain poorly defined. This gap largely reflects the lack of well-characterized immortalized cell models harboring distinct HPV16 variants for comparative studies. Identifying host factors that promote sub-lineage–specific viral persistence, genome amplification and pathogenic outcomes is therefore critical for the development of targeted antiviral and cancer preventive strategies.

In this study, we established organotypic epithelial raft cultures using normal immortalized keratinocytes (NIKS) harboring HPV16 sub-lineages A1 and D3. DNA and RNA in situ hybridization revealed that HPV16 D3, compared to A1-infected rafts undergo viral genome amplification in superficial epithelial layers, accompanied by late L1/L2 transcript expression and persistent E6/E7 transcript expression in superficial layers. Consistent with these molecular differences, D3-infected epithelia displayed basal layer expansion, increased suprabasal proliferation, and impaired stratified differentiation. These phenotypes recapitulate key features of the upward expansion of p16+ and Ki67+ cells characteristic of cervical intraepithelial neoplasia (CIN) grades 2 and 3.

Collectively, our findings identify the HPV16 D3 as a sub-lineage prone to a productive life cycle, and a model system wherein candidate sub-lineage–specific mechanisms that promote a more aggressive disease course can now be investigated.

Contribution of [68Ga]68Ga-RGD PET in the evaluation of adulthood gliomas associated with suspected viral aetiology

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Objective: 68Ga-[integrin αvβ3] PET/CT has a Tripeptide Arg-Gly-Asp (RGD) sequence, which exhibits high binding affinity and specificity for integrin αvβ3. This prospective study aims to evaluate the role and contribution of 68Ga-[integrin αvβ3]PET/CT in staging and restaging primary brain cancer specifically glioma patients.
Approximately 20% of cancer aetiology can be attributed to viruses. To date six herpes viruses, human adenoviruses (A - G) and other neurotropic viruses e.g. EBV (Epstein-Barr Virus), HSV-2 (Herpes Simplex) and HHV-6 (Human Herpesvirus 6) have been identified in glioma patients using PCR (Polymerase Chain Reaction) assay used with immunolabeling.
Additional studies have shown definitively that HHV-6 integrates into the subtelomeric regions of the host's chromosomes by homologous recombination; this gives it the ability to be inherited therefore explaining familial gliomas even though the pattern is not yet clear. Although PET is a molecular imaging tool and can detected aberrations at a molecular level prior to morphology; this tool can be used in a non-invasive manner to either use vectors or detect infections and inflammatory conditions.

Materials and Methods: In this prospective and ongoing study, 25 patients diagnosed with Primary brain cancer of glioma origin, who had previously undergone MRI (Magnetic Resonance Imaging) and a [68Ga]-RGD PET imaging, were included. 68Ga-RGD PET/CT imaging was performed prior to surgery and within a week of an MRI brain scan. Written informed consent was obtained from all patients. The primary tumor uptake values obtained from 68Ga-RGD PET/CT were measured and compared with normal scalp background, normal liver parenchyma, liver metastases, blood pool, and distant organ metastases. These parameters were then compared with MRI i.e. morphological imaging. Consent for HIV testing was not granted in all patients however a previous study demonstrated a prevalence of 66,6% in female patients.

Results: A statistically significant increase in all metabolic parameters was observed in high-grade glioma cases imaged with 68Ga-RGD PET ($p < 0.05$ for SUVmax, SUVmean, SUVpeak, TLG), indicating possible recurrence or residual tumour. Spearman's test indicated a statistically significant correlation ($p = 0.002$), rho = 0.6 between PET and MRI. The volume of the lesions on MRI vs PET in 25 patients demonstrated a linear correlation ($p = 0.001$; rho = 0.603). Additionally Spearman's rank correlation was used to assess the relationship between MRI lesion and volume and PET metabolic parameters because of the "small" sample size ($n = 25$) and the likely non-normal distribution of imaging variables.

Discussion and Conclusion: These cases may illustrate a use of 68Ga-RGD PET as a surrogate marker not only for detection of glioma recurrence but an avenue for viruses to be used as vectors for the therapeutic management of gliomas in order to give better outcomes. Epigenetics and genetics should be in the primary multidisciplinary team in order to assess the familial patterns of inheritance. Viral testing should be considered standard in these patients.

Hyperactivating the p53 and RB1 tumour suppressor gene for cervical cancer therapy

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Introduction: Cervical cancer is primarily caused by persistent infection of Human papillomavirus (HPV). The E6 and E7 oncoproteins produced by HPV mainly contribute to the carcinogenic process by dysregulating the host p53 and pRb tumour suppressor pathways, respectively. CRISPR activation (CRISPRa) is a genetic tool in which catalytically inactive cas9 (dcas9) is fused with transcriptional activators to induce gene expression. Using CRISPRa to activate the tumour suppressor genes is a novel concept in cervical cancer gene therapy. Here we use a doxycycline-inducible second-generation CRISPRa system to overexpress p53 and pRb.

Aim: To investigate the use of CRISPRa to overexpress the p53 and Retinoblastoma gene (RB1) to overcome the effects of E6 and E7 and reestablish normal cell cycle control.

Methodology: Four guide RNAs (gRNA) were designed to activate either the p53 or RB1 gene and loned into a doxycycline-inducible cassette in a third-generation lentiviral system. The inducible gRNA cassette was transduced into HPV-positive HeLa cells expressing dCas9. Finally, induction was performed using doxycycline to activate the CRISPRa system. Various protein markers indicative of p53 or pRB activation, such as p21. MDM2, Cyclin E1 or Cyclin A2 expression, were measured as an indicator of activiation and the restoration of apoptosis and/or the cell cycle.

Results: Hyperexpression of p53 induced a 15-fold increase in protein levels over controls and led to HPV+ cervical cancer cell killing and reduced cell proliferation. Hyperexpression of pRb led to a more modest increase (5 fold) at 24 hours post doxycycline induction. Reduced expression of cyclin E1 and Cyclin A2 were observed in pRB overexpressed HeLa cells.

Conclusion: The doxycycline-inducible CRISPRa system effectively overexpressed both p53 and pRb, demonstrating its suitability for targeted gene activation. Elevated levels of pRb facilitate the re-establishment of normal cell cycle regulation while p53 overexperssion led to cell death.

Key words: Human papillomavirus, E6/E7 oncoproteins, p53/pRb, Cervical cancer, CRISPRa

Dual targeting of HPV E6 and cancer mutant p53 by a DARPin defines a new p53 rescue strategy

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Background: The tumour suppressor p53 is the most frequently inactivated protein in human cancers, either through mutation or viral interference. In high-risk HPV-associated cancers, the viral oncoprotein E6 promotes degradation of p53 via E6AP. In contrast, most non-viral tumours harbour missense mutations within the p53 DNA-binding domain (DBD), leading to structural instability. Notably, a substantial fraction of these alterations represents temperature-sensitive mutants that retain a latent capacity for refolding into a functional conformation, defining a therapeutically actionable vulnerability. A unified strategy to restore p53 activity across both contexts remains a major unmet need.

Objectives: To develop a single therapeutic strategy that reactivates p53 across HPV-driven and p53-mutant cancers, and to establish a broadly applicable, mutation-class–directed framework for p53 rescue.

Methods: Using ribosome and phage display, we identified a designed ankyrin repeat protein (DARPin) targeting the p53 DBD. Biochemical, structural, and cell-based assays were performed to characterize binding, specificity, and functional reactivation of p53 in HPV-positive and p53-mutant cancer models. p53 mutant rescue was assessed across a panel of cancer-associated variants. Translational potential was evaluated using mRNA/lipid nanoparticle (LNP)-based delivery strategies.

Results: The p53-DARPin binds p53 with high affinity at the E6/E6AP interaction interface, thereby blocking viral-mediated degradation and restoring p53 activity in HPV-positive cells. In HeLa and SiHa models, this induces canonical p53 target genes and reduces cell viability. Beyond viral contexts, functional screening demonstrates that the p53-DARPin stabilizes and reactivates the majority of temperature-sensitive mutants by restoring DBD folding, while DNA-contact mutants remain inactive, consistent with their mechanistic defect. Reactivated p53 induces growth-suppressive transcriptional programs and robust antiproliferative effects across cancer cell lines.

Conclusion: The p53-DARPin enables dual and mechanistically distinct reactivation of p53, establishing a broadly applicable p53 rescue strategy. By simultaneously blocking HPV E6-mediated degradation and stabilizing mutant p53, this strategy provides a unified therapeutic framework for both viral and non-viral cancers, with strong potential for combination therapies, including chemo- and radio-sensitization.

A novel BK polyomavirus replication assay reveals strain-dependent sensitivity to TNF-α and broad antiviral activity of bexarotene

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No approved therapies exist for BK polyomavirus (BKPyV) reactivation, a leading cause of nephropathy after kidney transplantation. Antiviral development has been impeded by the absence of reliable in vitro replication assay for the clinically predominant archetype (ww) strains in primary renal proximal tubule epithelial cells (RPTEC); most prior studies relied on laboratory-adapted strains with rearranged non-coding control regions (rr-NCCR).

Here, we optimized RPTEC culture conditions to support efficient replication of patient-derived ww-BKPyV while preserving archetype NCCR integrity. Using this assay, we found strain-dependent effects of the inflammatory cytokine TNF-α: it enhanced replication of rr-BKPyV, consistent with prior reports, yet unexpectedly suppressed ww strain replication. By contrast, the TH1 cytokine IFN-γ inhibited both ww and rr strains, even in the presence of TNF-α. Mechanistic analyses using siRNA knockdown, as well as chimeric and mutant viruses, demonstrated that TNF-α responsiveness is governed by NCCR architecture, and that enhancement of rr-BKPyV replication requires NF-κB p65 signaling.

We next applied this assay to screen antivirals against both ww and rr clinical isolates, focusing on CDK inhibitors and retinoids. A viral dynamics model quantifying net antiviral benefit (NAB), enabled discrimination of genuine antiviral activity from cytostatic effects. Bexarotene, an approved therapy for cutaneous T-cell lymphoma, exhibited the most favorable profile: a positive NAB across a broad concentration range, an EC50 comparable to peak plasma levels in patients, and consistent activity against all ww and rr isolates tested.

These findings have important therapeutic implications. The differential effect of TNF-α suggests that its blockade—previously proposed as a treatment strategy—may undermine natural control of ww strains by TNF-α and prove detrimental in patients with archetype-dominated infections. In contrast, bexarotene emerges as a compelling repurposing candidate for BKPyV-associated disease. More broadly, our work highlights the importance of using clinically relevant ww strains in antiviral testing, now made tractable by this improved replication assay.

Discovery of Phospho-BRD4 IDR-Targeting Compounds as Anti-HPV and Anticancer Agents

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Drug development based on domain targeting often elicits strong side effects due to off-target inhibition of structurally and functionally related proteins. A new strategy of therapeutics development is to target an intrinsically disordered region (IDR) of a protein important for HPV genome replication. In our earlier study, we found phosphorylation of bromodomain-containing protein 4 (BRD4) at its N-terminal phosphorylation sites (NPS), situated downstream of the second bromodomain (BD2) and regulated by casein kinase II (CK2) and protein phosphatase 2A (PP2A), is required for BRD4 binding to acetylated chromatin and recruitment of replication and transcription factors to targeted genomic loci, including HPV origin of replication and enhancer/promoter regions (Wu et al., Molecular Cell 49: 843-857, 2013). This NPS phosphorylation is required for BRD4-potentiated HPV DNA replication. Two amino acid-like peptoid inhibitors (DC-1 and DC-2) binding to phospho-NPS effectively block phosphorylation-dependent BRD4 function (Wu et al., Cell Reports 16: 1733-1748, 2016). This proof-of-concept prompted us to conduct a high-throughput screen of a 200,000-compound library to identify small-molecule inhibitors capable of disrupting phospho-BRD4 interaction with HPV16-encoded E2 protein. Thirty-seven compounds were identified, with some potently inhibiting HPV DNA replication at different stages of the viral life cycle with minimal perturbation of the cellular transcriptome and chromatin landscape in normal keratinocytes (Wu et al., Molecular Cell 84: 202-220, 2024). Chemical modification was further conducted to improve the potency and selectivity of several phospho-BRD4-targeting compounds in suppressing HPV DNA replication and cancer progression. These newly identified BRD4 IDR inhibitors (e.g., compounds 14 and 90) represent a new class of epigenome-targeting compounds acting through modulation of BRD4 interaction with chromatin regulators without globally and non-selectively blocking BRD4 association with genomic loci as often seen with the widely used BET bromodomain inhibitors such as JQ1 and I-BET-derived compounds, thus significantly reducing off-target inhibition and side effects during antiviral and anticancer treatment.

Disrupting the interaction between Polo-like kinase 1 and HPV oncoproteins: a previously unidentified therapeutic horizon for HPV?

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Human papillomavirus oncoproteins, E6 and E7, are known to promote carcinogenesis through interacting with a pan of strategic interacting partners. Some of which are in their degradation targets, and some are crucial for the viral protein post-translational modification and stability.

From the virus-host interactome, several serine-threonine kinases have been known to interact with human papillomavirus (HPV) oncoproteins, for instance protein kinase A and B, as well as Aurora kinases. In the current study, through in silico modelling and virtual screenings, a series of in vitro interaction assays, we identified Polo-like kinase-1 (Plk1), a downstream target of Aurora kinases, is also interact with E6 and E7. Intriguingly, E6 interaction increases Plk1 kinase activity in a greater extend than E7. In turn, in the presence of Plk1, half-life of both E6 and E7 are extended dramatically. To further elucidate the dynamic of Plk1 and E6/E7 interaction, we adopted gene silencing and pharmacological inhibition approaches. We observed that both approaches increased pRB and reduced E7 levels. From high-throughput virtual and in vitro screenings, we found that a compound that may exhibit anti-HPV property. Treatment using this compound resulted to marked suppression cancer hallmarks of HPV-positive cells, including cell proliferation, anchorage independent growth, migration and invasion through Matrigel. From the cell-derived HPV-xenograft model, we observed that treatment using Plk1 inhibitor drastically reduced the tumour formation. Although showing plausible selectivity and efficacy toward HPV-positive cancer cells, treatment regimen requires further investigation.

Integrative multi-omics reveals HPV-driven ganglioside metabolism as a candidate for drug repurposing

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High-risk human papillomavirus (HR-HPV) infection of stratified squamous epithelium (SE) is responsible for ~5% of all human cancers, including squamous cell carcinomas (SCCs) of the cervix, head, neck, and anogenital tract. Despite the availability of prophylactic vaccines, HPV-associated disease burden remains high due to limited vaccine uptake, long latency between infection and tumorigenesis, and the absence of approved antiviral therapies. Understanding early HPV-driven molecular and metabolic alterations in differentiating SE is critical for identifying therapeutic vulnerabilities that may be targeted to suppress infection and prevent disease progression.

We identified HPV-regulated pathways amenable to therapeutic intervention by integrating bulk transcriptomic and steady-state metabolomic data generated from HPV16-infected and control 3D organotypic SE raft cultures. Pathway analysis revealed significant upregulation of ganglioside lipid metabolism in HPV16+ rafts. Targeted lipidomic analyses by LC–MS/MS confirmed increased levels of multiple ganglioside species, including GM3 and GD1a, in HPV-infected tissues. Consistent with these findings, transcripts encoding key ganglioside biosynthetic enzymes were elevated, supporting coordinated upregulation of this pathway by HPV infection. Eliglustat, a clinically relevant FDA-approved glucosylceramide synthase (GCS) inhibitor that suppresses glycosphingolipid synthesis, including gangliosides, was tested for its ability to suppress HPV-associated phenotypes. Eliglustat treatment of HPV16+ 3D spheroids and organotypic rafts reduced HPV-dependent aberrant cellular proliferation and restored tissue organization to levels observed in uninfected controls.

Extending these findings to in vivo models, lipidomic analyses demonstrated increased ganglioside levels in the female reproductive tract of both HPV16 E6/E7 transgenic mice and mice persistently infected with murine papillomavirus (MmuPV1), supporting the relevance of this pathway across complementary models of papillomavirus-associated disease. Building on these observations, ongoing studies in collaboration with the Spurgeon laboratory are evaluating the therapeutic efficacy of Eliglustat in persistently MmuPV1-infected mice. These studies will assess reductions in viral gene expression, genome copy number, and disease severity within the female reproductive tract, providing critical preclinical data for glycosphingolipid pathway inhibition as an antiviral and cancer-preventive treatment strategy.

Organoid models for assessment of novel therapeutics against BK Polyomavirus

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BK polyomavirus (BKPyV) is a widespread human DNA virus that presents a significant threat to kidney transplant recipients, leading to polyomavirus-associated nephropathy (PVAN) in 1–10% of cases and resulting in graft loss in approximately 50% of affected individuals. As no effective antivirals exist, treatment relies on reducing immunosuppression, increasing the risk of graft rejection. Therefore, identifying host-directed antiviral strategies is urgently needed.

We have established advanced 3D human kidney organoid models to study BKPyV infection, including adult stem cell-derived kidney tubuloids (KTs) and KTs-on-a-chip, that recapitulate the cellular architecture and complexity of the renal epithelium. BKPyV efficiently infected and spread throughout the nephron epithelial cells in both systems.

Using a genome-wide CRISPR/Cas9 knockout screen in renal proximal tubule epithelial cells, we identified the enzyme Methionine Adenosyltransferase 2A (MAT2A) as potential critical proviral factor. Pharmacological inhibition of MAT2A significantly reduced BKPyV replication in our 3D models, delaying the expression of early (LTAg) and late (VP1) viral proteins and impairing viral production. Furthermore, combination treatment with MAT2A inhibitors and the CFTR inhibitor glibenclamide, which prevents viral entry, demonstrated a synergistic antiviral effect in kidney tubuloids and KTs-on-a-chip.

Together, our findings identify MAT2A as a promising host target for therapeutic intervention against BKPyV. Moreover, these results establish kidney organoid models as powerful tools for studying BKPyV pathogenesis and for preclinical evaluation of antiviral strategies, contributing to the development of effective host-directed therapies.

Development of an effective in vivo siRNA treatment for Merkel cell carcinoma

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Introduction: Merkel cell carcinoma (MCC) is a rare but highly aggressive neuroendocrine skin cancer with high recurrence and mortality. ~80% of cases are driven by Merkel cell polyomavirus (MCPyV), where integrated viral genomes constitutively express truncated Large T (LT) and Small T (sT) antigens that inactivate tumour suppressors such as pRB and p53. Targeting viral oncogene expression using siRNA represents a rational therapeutic strategy.

Aim: To develop and evaluate siRNA therapeutics targeting MCPyV T antigen in vitro and in vivo using stealth lipid nanoparticles (sLNPs).

Methodology: Four siRNAs targeting distinct regions of the T antigen transcript were designed and tested in MCPyV+ and – MCC cell lines. Lead candidates were formulated into sLNPs and systemically administered (100 µL; 20 µg) in BALB/c Foxn1^nu/Arc mouse xenograft models (P055 and MCC13). Tumour growth and final weights were assessed.

Results: Four siRNAs (si-sT, si-LT, si-sLT, si-RB) reduced T antigen mRNA to 22–58%. All increased G0/apoptotic populations, reduced clonogenic survival by up to 80% at two weeks, and impaired cell migration, with wound closure reduced by up to 60% at 48 h. In vivo, we injected MCPyV+ and – MCC cell lines to establish tumours (day 63) before injection IV with siRNAs loaded in LNPS. Control groups (empty sLNP, non-targeting siRNA) showed aggressive growth while sLNP-LT moderately reduced MCPyV+ tumours but not MCPyV– tumours. sLNP-RB produced the strongest response, reducing MCPyV+ tumours up to 80% compared to controls.

Conclusion: siRNA-mediated targeting of MCPyV T antigen effectively suppresses oncogene expression, induces apoptosis, and inhibits tumour growth. LNP delivery, particularly with RB-targeting siRNA, achieved significant tumour regression in MCPyV-positive tumours, supporting this approach as a promising, sequence-specific therapy for MCPyV-driven MCC.

Keywords: Merkel cell carcinoma; Merkel cell polyomavirus; T antigen; siRNA therapy; stealth lipid nanoparticles.

A Phenotypic Screening Platform Identifies Candidate Modulators of HPV16 E6-Driven Basal Epithelial Homeostasis for Viral Reservoir Clearance

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Persistent infection with high-risk human papillomavirus (HPV) is maintained in the basal epithelial layer, yet there is currently no effective therapeutic strategy to eliminate HPV-infected basal cell reservoirs. We previously demonstrated that keratinocytes expressing HPV16 E6, but not E7, show increased proliferation at high cell density, reach a higher saturation density, exhibit delayed commitment to differentiation, and acquire a competitive advantage over normal keratinocytes in the basal layer. These findings suggest that HPV16 E6 plays a central role in sustaining the basal-layer fitness of infected cells.

To identify compounds that modulate this persistence-associated phenotype, we established a quantitative phenotypic screening platform using 2D and 3D epithelial model systems based on normal immortalized keratinocytes. A cell-density response assay was used to measure high-density growth, contact inhibition, and commitment to differentiation/delamination, while a high-cell-density competition assay was used to evaluate the relative fitness of HPV protein-expressing keratinocytes against normal keratinocytes in the basal layer.

Using these assays, a preliminary screen of FDA-approved small molecules identified candidate compounds that suppress HPV16 E6-driven modulation of basal epithelial homeostasis. Among these, lopinavir was selected for further analysis. Single-cell RNA sequencing was then used to define the transcriptional response induced by lopinavir and to identify compounds with similar differential gene expression profiles. This approach yielded additional candidate compounds, including Src inhibitors, that may interfere with HPV16 E6/E7-associated regulation of basal epithelial homeostasis.

Together, these findings support a therapeutic concept in which drug combinations, such as lopinavir with Src inhibitors, may reduce the enhanced basal-layer fitness conferred by HPV16 infection. By rendering infected keratinocytes less competitive than their normal counterparts, this strategy may promote selective depletion of persistent HPV-infected basal cell reservoirs and contribute to the development of novel treatments for HPV-associated disease.

Targeting HPV oncoproteins using novel antibody therapeutics

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Human papillomavirus (HPV)-related cancers depend critically on the continuous expression of the viral oncoproteins E6 and E7, which disable the endogenous tumor suppressors p53 and Rb. Due to their indispensable role for the onset and maintenance of HPV-related cancers, these viral oncoproteins thus represent a major yet clinically untapped therapeutic vulnerability. Antibodies have been traditionally considered to target predominantly, if not exclusively, cell surface proteins and elicit their effector functions by blocking functional interactions or recruitment of additional immune components. Here, we evaluated the anti-cancer activity of HPV E6-specific human monoclonal antibodies (hmAbs) we previously generated from tumor-infiltrating B cells of a HPV+ head and neck cancer patient.

Treatment with E6-specific hmAbs in a dimeric IgA (dIgA) format elicited potent anti-cancer activity against multiple HPV+ HNC and cervical cancer cell lines. Notably, this activity was strictly dependent on the dimeric nature of the IgA hmAbs, with neither IgG nor monomeric IgA versions of the same hmAbs displaying anti-cancer effects. The dIgA-mediated effect depended on the expression of E6 and on targeting distinct epitopes. Mechanistically, treatment with E6-specific dIgA resulted in the depletion of intracellular E6, restoration of p53, and ultimately apoptotic cell death of HPV+ cancer cells. Genetic deletion of either p53 or the polymeric immunoglobulin receptor (pIgR), which initiates transcytosis upon binding to dIgA, completely abrogated the anti-cancer activity of dIgA. Consistent with our in vitro findings, E6-specific dIgA also significantly suppressed tumor growth in vivo.

Overall, our findings reveal an unexpected anti-cancer activity of dIgA hmAbs targeting an intracellular HPV oncoprotein and introduce a novel class of HPV-specific therapeutics with the potential to provide highly selective and low-toxicity treatment options for HPV-related cancers.

HPV-Specific Reactions of Cancer Cells to the SHetA2 Investigational Drug

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The investigational new drug, SHetA2, was identified out of a screen of heteroarotinoid structures for its low micromolar potency of killing cancer cells while having reduced effect against non-cancer cells. SHetA2 is completing a Phase 1 clinical trial in the US (NCT04928508) and entering planned Phase 2 clinical trials for treatment of cervical intraepithelial neoplasia 2/3 in India and advanced and recurrent endometrial cancer in the US.

SHetA2 binds the peptide binding domain of specific heat shock protein 70 kDa (HSP70) proteins, which hydrolyse ATP to fold newly synthesized and misfolded proteins and support the stability, function and intracellular localization of multiple proteins and protein complexes. Many HSP70s become transiently elevated during stress and confer cell survival. Their chronic elevation during evolution of cancer causes a vulnerability that explains the differential toxicity of SHetA2. Most types of cancers exhibit similar responses to SHetA2; however, cancers infected with high-risk human papillomavirus (HR-HPV) display unique characteristics.

While most cancer types have mutations in TP53 and retinoblastoma (RB1) genes, HR-HPV-driven cancers do not, because HR-HPV E6 and E7 proteins drive carcinogenesis through their degradation/inactivation of p53 and pRb, respectively. We demonstrated that SHetA2 inhibits expression of the polycistronic E6/E7 mRNA but reduces levels of the E7 protein only. To understand this difference, we evaluated the interactions of E6 and E7 with the Hsp70/hsc70 proteins and found that only E7, and not E6, formed complexes with HSP70/hsc70 and that SHetA2 disrupted these complexes causing the release of E7 thereby leaving it vulnerable to proteasomal degradation. While most cancer types undergo cell cycle arrest, mitophagy and decreased mitochondrial metabolism and glycolysis upon SHetA2 treatment, HR-HPV infected cells differ in that they upregulate glycolysis in response to SHetA2 treatment. Combination of SHetA2 with glycolytic inhibitors enhances the effect of SHetA2 against HR-HPV-infected cancer cells. Another difference in non-HR-HPV and HR-HPV cancer cells is that their apoptotic response to SHetA2 is caspase-dependent and -independent apoptosis, respectively.

In conclusion, Hsp70/hsc70 proteins support HR-HPV E7-driven carcinogenesis making HR-HPV cancer cells vulnerable to SHetA2. The efficacy of SHetA2 can be improved by combining the low toxicity drug with glycolysis inhibitors.

Defining the Mechanisms of MEK Inhibitor-Induced Tumor Regression Using a Preclinical Model of Human Papillomavirus Precancer

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There are currently no effective antivirals or curative therapies for HPV-induced warts, condylomas, or precancers. We previously reported that MEK inhibitors (MEKi) have potent antiviral activities against oncogenic HPVs in vitro and promote tumor regression in an athymic mouse papillomavirus (MmuPV1) infection-induced model of HPV precancer. Understanding the mechanisms of MEKi-induced tumor regression is essential to determine whether regression reflects on-target suppression of viral oncogene-driven disease, to identify biomarkers and resistance pathways, and to guide rational translation of this strategy for HPV cancer interception.

Thus, athymic mice bearing MmuPV1 tumors were orally treated with vehicle or the MEKi, trametinib. Tumor growth was monitored longitudinally, and endpoint tumors were analyzed using histopathology, in situ hybridization, immunohistochemistry, spatial transcriptomics, and single-cell RNA sequencing.

Trametinib induced robust, sex-independent tumor regression. Regressing tumors exhibited decreased expression of MEK/ERK-responsive genes, reduced viral oncogene levels, and altered basal/suprabasal keratinocyte differentiation programs. Ki67 staining revealed that MEK inhibition reduces the absolute tumor proliferative burden while not altering proliferative index. Regressing tumors showed increased cleaved caspase-3-positive basal keratinocytes, basal compartment contraction, and reduced basal cell-ECM adhesion, consistent with anoikis-associated apoptosis and delamination.

Growing tumors appeared to be enriched for stromal Arg1+ cells, whereas regressing tumors exhibited increased intratumoral NK cells, along with elevated epithelial Cxcl14 mRNAs. A short 7-day course of trametinib reduced tumor volume by ~50% and was associated with stromal remodeling and early innate lymphocyte recruitment. Spatial transcriptomic and single-cell RNA-sequencing analyses suggest coordinated epithelial stress responses, inflammatory signaling, and immune cell participation during tumor regression.

Together, these data suggest that MEK inhibitors promote regression of papillomavirus-induced tumors through epithelial reprogramming, basal cell apoptosis, and innate lymphocyte activation. This supports their potential as therapies for benign and precancerous HPV-associated disease, particularly in immunocompromised populations.

Mechanisms of chemoradiotherapy resistance and sensitivity in HPV-positive anal squamous cell carcinomas

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Anal squamous cell carcinomas (ASCC) are HPV-driven malignancies with rising incidence and substantial variability in chemoradiotherapy (CRT) response. Due to historical absence of anal cancer cell line models, the molecular biology underpinning ASCC is broadly underexplored. Although HPV-positive status is typically associated with favourable prognosis across mucosal squamous cell carcinomas, more than 90% of ASCC are HPV-positive, meaning that most recurrent and treatment-resistant tumours arise within HPV-positive disease. Findings from recent PLATO clinical trials demonstrate that reduced-dose radiotherapy lowers acute toxicity and improves CRT-compliance in good-prognosis disease. However, there remains an unmet research need for biological markers to indicate suitability for dose de-escalation. This highlights the need to characterise molecular indicators of CRT sensitivity within HPV-driven ASCC.

To address this, a subset of pre-treatment FFPE biopsies from the PLATO cohort were subjected to bulk whole-transcriptome sequencing and stratified by clinical CRT response. Differential expression analysis uncovered distinct transcriptomic profiles between CRT-resistant and CRT-responsive disease. HPV genotyping confirmed that CRT responsive tumours were predominantly HPV16-positive, with occasional low-risk or HPV-negative cases, and all CRT-resistant tumours harboured high-risk HPV subtypes. This recapitulated the need to examine molecular heterogeneity within HPV-driven disease beyond HPV status alone.

Gene-set enrichment analysis revealed consistent suppression of multiple DNA damage-response programmes in CRT-responsive tumours, including double-strand break repair, excision repair, and replication-coupled repair elongation pathways. This indicates that CRT-responders possess an intrinsically weaker capacity to detect, process, and repair radiation-induced DNA lesions. In parallel, MYC target genes were significantly suppressed in CRT-responsive tumours, a pattern that is mechanistically consistent with reduced expression of BRD4, a key co-activator of MYC and regulator of transcriptional elongation. Finally, reduced PI3K/Akt/mTOR signalling in CRT-responsive tumours aligns with prior evidence that PI3K/mTOR activity promotes anal carcinogenesis.

Using a panel of HPV-negative and HPV-positive cell lines with divergent CRT sensitivities, we functionally evaluate candidate pathways identified in the transcriptomic analysis. Further, we explore how HPV-associated DNA repair capacity modulates cellular response to CRT-mimetic treatments in ASCC. Together, these data provide a framework for defining biomarkers of CRT response and support the development of biologically informed treatment de-escalation strategies in HPV-associated ASCC.

Adenovirus regulates the RAD18-HLTF-USP1 axis to promote the monoubiquitylation of PCNA during infection

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Adenoviruses (Ad's) have evolved to inhibit cellular proteins and pathways that possess inherent antiviral activity and utilise cellular proteins and pathways that possess proviral activities. Ad typically inactivates antiviral cellular proteins and signalling pathways through ubiquitin-targeted proteolysis, SUMOylation, gene repression and/or chromatin regulation. As such, Ad E1B-55K and E4orf6 proteins associate with Cullin Ring Ligase (CRL) 2 or 5, to target proteins such as p53 and MRE11 for protein degradation, whilst E4orf3, similarly targets MRN components to PML nuclear tracks for inactivation through SUMOylation. E4orf3 also has the capacity to target proteins for SUMO-targeted ubiquitylation and proteasome-mediated degradation. Proviral proteins are typically recruited to viral replication centres/compartments (VRCs) where they participate with viral proteins to promote viral replication. HLTF is a dual DNA helicase/translocase and ubiquitin ligase that promotes the K63-linked polyubiquitylation of PCNA on K164 to promote template switching during the replication of damaged DNA templates, whilst RAD18 promotes the monoubiquitylation of PCNA on K164 to promote the translesion synthesis of damaged DNA templates; USP1 functions as a deubiquitylase for ubiquitylated PCNA. In the present study, we have determined that Ad5 and Ad12 target both HLTF and USP1 for degradation during infection in order to promote the RAD18-dependent monoubiquitylation of PCNA at VRCs. These findings suggest that Ad targets the RAD18-HLTF-USP1 axis specifically during infection to allow for the accumulation of monoubiquitylated PCNA at VRCs. These findings will be presented and their implications for Ad infection considered.

Altered mitochondrial dynamics during HPV16 infection in tonsillar crypt and surface epithelium

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Mitochondria serve a vital role not only in energy production, but also in facilitating metabolic pathways and regulating cell homeostasis. Aberrations in mitochondrial processes are implicated in many diseases including cancer. Mitochondrial dysfunction is a characteristic of high-risk human papillomavirus (HR-HPV) infections, which are linked to the majority of anogenital and oropharyngeal cancers. Previous single cell RNA sequencing (scRNA-seq) analysis from our lab identified changes in gene expression of HPV18(+) cervical monolayer cultures. Indeed, HPV(+) cells have higher mitochondrial activity compared to uninfected control cells, prompting us to examine mitochondrial dynamics more closely.

We utilized organotypic raft cultures to recreate the 3D architecture of stratified squamous tonsillar epithelial tissue in vitro. Donor-derived primary tonsillar epithelial cells recapitulate the morphologically distinct crypt and surface epithelia and were used to generate HPV16-positive and control 3D tissue equivalents. We assessed mitochondrial dynamics by quantifying colocalization of the outer membrane protein TOMM20 and the fission GTPase DRP1 using immunofluorescence confocal microscopy. Relative to controls, HPV16-positive tissues exhibited increased TOMM20–DRP1 colocalization, consistent with elevated mitochondrial fission. Quantitative image analysis further revealed differences in signal intensity between tonsillar crypt and surface epithelia, indicating compartment-specific regulation of mitochondrial dynamics.

A significant majority of HPV-associated oropharyngeal squamous cell carcinomas (OPSCCs) originate from the tonsillar crypt tissues. Preliminary findings of this study suggest differences in mitochondrial dynamics between surface and crypt tonsillar tissues, which may contribute to their cancer risk. To resolve these differences across the differentiation spectrum, we are analyzing single-cell RNA sequencing data to define gene expression programs within these heterogeneous tissues. We will further investigate the expression of Reactive Oxygen Species Modulator 1 (ROMO1) and oxidative stress-associated gene sets — defining how HPV reprograms the metabolic state of differentiating cells.

APOBEC3A/B-mediated oncogene regulation in HPV-induced cell transformation

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In persistent HPV infections, a group of cytosine deaminases, APOBEC 3 (A3), is involved in the accumulation of specific mutational signatures in the host cell genome, leading to high levels of DNA damage and oncogenesis. Two members of this family, A3A and A3B, have been associated with a high mutational burden in HPV-related cancers. On the other hand, the role of the editing-independent activity of A3A and A3B in human tumorigenesis is still largely unknown.

We first analysed the gene expression profiles from head and neck cancer patients in The Cancer Genome Atlas (TCGA) and identified genes that correlated with either A3A or A3B. Distinct correlation patterns suggest that these enzymes play different roles in HPV-associated pathologies. This analysis was complemented by the RNASeq analysis of HFK (human foreskin keratinocytes) and HPV16-positive HFK16 cell lines. A panel of oncogenes whose expression corelated to A3A or A3B was then selected for biochemical and functional studies, including protein-protein interactions, intercellular localisation and cell transformation potential.

Among the oncogenes examined, p16 (INK4A/CDKN2A) was identified as a promising candidate, as it is a well-established biomarker for cervical cancer and HPV-positive HNSCC. A3 proteins regulate p16 at both the transcriptional and protein levels without altering its intracellular localisation. Functional analyses showed that A3-mediated p16 regulation affects the viability and migratory capacity of HPV-positive cell lines. These findings indicate that APOBEC3 proteins contribute to HPV-induced cell transformation also through non-editing mechanisms, including the modulation of host oncogene expression. Overall, the results support a role for editing-independent A3 activity in cell transformation, particularly in the context of HPV infection.

Biophysical and computational analysis of HPV16 E7 interactions with host trafficking adaptor proteins

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Human papillomavirus type 16 (HPV16) is the most cancer-causing high-risk HPV type, responsible for more than 50% of cervical cancers. It's one of the proteins, E7, a multifunctional oncoprotein that promotes cellular transformation through interactions with numerous host factors. While several cellular pathways targeted by E7 have been described, the mechanistic basis of its engagement with components of the host trafficking machinery mediated by endocytic proteins still need to be unfold to understand the cellular transformation.

In this study, we explore the molecular details of the interaction between full length HPV16 E7 protein with host adaptor proteins (AP2 and coatomer complex 1) using biophysical and bioinformatic approaches. The outcome supports the direct association of N-terminal region of E7 with Mu subunit of AP2 with a binding in the range of nanomolar range. This builds a structural understanding on how the E7 manages to do conformational changes implicating the functional changes in the host adaptor proteins for the viral survival and infection. In parallel, we also investigated the WD40 repeat domain of the coatomer complex subunit alpha (COPA), a scaffold protein involved in vesicular trafficking, to assess whether E7 targets these proteins and manipulates these cargo transport pathways for its survival. Biophysical characterization combined with computational analyses is being used to evaluate binding interactions with potential binding interfaces.

These studies aim to provide a framework for understanding how HPV16 E7 interfaces with host trafficking proteins and to evaluate the potential targets for therapeutic intervention targeting E7–host protein interactions.

Comparing the ability of HPV oncoproteins to promote basal epithelial identity

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Human papillomavirus (HPV) E6 and E7 interact with host cell proteins to dysregulate cellular signaling pathways. High-risk E7 proteins degrade PTPN14, thereby activating the YAP1 oncoprotein and repressing epithelial differentiation. We previously found that cells expressing high-risk HPV E7 are retained in the basal layer of an engineered stratified epithelium dependent upon their ability to degrade PTPN14.

We sought to extend our findings on high-risk HPV E7 by determining whether HPV E7 proteins from additional HPV genotypes promote basal epithelial identity. Similar to high-risk HPV E7, low-risk and cutaneous HPV E7 degrade PTPN14 to varying degrees. We compared the ability of diverse HPV E7 to promote basal cell retention using our previously established cell fate assay. All of the E7 proteins tested had some capacity to promote the retention of cells in the basal epithelial compartment, but cells expressing high-risk HPV E7 were more likely to remain associated with the basal layer than those expressing low-risk or cutaneous E7. We hypothesize that the increase in basal epithelial identity upon HPV E7 expression depends upon YAP1 activation. YAP1 localizes to the nucleus when active, thus we will compare the ability of several high-risk and low-risk HPV E7 to promote YAP1 nuclear localization in three-dimensional cultures.

Like HPV E7, HPV E6 has been reported to activate YAP1 and to promote basal epithelial identity. We are now testing the relative contribution of high-risk HPV E6 and E7 to basal cell retention in our cell fate assay. Our long-term goal is to determine the cellular signaling pathways activated by HPV E6 and E7 proteins that drive persistence in the basal epithelial layer. Defining the mechanisms by which HPV oncoproteins promote basal epithelial identity is relevant to virus biology and human disease, since blocking the retention of infected cells in the basal epithelial layer could inhibit HPV persistent infection.

Defining the Role of Epithelial Wounding in Persistent Papillomavirus Infections

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Papillomaviruses (PVs) are epitheliotropic tumor viruses that cause benign and malignant disease. Oncogenic human PV (HPV) infections lead to ~4.8% of the global cancer burden, and persistent infection is required for HPV-driven carcinogenesis. Epithelial wounding is essential for PV infection in vivo because it permits virion access to basal keratinocytes. However, whether the wound response actively promotes infection, persistence, and tumorigenesis beyond this physical access remains poorly understood. Defining wound-associated cellular and microenvironmental mechanisms that facilitate PV infection could identify new strategies to prevent persistent HPV infections prior to neoplastic progression.

Because HPVs are species restricted and in vitro systems lack the intact epithelial injury microenvironment, we are using the mouse PV (MmuPV1) model to determine how epithelial wound responses regulate infection. Our preliminary data show that standardized MmuPV1 inoculation into wounded tail skin reproducibly induces ~40 mm^3 tumors within six weeks in athymic mice, which lack mature T cells but retain functional innate lymphocyte populations. In contrast, comparable inoculation of NOD-SCID-gamma (NSG) mice, which lack key innate lymphocyte populations, produces only subclinical infections or small lesions of $\leq$5 mm^3 over the same interval. These findings suggest that innate lymphocytes promote PV infection, lesion outgrowth, or both. Consistent with this model, epithelial "priming" wounds delivered 2 to 3 days before re-wounding and MmuPV1 inoculation accelerate tumor development and increase tumor volume compared with wounds inoculated immediately. In addition, spatial transcriptomic analysis of growing tumors reveals increased Arg1 expression, implicating wound-associated inflammatory and repair programs in PV-driven tumorigenesis.

We hypothesize that epithelial wounding activates innate lymphocytes, macrophage-like inflammatory programs, and fibroblasts to produce soluble factors that increase keratinocyte susceptibility to PV infection and lesion outgrowth. To test this hypothesis, we will define the cell types and activation states induced in wounded epithelium and perform functional infection studies using keratinocytes co-cultured with activated wound-associated cell populations. We predict that M2-like macrophages and activated fibroblasts will enhance MmuPV1 infection in keratinocytes. This work will identify wound-induced cellular interactions that promote PV infection and address a major gap in understanding how epithelial injury shapes PV persistence and disease initiation.

Expanding Roles for CircRNA in the HPV Life Cycle

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High-risk human papillomaviruses (hrHPV), including HPV16, produce circular RNA that encompass the E7 oncogenic open reading frame (ORF) called circE7. A single N^6-methyladenosine (m^6A) modification (m^6A-SA-418) promoted circE7 backsplicing and inhibited linear E6*I (226^409) forward splicing. Viral episomes containing con-coding point mutations that abolish the m6A-SA-418 motif (Mut2) inhibited circE7 formation and viral replication, but increased keratinocyte resistance to differentiation. Both E6*I and circE7 isoforms are only found in hrHPV, suggesting that the evolution of the early region and circE7 backsplicing are linked. Indeed, analysis of RNA-seq data from condyloma acuminata, which are infected with low-risk HPV, did not reveal the presence of any circE7 isoforms. Unexpectedly, analysis of these RNA-seq datasets identified a previously undetected circRNA, called circE4, which encompassed the coding region of the E4 gene. Expression vectors containing the predicted backspliced region for circE4 for both HPV11 and HPV16 were tested for their ability to generate circE4 in vitro. RNA prepared from a retrospective cohort of high-risk (n=11) and low-risk (n=10) HPV-induced skin lesions was assessed for the presence of both endogenous and HPV-derived circRNA by RT-PCR. Our study expands our understanding of the regulation and function of circRNA in the HPV life cycle. More broadly, we suggest that circRNA from HPV and human polyomaviruses may play important roles in the expression of overprinted ORFs.

Exploring the Interaction between Aurora Kinases with HPV on the disease promotion

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Human papillomaviruses (HPVs) are double-stranded DNA viruses that cause lesions in both the mucosal tissues and cutaneous tissues. To date, the genomes of nearly 450 distinct HPV types have been isolated and sequenced. HPV viruses exploit the host cell division machinery by hijacking key proteins involved in cell cycle progression and producing viral particles in terminally differentiated cells.

Aurora protein kinase family is believed to be important in regulating mitosis, cell proliferation and activation of cell signalling cascades. Among the Aurora kinases, is one of the key mitotic regulators that frequently aberrantly expressed in human cancers. In HPV context, we found that Aurora A preferentially interacts with the E6 protein encoded by oncogenic HPV types, including HPV16 and HPV58. Oncogenic mucosal HPVs, most of which belong to the alpha (α) genus, have been the primary focus of research, whereas cutaneous HPVs remain relatively insufficiently studied. Therefore, we sought to determine whether the E6 proteins of cutaneous HPVs could form a similar interaction with Aurora kinases.

Based on previous research via in silico modelling, we hypothesized that E6-encoded by cutaneous HPV can interact with Aurora A. To validate this, we performed GST pull-down, co-immunoprecipitation (co-IP), and immunofluorescence assays. The results showed that cutaneous HPV8/38 E6 can interact with Aurora-A. From a series of in silico and in vitro screenings, we identified a library of inhibitors. Among the inhibitors, we observed that treatment with compound CUHK-CI induced a dramatic reduction in E6 protein. The specificity, drug-ability and efficacy of CUHK-CI in dampening oncogenicity requires to be further validated. Collectively, the current findings advance our understanding of the mechanism of the disease promotion of cutaneous HPV, offer the novel horizon for the development of broad-spectrum antiviral strategy.

Functional genomics screening of host genes involved in long-term maintenance of HPV-immortalized cell

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Infection with high-risk human papillomavirus (HPV) genotypes such as HPV16 and HPV18 can cause cervical, oropharyngeal, and other anogenital cancers. The HPV E6 and E7 oncoproteins drive HPV-mediated carcinogenesis. Expression of high-risk HPV E6 and E7 is necessary and sufficient to immortalize primary human epithelial cells. Cellular immortalization is a prerequisite for oncogenic transformation. Inhibiting the activities of E6 and E7 is therefore a promising pathway to treating HPV-positive cancers.

Our goal is to identify genes that are required for the viability of primary human epithelial cells immortalized by the high-risk HPV E6 and E7 oncogenes. Here, we performed genome-wide screens to identify cellular genes that promote or inhibit the growth of epithelial cells expressing HPV16 E6 and E7 oncogenes in cell culture. These screens used CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) technology to inhibit or activate a single gene per cell using a genome-wide library of single guide RNAs (sgRNAs). Cells that expressed HPV16 E6 and E7 were transduced with the genome-wide libraries and subsequently cultured for approximately one month. The relative abundance of CRISPRi and CRISPRa sgRNAs in the cell pools was determined by sequencing at multiple time points post-transduction. Replicate CRISPRi screens identified 540 growth-inhibitory and 42 growth-promoting sgRNAs. Out of the growth-inhibitory sgRNAs, 395 target genes classified as common essential genes in the Broad Institute Cancer Dependency Portal (DepMap). Replicate CRISPRa screens identified 51 growth-inhibitory and 11 growth-promoting sgRNAs.

We selected 12 growth-inhibitory sgRNA candidates identified in the CRISPRi screens and cloned them into a second, all-in-one CRISPRi vector for validation experiments. We are assessing how these candidate sgRNAs impact cell growth in primary epithelial cells expressing HPV16 E6 and E7 and in HPV16-positive cancer cells. In the short term, we will determine the effects of individual sgRNAs on cell growth and whether these effects are specific for cells that express HPV16 oncogenes. In the long term, we aim to develop a verified list of candidate target genes for subsequent mechanistic studies and future investigation into potential novel small molecular therapeutics for the treatment of HPV-positive cancers.

High-Risk α-HPV E6 Oncoproteins Interact with MAML1 and Modulate Notch Signaling to Delay Keratinocyte Differentiation

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α- and β-human papillomaviruses (HPVs), two of the most extensively studied genera due to their significant impact on human health, display distinct biological properties. High-risk α-HPVs are strongly associated with a wide range of human malignancies, whereas β-HPVs primarily act as cofactors in the development of cutaneous cancers. Despite these differences, both genera encode the E6 oncoprotein, which is essential for the establishment and maintenance of viral infection and contributes to cellular transformation and carcinogenesis. The E6 oncoproteins contain an LXXLL motif that mediates key protein–protein interactions, enabling α-E6 to form a complex with E6AP and β-E6 to interact with MAML1. Although this interaction pattern was initially considered a defining distinction between α- and β-HPVs, we have previously demonstrated that α-E6 oncoproteins are also capable of binding MAML1, suggesting a formerly unrecognized and functionally relevant overlap between these viral groups. We now further extend these findings by demonstrating a physical interaction between MAML1 and high-risk α-E6 oncoproteins, indicating that this association is not restricted to β-HPVs as previously assumed. In addition, we show that expression of high-risk α-E6 leads to a delay in keratinocyte differentiation, a key process in viral life cycle progression. This effect is accompanied by altered expression of Notch-responsive genes, consistent with attenuation of Notch signalling. Notably, these changes mirror those observed in the presence of β-E6, supporting the idea that α- and β-E6 proteins converge functionally on the Notch pathway. Together, these results point to a shared strategy among HPV types to interfere with differentiation programs, thereby creating a cellular environment conducive to viral persistence.

HPV L1-specific T-cell responses dominate across the clinical spectrum from asymptomatic infection to cervical cancer

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Persistent infection with high-risk human papillomaviruses (HPV) strongly associates with cervical cancer development, yet the immune features associated with viral clearance versus progression remain incompletely defined. T-cell responses are central to antiviral immunity, but the antigenic targets that characterize protective versus non-protective responses in human HPV infection are not fully understood.

As a segment of a broader consortium investigating immune correlates of HPV persistence and clearance, we profiled HPV-specific T-cell responses in a clinically annotated cohort of women spanning asymptomatic infection, cervical intraepithelial neoplasia (CIN), and invasive cervical cancer. We expanded peripheral blood mononuclear cells (PBMCs) in vitro by stimulating them with overlapping peptide pools representing all HPV proteins, and then assessed antigen-specific T-cell reactivity by measuring CD137 expression and IFNg secretion. Across all clinical groups, we observed a consistent immunodominance pattern: the strongest responses targeted the L1 capsid protein, followed by the E1 replication protein. Notably, this hierarchy was preserved even in individuals with invasive cervical cancer.

Given that L1 is predominantly expressed during productive infection and is often reduced after viral integration, the persistence of L1-directed responses suggests that dominant systemic T-cell reactivity may not closely correspond to the antigenic drivers of disease progression. These findings highlight antigenic skewing as a feature of HPV-specific immunity across disease states.

Ongoing work within the consortium will eventually unite tumor-infiltrating lymphocyte analyses to determine how T-cell function relates to viral persistence and clinical outcome.

HPV16 E6–Driven Upregulation of lncRNA NBAT1 in Head and Neck Squamous Cell Carcinoma

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Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides that lack protein-coding potential yet play critical roles in regulating diverse biological processes, including epigenetic modification, transcription, and translation. Increasing evidence highlights their involvement in pathological conditions, particularly cancer. The HPV oncoproteins E6 and E7 have been shown to dysregulate the expression of more than 1,400 lncRNAs; however, the functional relevance of many of these transcripts remains poorly understood. Among them, lncRNA NBAT1 has emerged as a candidate of interest. NBAT1 has been reported to function as a tumor suppressor in several cancer types, while exhibiting oncogenic properties in others. Its expression is regulated by p53, and decreased NBAT1 levels have been associated with cytoplasmic accumulation of p53. This study aimed to investigate the role of NBAT1 in HPV-associated head and neck squamous cell carcinoma (HNSCC).

We analyzed NBAT1 expression in a cohort of patient samples and observed significantly increased expression in HPV-positive HNSCC tumors, which correlated with HPV16 E6 expression. This pattern was further supported by analyses in HNSCC and keratinocyte cell lines. Matched normal oral keratinocytes and oral cancer cell lines, with and without HPV16, demonstrated elevated NBAT1 expression in HPV16-positive models. Moreover, ectopic expression of E6 in HPV-negative HNSCC cells resulted in a significant upregulation of NBAT1. The subcellular localization of TP53 was assessed in the context of NBAT1 overexpression, and functional assays revealed that NBAT1 influences cellular proliferation.

These findings suggest that NBAT1 is an HPV-responsive lncRNA whose expression is driven, at least in part, by E6 activity. Its association with p53 localization and proliferative capacity indicates a potential role in modulating key oncogenic pathways in HPV-associated HNSCC. Further investigation is warranted to clarify whether NBAT1 functions predominantly as a tumor suppressor or oncogene in this context and to assess its potential as a biomarker or therapeutic target.

HPV16 exploits the DNA nucleases DNA2 and FEN1 during genome amplification

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Persistent infection with high-risk human papillomavirus type 16 (HPV16) requires faithful replication and maintenance of episomal viral genomes in an environment of chronic replication stress. While HPV16 is known to activate host DNA damage response pathways to support viral DNA replication, the host factors that preserve viral genome integrity during episomal replication remain incompletely defined.

Here, we identify the host nucleases DNA2 and flap endonuclease 1 (FEN1) as previously unrecognized components of the HPV16 life cycle that promote episomal genome stability. Although HPV16 does not broadly alter DNA2 or FEN1 transcription, both factors are recruited to viral chromatin and associate with HPV16 replication compartments. Depletion of DNA2 or FEN1 does not significantly impair overall viral DNA synthesis but instead results in increased replication-associated defects and reduced replication fidelity. Loss of either nuclease leads to destabilization of episomal HPV16 genomes, with viral genome linearization consistent with integration. Notably, DNA2 and FEN1 do not form stable complexes with the core viral replication proteins E1 or E2, but instead associate indirectly within viral replication compartments, consistent with roles in resolving replication-associated DNA structures.

Together, these findings demonstrate that HPV16 co-opts host replication stress response nucleases to preserve replication fidelity and maintain episomal viral genomes. This work identifies DNA2 and FEN1 as key determinants of HPV16 genome fate and provides new insight into how host DNA repair pathways influence viral persistence.

HPV18 increases cytoplasmic PRMT1 and alters the cellular methylome

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Human papillomavirus (HPV) oncogenes, E6 and E7, have been found to reprogram the cellular environment to enable viral establishment and long-term maintenance. This process requires precise coordination of viral gene expression, which is achieved in part through alternative splicing of the viral polycistronic mRNA. Therefore, understanding regulation of viral mRNA splicing has important implications for the viral lifecycle, transmission, and cancer development.

We previously used single cell genomics to identify Protein arginine N-methyltransferase 1 (PRMT1) as being an important regulator of HPV infection, establishment, and long-term maintenance. PRMT1 is the main cellular enzyme responsible for asymmetric dimethylation of cellular proteins within human cells and is upregulated in HPV(+) cells. Mechanistically, we demonstrated that PRMT1 inhibition results in dysregulated viral splicing and reduced mRNA stability.

Interestingly, in addition to upregulating PRMT1 mRNA and protein, HPV18(+) cells express an alternatively spliced isoform of PRMT1. This alternative v2 isoform is largely identical to the predominant v1 isoform but encodes an additional exon encoding for a nuclear export sequence. Consistent with this, PRMT1 shifts from predominantly nuclear localization in control cells to increased cytoplasmic localization in HPV18(+) cells. Importantly, v2 isoform-specific knockdown phenocopies PRMT1 inhibition on HPV18 splicing, indicating that HPV18 regulates viral splicing by altering the localization of PRMT1.

To determine cellular targets of cytoplasmic PRMT1, we used methylation-specific immunoprecipitation combined with mass spectrometry (AP-MS) to identify cellular proteins that are differentially methylated in HPV18(+) HFKs compared to controls. These HPV18-specific PRMT1 targets define the host pathways rewired by cytoplasmic PRMT1 and provide a framework for linking PRMT1-dependent methylation to viral splicing control.

HPV8-E6 drives coordinated transcriptional and epigenetic reprogramming of keratinocytes

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Background: HPV of genus betapapillomavirus (e.g. HPV8) are key cofactors in cutaneous squamous cell carcinoma (cSCC), yet how they reprogram keratinocytes remains unclear. We investigated whether HPV8 oncogenes drive coordinated transcriptional and epigenetic rewiring that promotes tumour initiation.

Methods: We applied integrated multi-omics profiling in N/TERT keratinocytes expressing HPV8-E6, E7, or both, combining RNA-seq, small RNA-seq, and whole-genome bisulfite sequencing. Data were integrated with pathway, transcription factor, and miRNA–target analyses, and linked to methylation-defined cSCC subtypes.

Results: HPV8-E6 emerged as the dominant reprogramming factor, inducing widespread transcriptional changes, while E7 had minor, complementary effects. E6 potently suppressed epidermal differentiation programs, targeting key lineage regulators. In parallel, E6 reshaped the miRNA landscape, with reciprocal miRNA–mRNA networks indicating active post-transcriptional control. At the epigenetic level, E6 drove extensive redistribution of DNA methylation across promoters, enhancers, and gene bodies. Hypermethylation silenced developmental regulators, whereas hypomethylation increased accessibility of AP1- and STAT3-associated sites. Integrated analyses identified an E6-driven FOSL2-centered regulatory axis linking methylation changes to differentiation control. Importantly, betaHPV DNA was enriched in actinic keratoses and cSCC with stem cell-like (Epi-SC-like) methylation profiles.

Discussion: HPV8-E6 orchestrates a multilayered reprogramming program that converges on loss of keratinocyte identity. By coupling transcriptional repression, miRNA remodelling, and methylome reorganization, E6 establishes a plastic, progenitor-like state permissive for transformation. The enrichment of betaHPV in stem cell-like tumours links this mechanism to clinically relevant disease subtypes. These findings position epigenetic reprogramming as a central driver of betaHPV-associated carcinogenesis and a potential therapeutic vulnerability.

Independent proviral and antiviral host factors recognise the same capsid protein in divergent human herpesviruses

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Intrinsic cellular factors that inhibit herpesvirus infection remain incompletely defined. Here, we identify TRIM5α as a restriction factor for herpes simplex virus type 1 (HSV-1). TRIM5α-mediated restriction requires its ubiquitin ligase activity, PRY–SPRY domain, and the ability to oligomerize. Mechanistically, we show that TRIM5α directly engages capsid protein VP19C and promotes the stability of the VP19C–VP23 complex and its nuclear accumulation. VP19C also activates NF-κB synergistically with TRIM5 and independently. HSV-1 counteracts this host defense by triggering proteasome-dependent TRIM5α degradation. In addition, we show that cyclophilin A (CypA), which is incorporated into HSV-1 virions, also binds to VP19C, but enhances infection. As with HIV-1 and orthopoxviruses, the proviral activity of CypA is disrupted by cyclosporin A (CsA), but unlike the situation with these other viruses, the proviral activity of CypA is independent of TRIM5α. Notably, CsA and its non-immunosuppressive derivatives also exhibit anti-HSV-1 activity in neuronal cell lines, suggesting a potential therapy for HSV-1 encephalitis. TRIM5α and CypA also interact with orthologs of VP19C in other alpha, beta and gamma human herpesviruses. These findings reveal two distinct host pathways acting on the herpesvirus capsid and provide a foundation for comparing how TRIM5α and CypA modulate infection of unrelated virus families, offering new directions to identify shared principles of host recognition and viral evasion.

Investigating the Molecular Mechanism of Merkel Cell Polyomavirus Nuclear Entry

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Merkel cell polyomavirus (MCPyV) is a small DNA virus, and the only polyomavirus definitively linked to human cancer. MCPyV is the causative agent of Merkel cell carcinoma (MCC), an aggressive and often lethal form of skin cancer. Despite its clinical relevance, the early steps of MCPyV infection remain poorly understood. Like other DNA viruses, MCPyV must access the host nucleus to initiate gene expression and replication of its viral genome. However, unlike the well-studied archetype, SV40 polyomavirus, which enters the nucleus through the nuclear pore complex, MCPyV exploits mitotic nuclear envelope breakdown (NEBD) for nuclear entry. This unique requirement raises a fundamental question: how is MCPyV positioned at the nuclear periphery to gain access to the nucleus during mitosis?

Nesprin-2 is a core component of the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex that resides at the outer nuclear membrane and physically connects the cytoskeleton to the nuclear envelope, playing a central role in nuclear positioning and organization. Previous studies have shown that SV40 interacts with Nesprin-2 to facilitate nuclear targeting, suggesting that nuclear envelope–associated scaffolding proteins may serve as platforms for viral positioning. Based on both its strategic localization and its role in organizing the nuclear periphery, we hypothesize that MCPyV utilizes Nesprin-2 as a docking platform to position incoming capsids at the nuclear envelope prior to NEBD.

To test this, we use an MCPyV pseudovirus system expressing a GFP reporter to monitor infection. Preliminary data demonstrate that depletion of Nesprin-2 significantly impairs MCPyV infection, and that Nesprin-2 associates with the MCPyV VP1 capsid at 48 hpi as shown by co-immunoprecipitation. This project aims to define the molecular interaction between MCPyV capsids and Nesprin-2 and to determine how this interaction is regulated during the cell cycle.

Together, this work will define a host-dependent mechanism of viral nuclear targeting and establish a mechanistic framework for understanding MCPyV nuclear entry. These findings will provide insight into how DNA tumor viruses exploit mitotic processes and may reveal new host-directed therapeutic vulnerabilities.

MCPyV Large T Antigen–Host Chromatin Interactions Defined by Proximity Labelling

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The Merkel cell polyomavirus Large T antigen is the central regulator of polyomavirus replication, functionally replacing the host MCM helicase complex through its origin-binding domain (OBD) and ATPase/helicase activity. LT specifically recognizes GRGGC motifs within the viral origin of replication (ori), a 71 bp poly(T)-rich region containing eight pentanucleotide motifs. Binding to these sites promotes LT multimerization, DNA unwinding, and initiation of viral replication.

Beyond its role in viral replication, we and others have shown that LT also associates with the human genome, preferentially binding promoter regions enriched for GRGGC motifs. Similar chromatin tethering mechanisms have been described for other nuclear DNA viruses, including Epstein–Barr virus and Kaposi's sarcoma-associated herpesvirus, where viral proteins (EBNA1 and LANA) anchor viral genomes to host chromatin to support persistence without broadly altering host transcription.

Based on these observations, we hypothesize that (I) LT contributes to viral persistence by tethering the viral genome to defined host chromatin regions, and (II) LT–host DNA interactions are mechanistically distinct from viral ori binding, with implications for chromatin organization and pathogenesis.

To test this, we are developing complementary proximity-labeling strategies to define the LT-associated proteome in a DNA context. First, we employ a rapamycin-inducible system (tTA-FRB/FKBP-TurboID) to selectively label proteins at defined LT-bound DNA sequences. Second, we use TurboID–LT fusion constructs and DNA-binding mutants to distinguish DNA-dependent from DNA-independent interactions.

Mitochondrial targets of the adenovirus E4orf4 protein

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The adenovirus (Ad) E4orf4 protein is a multifunctional viral regulator, which contributes to temporal regulation of the progression of viral infection and to virus-induced inhibition of the DNA damage response. When expressed alone, E4orf4 induces a cancer-specific, caspase-independent mode of programmed cell-death. During infection, E4orf4 co-localizes with viral replication centers, and when expressed alone it has been reported to first localize to the nucleus and then to cytoplasmic and membrane regions.

Proteomic assays revealed an interaction between E4orf4 and mitochondrial proteins. To investigate whether E4orf4 enters this organelle, we utilized methods including protease protection assays and electron microscopy to reveal that a subpopulation of E4orf4 molecules resides within mitochondria, both during virus infection and when E4orf4 is expressed alone. E4orf4 associates with proteins belonging to various complexes of the electron transfer chain (ETC) in the mitochondria, as part of large complexes, Specifically, E4orf4 is closely associated with proteins of complex 3 and 4, altering their activity. Additionally, interactions with several mitochondrial proteins such as the VDAC ion channel and translocase of the outer mitochondrial membrane protein (TOMM) were also observed.

Association with mitochondrial complexes likely has important consequences both for Ad replication and for cancer-selective cell death. The E4orf4 interaction with mitochondria will be discussed, and its functional consequences will be described in another abstract by Amir Basis.

Novel Insights into HPV-Mediated Carcinogenesis

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Most people will be infected with Human papillomaviruses (HPVs) at some stage of their lives. The majority of these viruses cause benign warts and are not considered tumorigenic. Only persistent infection with high-risk (HR) types, such as HPV 16 and 18, can induce development of various cancers, with cervical cancer being the principal disease. Additionally, HR HPVs are also associated with a subset of anogenital and head and neck cancers. HPV-induced tumorigenesis depends on the uncontrolled expression of two main viral oncoproteins, E6 and E7, which target a number of cellular proteins, including p53 and pRB, the major tumor suppressor proteins, which are involved in the control of the cell cycle and apoptosis. Furthermore, HR E6 oncoproteins also interact with a number of PDZ-domain containing proteins, which act as cellular tumor suppressors and play a role in cell polarity regulation. E6s bind to these targets via their PDZ-binding motifs (PBMs) located at their extreme C-termini and, in most instances, ultimately direct their degradation in a proteasome-dependent manner.

In this study, we identified a novel E6 interacting partner, which has been recently shown to be strongly associated with promotion and progression of a number of human cancers. Our preliminary data demonstrate that HR E6 oncoproteins (16, 18, 33) interact with the novel cellular substrate in a PBM/PDZ dependent manner, with varying affinities. Here we show that this interaction results in a significant reduction in the protein expression levels of the novel interacting partner. Notably, this downregulation was not observed in the presence of a proteasome inhibitor in cell lines derived from HPV-positive cervical and head and neck tumors, suggesting that this PBM/PDZ-dependent interaction is likely to be proteasome-regulated. Thus, our results provide new insights into the complexity of the E6 interactome and are likely to reveal novel mechanisms by which HPV contributes to malignant transformation, improving our understanding of HPV-induced carcinogenesis.

Pharmacological Induction of Matrix Metalloproteinase Inhibitors in Human Papillomavirus-Transformed Cells

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Human Papillomavirus mediated carcinogenesis relies on the sustained overexpression of viral oncoproteins. Besides being directly involved in the cellular transformation process, these viral factors are also important in other scenarios required for cancer development, such as the induction of alterations in the tumor microenvironment. We have previously shown that the levels of the matrix metalloproteinases inhibitors RECK and TIMP-2 are negatively regulated in primary human keratinocytes expressing HPV16 E6/E7 and cervical cancer derived cell lines. Besides, we observed an inverse correlation between RECK levels and cervical disease progression in clinical samples. Altogether, these observations indicate that the disruption of extracellular matrix homeostasis by viral factors may contribute to disease establishment and progression. Furthermore, the low mutational rate of RECK and TIMP-2 (approximately 1%) in cervical cancer suggests their regulation is governed by epigenetic and post-transcriptional events, making them possible targets for pharmacological approaches. However, no pharmacological approaches that target components of the extracellular matrix in HPV-related pathologies have been explored.

In this work, we evaluated the induction of RECK and TIMP-2 proteins in the cervical cancer derived cell lines SiHa (HPV16+) and SW756 (HPV18+) by disulfiram, 5-azacytidine, and doxorubicin. The cells were treated with different concentrations of the drugs for 72 and 120h, and the levels of RECK and TIMP-2 were determined by Western blot. The effect of these compounds on cell viability and toxicity was determined using Alamar Blue colorimetric assay. In our experimental conditions, 5-azacytidine and doxorubicin were capable of inducing RECK and TIMP-2. On the other hand, disulfiram did not affect the levels of these proteins. Induction occurred at concentrations near the half-maximal inhibitory concentration (IC_{50}), a result that corroborates previous data, since RECK overexpression is related to a greater sensitivity of these cells to treatment. These data suggest that pharmacological modulation of RECK and TIMP-2 may represent a potential strategy to restore extracellular matrix remodelling in cervical cancer.

Phosphorylated HPV-16 E7 Perturbs Vangl1 Trafficking to Drive Polarity Loss

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High-risk human papillomaviruses (HPVs) rely on sustained expression of the E6 and E7 oncoproteins to drive malignant transformation. Although the high-risk E7 protein is known to be multifunctional, the mechanistic consequences of its Casein Kinase II (CKII)–mediated phosphorylation remain of oncogenic importance. In this study, we identify Vangl1, a core scaffold of the planar cell polarity (PCP) pathway, as a novel, phosphorylation-dependent interactor of HPV-16 E7. CKII phosphorylation is essential for Vangl1 recruitment, and this interaction is largely selective for HPV-16 E7.

Using CaSki cells, we show that E7 expression profoundly disrupts Vangl1 homeostasis, generating a phospho-dependent imbalance. This retention parallels E7 stability, revealing a reciprocal oncogenic stabilization loop. Mechanistically, E7 co-opts the clathrin adaptor AP1M1 to impair Vangl1 trafficking and subcellular localization, further amplifying PCP dysregulation. Functionally, Vangl1 depletion phenocopies E6/E7 loss in CaSki spheroids, leading to disrupted spheroid architecture, reduced invasiveness, and increased chemosensitivity. These findings position Vangl1 as a central effector of E7-driven cervical transformation and highlight a previously unrecognized PCP-based mechanism contributing to HPV-mediated oncogenesis.

Together, this work uncovers a CKII-phosphorylation-dependent E7–Vangl1 axis that reshapes polarity signalling, proteostasis, and invasive behavior, offering new mechanistic insight and potential therapeutic targets for HPV-associated cancers.

Presence of koilocytes in actinic keratosis identifies those at high risk of developing keratinocyte carcinoma

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Background: Keratinocyte carcinoma (KC) incidence continues to rise despite current prevention strategies. Although effective treatment of actinic keratoses (AKs) reduces the risk of subsequent KC, their high prevalence limits the feasibility of treating all individuals. Recently, we identified a subset of HPV8 associated AKs, characterized by the presence of koilocytes, underscoring the need for improved stratification to identify patients at highest risk of KC.

Objectives: To determine the association of HPV8 with subsequent KC and to identify specific treatment strategies.

Methods: Patients with a prior history of pathologist-proven AK were recruited. Histological samples were analysed for HPV8 and assessed for the presence of koilocytes, and medical records were reviewed for KC. HPV8-associated AKs, cell lines and mouse models were interrogated for expression of Src family kinases. The in vitro and in vivo biological effects of Src inhibition were determined using small interfering RNA and tirbanibulin.

Results: Sixty-one patients with AK and no history of antecedent KC were divided into those with ($n = 31$) and without HPV8 ($n = 30$) infection. Using multivariable Cox regression adjusted for sex, age and body site, patients with HPV8-associated AKs (defined by the presence of koilocytes) had a greater risk of subsequent KC (hazard ratio 5.5, 95% confidence interval 2.3–12.9; $P < 0.001$). Independently, HPV8-associated AKs were associated with invasive squamous cell carcinoma [odds ratio (OR) 32.0; $P < 0.001$] and basal cell carcinoma (OR 4.5; $P < 0.01$), and included all nine patients with multiple KCs. These lesions showed elevated expression of phosphorylated Src kinase. Src inhibition reduced cell proliferation and blocked colony-forming efficiency. In vivo, Src inhibition restored epidermal thickness and hindered the development of skin tumours.

Conclusions: The presence of koilocytes in AK histology identifies a subset of patients at risk of multiple KCs. Targeting Src kinase inhibits HPV8-associated keratinocyte proliferation and prevents skin tumour formation in vivo, suggesting a potential therapeutic strategy.

Recent Trends in HPV-Positive Oropharyngeal Squamous Cell Carcinoma in Northern Israel

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The epidemiology of oropharyngeal squamous cell carcinoma (OPSCC) has shifted globally due to the rising contribution of human papillomavirus (HPV) . While HPV-related OPSCC predominates in North America and Northern Europe, historical data from Israel suggested a substantially lower prevalence, yet contemporary trends and outcome data from Israel remain limited. This study evaluates temporal trends, clinicopathologic characteristics, and survival outcomes of HPV-related OPSCC in the heterogenic society of northern Israel.

We conducted a retrospective cohort study of 130 adult patients with histologically confirmed OPSCC treated at a single tertiary referral center between 2012 and 2024. HPV status was assessed, and patients were staged according to AJCC 8th edition criteria. Temporal trends in p16 positivity were analyzed across predefined periods. Overall survival (OS) was evaluated using Kaplan–Meier analysis and Cox proportional hazards modeling, including a combined variable of p16 status and T stage.

Our results revealed that HPV-related OPSCC now accounts for the vast majority of OPSCC cases in northern Israel, with proportions comparable to the highest reported worldwide. Interestingly, HPV16 positivity was strongly associated with earlier stage at presentation and improved survival. Our result's implications and alignment with current knowledge of HPV positive OPSCC epidemiology will be presented and highlighted from a clinical point of view.

Regulation of HPV-16 Infection by Ubiquitination of the L1 Major Capsid Protein

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The major capsid protein L1 of human papillomavirus plays an essential role in virion assembly and host cell entry, yet the contribution of its post-translational modifications to the viral life cycle remains incompletely defined.

Here, we provide the first evidence that HPV16 L1 is ubiquitinated at multiple conserved lysine residues (K20, K64, K152, K217, K437, K452, and K454), as identified by mass spectrometry. Functional characterisation revealed that substitution of K64 and K152 abolishes pseudovirion formation, whereas mutations at other lysine residues still permit particle assembly but result in markedly reduced infectivity across several epithelial cell types. In addition, pre-treatment of HPV16 pseudovirions with an anti-ubiquitin antibody significantly impairs their ability to infect host cells. While ubiquitination is not required for initial viral attachment, residues K452 and K454 were found to be critical for efficient entry, influencing intracellular trafficking and capsid processing.

Collectively, these findings uncover a previously unrecognised connection between the ubiquitin conjugating system and HPV capsid proteins, suggesting that L1 ubiquitination contributes to multiple stages of infection. This work highlights a novel regulatory mechanism that may inform future antiviral strategies targeting early steps of HPV infection.

Spatial Mapping of Cervical Lesions Using Cervical Cell Lift in a Japanese Cohort: Diagnostic Concordance with Histology

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Cervical biopsy can only sample small parts of a lesion, while cytology loses spatial information entirely. Cervical Cell Lift (CCL) is a non-invasive technique which uses a nitrocellulose membrane to collect cervical cells in sheet form, so that spatial topology is preserved. We aimed to evaluate whether CCL maps reflect the location of histologically confirmed lesions in a Japanese screen-positive cohort.

We collected CCL samples from 50 women with abnormal cytology referred to Kumamoto University Hospital in Japan. Histological diagnoses ranged from CIN1 to CIN3, AIS, and invasive cancer. Each specimen was stained for MCM, which marks proliferating cells, and E4, which marks productive HPV infection. Positive areas were mapped onto a clock-face cervical schema and compared with biopsy and final histology. Case-level concordance was assessed using ±1 clock hour tolerance: Full concordance was defined as MCM-positive signal within ±1 clock hour of every biopsy-confirmed HSIL site, and each case was classified as Full, Partial or None. The primary analysis focused on cases with CIN3 or worse disease.

Of 41 patients with CIN3+ disease, 36 were evaluable; 4 cases were excluded because the scanning data were corrupted, and one AIS case was excluded because the biopsy site could not be located. Among the 36 evaluable cases, 27 (75%) showed Full concordance, 1 (3%) Partial, and 8 (22%) None, giving 78% any-concordance between CCL and biopsy. Detection was high in invasive cancer (8 of 9 cases with Full concordance). MCM-positive areas occasionally extended beyond the biopsied site, which may reflect either unsampled lesion extent or non-neoplastic proliferation in metaplasia or repair. The single pure AIS case showed no concordance, illustrating that deeper glandular lesions may require modified sampling approaches.

CCL provided spatially resolved mapping of HPV-associated cervical lesions in a Japanese cohort, with 78% of CIN3+ cases showing concordance with biopsy. CCL may serve as a non-invasive complement to biopsy, supporting diagnostic triage.

The Association of E6AP with E6 Proteins of African-Prevalent HPV Types Encountered in Cervical Cancer.

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High-risk human papillomaviruses (hrHPVs) are responsible for approximately 5% of cancers worldwide. While HPV-16 and HPV-18 are the most prevalent types globally, HPV-58 is commonly associated with cervical cancer in regions such as Africa and East Asia, yet remains underrepresented in molecular studies. The viral E6 oncoprotein contributes to malignancy by binding the cellular ubiquitin ligase E6AP (UBE3A), leading to the degradation of tumour suppressors and stabilization of E6 itself.

In this study, we investigated whether E6AP also regulates the stability of HPV-58 E6. GST pull-down assays and siRNA-mediated knockdown in HeLa and HaCat cells demonstrated that HPV-58 E6 binds robustly to E6AP. In HEK293 E6AP knockout cells, co-expression of either wild-type or catalytically inactive E6AP increased E6 protein levels, suggesting that E6AP stabilizes E6 independently of its enzymatic activity. Mutating the E6AP-binding motif disrupted this interaction and reduced the steady-state levels of E6.

These findings suggest that HPV-58 E6 relies on E6AP not only to degrade cellular targets but also to maintain its own stability. Compared with HPV-16 and HPV-18, HPV-58 E6 exhibits a partially distinct mechanism of E6AP engagement, highlighting type-specific differences in HPV oncogenic pathways. Understanding these differences may provide insight into regional variations in cervical cancer incidence and guide the development of targeted therapies for hrHPV infections.

The Role of Sp100 in Generating an Antiviral Chromatin Environment in HPV-infected cells

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Promyelocytic Leukemia Nuclear Bodies (PML-NBs) are dynamic subnuclear hubs that integrate transcriptional regulation, DNA damage response, and intrinsic immunity. Sp100, a component of PML-NBs, modulates the life cycle of several DNA viruses, including restriction of both the early and late phases of Human Papillomavirus (HPV) infection. Sp100 encodes several isoforms, which differentially regulate viral transcription. Sp100A has been shown to decondense chromatin to activate Cytomegalovirus transcription, whereas the longer isoforms (Sp100B/C/HMG) repress viral processes, including HPV transcription and replication. We have previously shown that Sp100 binds broadly across the HPV genome and propose that it marks viral chromatin for silencing rather than functioning at specific regulatory elements. However, the precise cellular function of Sp100 and the mechanisms through which it modulates HPV infection are yet to be fully determined.

In this study, we performed Sp100 ChIP-seq and RNA-seq in HPV31-positive CIN612-9E cervical keratinocytes to investigate the role of Sp100 in modulating the host chromatin environment. We identified at least two distinct patterns of Sp100 binding in the host genome. Following interferon treatment, Sp100 localized to large H3K27me3 associated regions of heterochromatin and we propose that this is due to one of the repressive Sp100 isoforms reinforcing silencing of lineage-inappropriate genes. We also observed Sp100 binding at the 5' ends of some transcriptionally active host genes and postulate that this could be due to Sp100A, which we recently showed recruits the histone chaperone HIRA to PML-NBs.

We have previously generated Sp100 knockout keratinocyte cell lines that exhibit both normal PML-NB formation and interferon responses. To decouple the activating and repressive functions of specific Sp100 isoforms, we stably expressed individual EYFP-Sp100 proteins in these knockout cells to map their global binding patterns following interferon induction. We propose that Sp100 reshapes the host chromatin landscape to restrict the HPV lifecycle through distinct, isoform-specific mechanisms. We predict that the longer, repressive isoforms bind the HPV genome to limit the early and late stages of infection, whereas Sp100A leverages its association with PML-NBs to coordinate host gene activity across distal genomic loci to establish an antiviral chromatin environment.

Uncovering PRMT1 as an Anti-Oncogenic Modulator of HPV-18 E6 in Cervical Cancer

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High risk human papillomaviruses (HPVs) contribute to cervical cancer through the actions of the E6 and E7 oncoproteins. Because HPV 18 E6 operates within a host environment that changes markedly across the cell cycle — particularly in the regulation of p53, DNA damage responses, and replication stress — its interactions with cellular proteins are likely to be phase specific. Although HPV 18 E6 is known to bind several host factors, many of its cell cycle dependent partners remain unidentified. To uncover new E6 associated proteins and determine whether their engagement varies with cell cycle state, we expressed HPV 18 E6 in HEK293 cells, synchronized the cells at the G1/S and G2/M phases, and performed mass spectrometry. Among the 463 proteins detected, PRMT1 emerged as a consistent potential interactor in both phases. We validated the interaction between HPV 18 E6 and PRMT1 using binding assays. To define the functional relevance of PRMT1 in HPV positive cells, we performed siRNA mediated knockdown of PRMT1 in HPV 18 positive HeLa and C 4 I cervical cancer cell lines. Reducing PRMT1 levels led to decreased p53 protein and a marked stabilization of HPV 18 E6, suggesting that PRMT1 normally supports p53 homeostasis while limiting E6 accumulation. When PRMT1 and HPV 18 E6 were simultaneously depleted, p53 levels remained low, indicating that PRMT1 influences p53 independently of E6. Together, these findings identify PRMT1 as a host restriction factor that maintains p53 stability and prevents excessive buildup of HPV 18 E6 across cell cycle states. Loss of PRMT1 therefore shifts the balance toward a more oncogenic environment in HPV 18 positive cervical cancer cells, highlighting PRMT1 as a key regulatory node in HPV biology and a candidate for further investigation.

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