Going Viral: Stealthy but deadly virus with a serious sweet tooth

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COVID-19 Outbreak

SARS-CoV2
2019-nCoV

STOP THE SPREAD OF GERMS
Help prevent the spread of respiratory diseases like COVID-19.

- Avoid close contact with people who are sick.
- Cover your cough or sneeze with a tissue, then throw the tissue in the trash.
- Avoid touching your eyes, nose, and mouth.
- Clean and disinfect frequently touched objects and surfaces.
- Stay home when you are sick, except to get medical care.
- Wash your hands often with soap and water for at least 20 seconds.
Emerging and Re-emerging Viruses

- Swine Flu
- Ebola virus
- Zika virus
- SARS Coronavirus
- MERS Coronavirus
COVID-19 Pandemic

• Declared a **pandemic** by WHO yesterday
• Virus emerged from a bat reservoir
• Cases as of March 12, 2020: **127,863**
• Countries/regions affected: **116**
• 80,932 cases in China, 70% recovered
• United States: **1,323**
• Death toll: **4,718**
• Fatality rate: 0.6-5% (Global average = 3.4%)
• Stable on surfaces for 3-9 days
• Prevention and control: hygiene and social distancing

Source: Johns Hopkins University
JC Polyomavirus Infection

Initial infection occurs via peroral transmission

Subsequent infection in the kidney

Immunosuppression

JCPyV spreads to the brain causing PML
JCPyV Infection in the Brain

- Capillary
- Neurons
- Oligodendrocytes
- Astrocytes
- Microglial

Michael Wilczek
JCPyV Causes PML

Healthy brain

PML brain

PML lesions

MRI Image: Courtesy of Dr. Avi Nath, NIH
PML

- Symptoms: partial paralysis and cognitive impairments
- 5% of HIV-1 positive patients
- 1:500 patients receiving immunomodulatory therapies
- Debilitating and fatal
- Limited treatment options

PML progression over 4 months

Bag et al. AJNR. 2010.
Laboratory Cell Culture Models for JCPyV Infection

- **Brain**: Human astrocytes and oligodendrocytes
  - VP1
  - Merge

- **Kidney**: Kidney cells
  - T Ag
  - Merge

- **Tissue Sections**:
  - Grey matter
  - White matter
  - Cortex
  - Medulla

**Authors:** Michael Wilczek
How does JCPyV select target cells?

Identification of a JCPyV Receptor

Angelina Palma
Feizi Lab, Imperial College of London

Glycan Array Screen

LSTc

- $\alpha2,6$-linked-lactoseries tetrasaccharide c
- Expressed on a glycoprotein or glycolipid
- Present on many glycan structures on human cells
- Receptor fragment for influenza A strains
Methods to Study JCPyV-LSTc Interactions

Incubate JCPyV with LSTc or controls

Add complex to cells

Measure viral infectivity

Colleen Mayberry
LSTc Inhibits JCPyV Infection

Infected cells (VP1+/visual field)

CONTROL LSTB LSTC

Treatment

Neu and Maginnis et al. 2010. Cell Host Microbe.

*p<0.05
Structural Studies: JCPyV-LSTc Interactions

Purification of VP1 pentamers

X-rays

X-ray detector

Crystals

Gal
GlcNAc
Gal
Glc
LSTc

3' Neu5Ac

Computer Graphics

Protein Model

Electron Density Map

Ursula Neu, Stehle Lab, University of Tübingen
JCPyV VP1 in Complex with LSTc

Ursula Neu, Stehle Lab, University of Tübingen

Neu and Maginnis et al. 2010. *Cell Host Microbe*. 
Specific Contacts Between JCPyV VP1 and LSTc

Key H-bonds with NeuNAc

LSTc L-shaped conformation

Neu and Maginnis et al. 2010. Cell Host Microbe.
Engineering Viruses with Mutated Genomes

Mutate viral genome in infectious clone

Transfect cells with infectious clones

Infectious virions produced?

Measure viral spread
Mutation of JCPyV Sialic Acid-Binding Sites Abolishes Viral Growth

Maginnis and Ströh et al. 2013. mBio.
Neu and Maginnis et al. 2010. Cell Host Microbe.
JCPyV has a Sweet Tooth for LSTc
How does JCPyV enter target cells?
Serotonin (5-HT) Receptors

- G-protein coupled receptors
- Mediates endocytosis of neurotransmitter 5-HT
- Functions in mood, sleep, and behavior
- Activates signaling events
- 15 known 5-HTRs
- 5-HT$_2$Rs (2A, 2B, 2C) are necessary for JCPyV infection
Analysis of JCPyV Interactions with 5-HT$_2$Rs by Super Resolution FPALM

Express photoactivatable 5-HT$_2$Rs in HEK cells

Infect cells with labeled JCPyV

Analyze interactions by FPALM

Kashif Mehmood, Jeanne DuShane
Matthew Parent, Sam Hess
Serotonin Receptor Endocytosis

- 5-HT₂R
- AP2
- Clathrin
- β-arrestin
- Dynamin
- Clathrin
- β-arrestin
Methods to Study Virus-Host Cell Interactions

- Inhibit cellular proteins with siRNAs or chemicals
- Infect with JCPyV
- Stain with Abs to detect JCPyV
- Quantify infection using microscopy

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Serotonin Scaffold Protein \( \beta \)-arrestin is Required for JCPyV Infection


\(*, p < 0.05\)
Confocal Microscopy to Study Viral Entry

JCPyV Nuclei

Colleen Mayberry
Serotonin Scaffold Protein \(\beta\)-arrestin is Required for JCPyV Entry


* \(, p<0.05\)
Disruption of $\beta$-arrestin Binding Motif in 5-HT$_{2A}$R Reduces JCPyV Entry


*, $p<0.05$
Model of JCPyV Internalization

- β-arrestin binding to 5-HT₂R
- CME protein recruitment
- Formation of clathrin-coated pit
- Initiation of clathrin-mediated endocytosis
- Internalization

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MAPK Signaling Pathway
Model of JCPyV Internalization and Signaling

- β-arrestin binding to 5-HT₂R
- Initiation of clathrin-mediated endocytosis
- CME protein recruitment
- Formation of clathrin-coated pit

β-arrestin binding to 5-HT₂R

- GRK
- Active
- Inactive
- β-arrestin

CME protein recruitment

- JCPyV
- Clathrin
- AP2

Formation of clathrin-coated pit

Internalization

MAPK activation

- Raf
- MEK
- ERK

Colleen Mayberry
In-Cell Western Assay to Detect Changes in Viral and Cellular Proteins during Infection
In-Cell Western Assay to Detect Changes in Viral and Cellular Proteins during Infection

Treatment

Infection

Fixation

IRDye

Detection

Jeanne DuShane
Michael Wilczek
Mason Crocker
MAPK ERK is Activated during JCPyV Infection

*P* < 0.001, p < 0.05

JCPyV Infection Requires Raf and MEK Activity

MAPK activation

Raf

MEK

ERK

MEK activation

% Infection

% Infection

Infection Timepoint (min)

Scr

bRaf siRNA

cRaf

Scr

MEK1/2 siRNA

*, p<0.05
Recap

Binding to LSTc

Cell Entry via 5-HT$_2$Rs

JCPyV-induced Cell Signaling

MAPK activation

- Raf
- MEK
- ERK
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