Zika Virus Diagnostics, Transmission, and Vaccine

Pei-Yong Shi
University of Texas Medical Branch
June 14, 2017
Flavivirus genome and proteins

- Capsid
- Pr peptide
- Envelope
- NS1
- Protease-Helicase
- Polymerase
- Methyltransferase

Diagram shows the genome with structural and non-structural proteins, including C, prM, E, NS1, 2A, 2B, NS3, 4A, 4B, NS5, and the enzymes polymerase, helicase, and methyltransferase.
An infectious cDNA clone of ZIKV

Shan et al. 2016. Cell Host & Microbe
Current Zika Diagnostics, Limitations, and Challenge

- **Detect virus**
  - Methods
    - Viral RNA: RT-PCR-based high-throughput assay
    - Viral protein: Immunohistochemistry assay
    - Virus isolation
  - Limitation: Short duration of viremia in patients

- **Detect antibodies**
  - Methods
    - ELISA: Viral envelope protein as the most immunogenic antigen
    - Virus neutralization assay (gold standard) needs safety containment and takes about a week to perform
  - Limitation: Antibodies against viral envelope are cross-reactive among flaviviruses

The challenge:
New serology assay is urgently needed to differentiate different flavivirus infections
Reporter Virus-based Neutralization Assay

- Gold standard of serology assay: the recommended confirmation test
- Assay turnaround time: <48 hours (current PRNT assay > weeks)
- High-throughput format with no limitation in testing large sample numbers
- Proof-of-concept achieved by testing patient specimens

**Zika virus**

- Incubate with patient serum
- Infect cells for 24 hours
- Read fluorescence in cells

**Dengue virus**

Diagnostic results
A reporter ZIKV

Shan et al. 2016. Cell Host & Microbe

Diagram: A reporter ZIKV with structural and non-structural regions.

B. Recombinant virus

WT

Day 4 p.i. plaque

C. EC<sub>50</sub> = 2.5 μM

Relative Rluc activity (% DMSO control)

Cell viability (% DMSO control)

Day 6 p.i. immunostaining

D. EC<sub>50</sub> = 3.6 μM

Log PFU/ml
Correlation of the reporter assay with the plaque reduction neutralization assay

Day 0
*Plating cells*

Day 1
*Infection*
ZIKV-Rlu + Serum

Day 2
*Data acquisition and analysis*
Luciferase substrates

*NT*$_{90}$ Calculation

Shan et al. 2017. EBioMedicine
What triggered the surge of recent epidemics and severe diseases?

- Viral evolution that increases mosquito transmission
- Viral adaptation that increases viremia, leading to transplacental infection
  - through enhanced viral replication
  - through increased antagonism of host restriction
- Introduction to a population with naïve immunity
- Co-infection with Chikungunya virus
- Previous infection and immunity of dengue virus
- Human genetic predisposition to disease
Post-epidemic ZIKV strain infects mosquitoes more efficiently than pre-epidemic strain (I)

Liu et al., 2017. Nature
Post-epidemic ZIKV strain infects mosquitoes more efficiently than pre-epidemic strain (II)
NS1 level determines mosquito infection rate (I)

ZIKV GZ01 strain -2016

ZIKV FSS13025 strain -2010
NS1 level determines mosquito infection rate (II)
NS1 A188V enhances mosquito infection (I)

(a) Zika virus E protein

(b) Zika virus NS1 protein

(c) Comparison of ZIKV NS1 ng/ml between FSS13025 and FSS13025-A188V

(d) Comparison of ZIKV pfu/ml (Log 10) between FSS13025 and FSS13025-A188V
NS1 A188V enhances mosquito infection (II)
Conclusions

- NS1 in mammalian blood facilitates mosquito infection
- Recent epidemic strains have accumulated an A188V mutation that enhances NS1 secretion, leading to enhanced mosquito infection
Zika vaccines

- Vaccine platforms
  - **Inactivated virus**: mouse and monkey efficacy demonstrated
  - **Subunit vaccine** (empty ZIKV particles expressed from DNA plasmid, mRNA, or viral vectors): mouse and monkey efficacy demonstrated
  - **Chimeric virus** using yellow fever or dengue vaccine or other virus vaccine strains to express ZIKV structural proteins
  - **Live attenuated virus**

- Inactivated vaccine and subunit vaccine: Multiple shots and periodic booster to maintain immunity; better safety compared with live attenuated vaccine
- Live attenuated vaccine: Single shot vaccine with potential life time immunity; higher safety barrier

Larocca et al., 2016, Nature; Abbink et al., 2016 Science; Dowd et al, 2016 Science; Pardi et al., 2017, Nature; Richner et al., 2017, Cell
A live-attenuated ZIKV vaccine with 3’UTR deletion

Mutant 10-del ZIKV is attenuated and protective in A129 mouse model

- Viremia after $10^4$ IFU s.c. vaccination. No disease or weight loss in vaccinated mice.
- The vaccinated mice were fully protected from viremia after $10^6$ PFU i.p. challenge.
Sterilizing antibody titers after vaccination

Antibody titer on day 28 post-vaccination

Antibody titer on day 28 post-challenge
Vaccine efficacy after single 100 IFU dose

- Viremia after 100 IFU dose
- Complete protection of viremia after $10^6$ PFU i.p. challenge
- Antibody titer on day 28 after 100 IFU vaccination
A129 mice were infected with $1 \times 10^4$ IFU WT and 10-del viruses. On day 28 post-infection, mouse spleens were harvested, stimulated, and assayed for T cell response.
Three-week old A129 mice were infected with $1 \times 10^4$ IFU WT or 10-del virus.
Neurovirulence in one-day old mice after i.c. inoculation
The vaccine candidate can not infect mosquitoes

Mosquito infection rate after $10^6$ PFU/ml blood meal
The vaccine protects pregnant C57BL/6 mice and their developing fetuses

- **10^5** IFU footpad vaccination to WT female C57BL/6 mice
- On day E5, inoculate 2 mg anti-Ifnar1
- On day E6, s.c. inoculate 10^6 FFU of mouse-adapted Dakar ZIKV
The vaccine protects against testis infection and oligospermia in male A129 mice

- 10^4 IFU s.c. vaccination to A129 male mice
- On day 28, s.c. challenged with 10^5 IFU of ZIKV PRVABC59
- On day 49, measure total and motile sperm counts
The vaccine protects rhesus macaques from ZIKV infection

- $10^3$ IFU s.c. vaccination
- On day 56, s.c. challenged with $10^3$ PFU of ZIKV PRVABC59
Conclusions

A single-dose of $10^3$ IFU of 3’UTR deletion vaccine candidate:
- Prevents infection in non-human primates
- Achieve protective immunity within 14 days post-vaccination in non-human primates
- Prevents in utero transmission in pregnant mice
- Protects testis damage and low sperm counts in mice
- Excellent safety profile in mice and non-human primates
Acknowledgement

Chao Shan, Xuping Xie, Camila Fontes, Tony Muruato, Jing Zou, Hongjie Xia

Xianwen Zhang, Yujiao Yang, Jing Zou, Hongjie Xia

Jannyce Nunes, Bruno Nunes, Daniele Medeiros, Coleman Baker

Wadsworth Center:
Susan Wong

Tsinghua University:
Gong Cheng

NIH:
Barney Graham

Washington University:
Michael Diamond

UTMB:
Scott Weaver
Shannan Rossi
Alan Barrett
Tina Wang
Robert Tesh
Nikos Vasilakis
Shelton Bradrick
Mariano Garcia-Blanco