

Embargoed until Nov. 13, 3 p.m. PST
Press Room, Nov. 12-16: (619) 525-6230

Contacts: Emily Ortman, (202) 962-4090
Kym Kilbourne, (202) 962-4060

Exploring How Zika Virus Attacks Cells in Developing Brains

Findings may aid development of diagnostic tools and vaccines to protect those at risk

SAN DIEGO — Zika virus rapidly emerged from obscurity to a scourge that dominates the news. Researchers are making progress as new discoveries provide insights into how Zika attacks the brain and how scientists may combat it. The findings were presented at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

The mosquito-borne Zika virus arose as a medical threat in October 2015, when northeastern Brazil reported an abrupt increase in the number of babies born with abnormally small heads, a condition called microcephaly. Since then, scientists have been working rapidly to find out just how harmful the virus might be. In the past year, scientists have linked Zika infection to neurological symptoms seen in infants, showing that Zika virus targets a type of neural stem cell that gives rise to the brain's cerebral cortex. Zika virus may also affect adults as it is also associated with Guillain-Barre Syndrome, a nerve-damaging disorder that can cause temporary paralysis. Scientists continue to determine just how Zika virus damages the nervous system.

Today's new findings show that:

- Patterns of gene activity in human neural stem cells show that Zika virus meddles with cellular machinery needed for cell division and alters the genetic program that guides cell differentiation (Patricia Garcez, abstract 495.25, see attached summary).
- Studies of mouse cells infected with Zika virus suggest the virus not only curbs production of proteins needed to grow new neurons, but also amps up production of proteins that spur development of non-neural brain cells called astrocytes (Olivia Lossia, abstract 28.02, see attached summary).

Other recent findings discussed show that:

- Tiny 3-D "mini-brains" grown in the lab, with the aid of a 3-D printer, allow scientists to view the effects of Zika virus exposure and test for drugs that might offer protection (Xuyu Qian, abstract 495.14, see attached summary).
- Neural stem cells from different donors and grown in the lab show that some may be more vulnerable to Zika infection than others. Researchers are now sorting out the ways the virus affects different cells (Erica McGrath, abstract 573.06, see attached summary).

"The speed at which Zika moved from being a relatively obscure disease to a global health threat illustrates just how vulnerable the world is to emerging infections," said press conference moderator Hongjun Song, PhD, of the Johns Hopkins School of Medicine. "Today's findings show the progress we've made in addressing some of the urgent questions about how Zika virus interacts with neural cells to interfere with their function." Song is also an author on one of the presented studies (abstract 495.14).

The research was supported by national funding agencies such as the National Institutes of Health, as well as other public, private, and philanthropic organizations worldwide. Find out more about Zika and the brain at *BrainFacts.org*.

Related Neuroscience 2016 Presentation

Special Lecture: Bitten: Understanding and Modulating Mosquito Attraction to Humans
Sunday, Nov. 13, 2016, 8:30-9:40 a.m., SDCC Ballroom 20

Abstract 495.25 Summary

Lead Author: Patricia Garcez, PhD
Federal University of Rio de Janeiro
Rio de Janeiro, Brazil

(+55) 21 996603096
ppgarcez@gmail.com

Zika Virus Hijacks Genetic Program That Signals Developing Cells to Multiply, Specialize

Stem cell studies show infection inhibits cell division, alters program for cell differentiation

Zika virus interferes with the cellular machinery controlling cell division and alters the expression of genes guiding the formation and development of neurons, according to findings released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Zika virus has been associated with microcephaly — a condition where a baby's head is abnormally small, often because the brain has not developed properly — and other fetal brain defects. But the mechanism used by the virus to attack cells is unknown. As key players in embryonic brain formation, neural stem cells can turn into any one of the three major cell types in the central nervous system.

In this study, scientists exposed human stem cells to a strain of Zika virus collected from Brazilian patients and coaxed the cells to become neurospheres, organized groups of neural cells resembling fetal brain tissue. Three days after exposure, the team screened the cells to identify the infected ones. Already, the Zika-infected cells appeared smaller in size compared to the healthy cells.

Researchers then identified which genes were active in infected and uninfected cells. The genes normally active during cell division, a multistep process by which a cell divides in two, weren't active in infected cells, thwarting their ability to multiply. In addition, genes driving cell specialization also were repressed in infected cells. As a result, these cells lacked the necessary genetic guidance to split in two and differentiate into the various types of brain cells.

“The findings provide insights into the molecular mechanisms of Zika infection,” said lead author Patricia Garcez of the Federal University of Rio de Janeiro. “The effect of the virus at these crucial points of brain development likely explains some of the consequences seen on brain formation and function.”

Research was supported with funds from the International Society of Neurochemistry.

Scientific Presentation: Tuesday, Nov. 15, 8-9 a.m., Halls B-H

15738. Zika virus impairs growth and alters cell cycle and neurogenic programmes

P. P. GARCEZ¹, J. MOTA DE VASCONCELOS³, R. MADEIRO DA COSTA⁴, E. C. LOIOLA⁴, R. DELVECCHIO², P. TRINDADE⁴, L. H. HIGA², J. M. NASCIMENTO⁵, J. J. VIANEZ³, A. TANURI², S. K. REHEN⁴;

²Inst. of Biol., ¹UFRJ, Rio de Janeiro, Brazil; ³Ctr. for Technological Innovation, Evandro Chagas Inst., Belém, Brazil; ⁴D'Or Inst. for Res. and Educ. (IDOR), Rio de Janeiro, Brazil; ⁵Univ. of Campinas, Campinas, Brazil

TECHNICAL ABSTRACT: Zika virus (ZIKV) has been associated with microcephaly and other brain malformations; however, the molecular effects of ZIKV over the central nervous system remain to be uncovered. Here we examined the deregulated genes expressed in neurospheres infected with ZIKV. Human Neural Stem Cells (NSC) were exposed to ZIKV Br_AB_ES- isolated from Brazilian patients. After 2 hours viral exposure (0.025 MOI), NSCs were submitted to a differentiation protocol to form neurospheres. Immunocytochemistry was performed 3 days after ZIKV exposure. To confirm ZIKV infection, we used flavivirus group antigen (clone 4G2) antibody. Apoptosis was measured with activated caspase immunofluorescence. Three days after infection, there was a significant increase in cell death observed in ZIKV infected neurospheres. After 12 days in vitro, neurospheres were completely depleted. In order to evaluate molecular effects of ZIKV infection, we performed RNA-seq of the infected and mock neurospheres after 3 days in vitro. mRNA transcriptional profile analyses revealed that ZIKV infection alters cell cycle regulators and downregulates neurogenic programmes, in addition to transcriptional regulation due to viral replication. Altogether, ZIKV infection modulated genes involved in different pathways inhibiting cell cycle and neurogenesis. The effect of ZIKV at crucial steps of brain development likely explains the consequences of the so-called congenital syndrome of ZIKV on brain formation and function. Our work provides insights into the molecular mechanisms of ZIKV infection. It implicates the ZIKV in progenitor proliferation, differentiation and survival, thus improving our understanding of human microcephalies.

Abstract 28.02 Summary

Lead Author: Olivia V. Lossia
Central Michigan University
Mount Pleasant, Mich.

(248) 416-3487
olossia@gmail.com

Study Suggests Zika Infection May Spur Production of Astrocytes

Infected cells curtail proteins needed to produce new neurons, increase proteins for growth of astrocytes

Zika virus damages and sometimes kills neural stem cells, but a study of infected mouse cells shows that Zika infection might actually spur production of another type of brain cell, non-neural “helper” cells called astrocytes. The findings were released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world’s largest source of emerging news about brain science and health.

Previous research shows Zika selectively targets neural stem cells in developing embryos and may also affect adult nerve cells. Mouse and cell culture models are hinting at how the virus causes harm, interfering with cell division and growth cycles. Researchers are beginning to study Zika infection by examining the way proteins secreted by cells change during the early stages of infection. Collectively known as the secretome, these proteins include growth factors, infection-fighting proteins, and bioactive molecules critical for differentiating stem cells into specific cell types.

In this study, over a period of seven days, researchers charted the genetic activity and protein output of mouse neural stem cells infected with Zika virus. They analyzed 17 genes that identify specific cells types and guide brain development. Seven days after infection, the activity of genes involved in building and maintaining new neurons abruptly slowed. At the same time, genes involved in building another type of brain cell — astrocytes — became busier. Astrocytes help maintain, support, and regenerate the central nervous system.

The protein profiles reflected these changes in gene behavior. Seven day after infection, overall protein production had slowed in the neural stem cells, including production of proteins playing a role in brain development and function. However, secretions increased for a protein called glia maturation factor-beta, which stimulates neural regeneration.

“Our research suggests that Zika virus infection in neural stem cells induces the differentiation of astrocytes, a cell type involved in resolving infections in the central nervous system,” said lead author Olivia Lossia of Central Michigan University. “We also found that Zika infection causes the secretion of proteins unique to infection, a finding that may represent biomarkers of fetal infection.”

Research was supported with funds from the College of Medicine, the Field Neurosciences Institute, and the John G. Kulhavi Professorship in Neuroscience at Central Michigan University, as well as by contributors to the Experiment.com crowdfunding campaign, “Does Zika virus replicate in neural cells?”

Scientific Presentation: Saturday, Nov. 12, 2-3 p.m., Halls B-H

11373. Determining the impact of Zika virus on neural stem cells and mature neurons

O. V. LOSSIA^{1,2,3}, M. T. OCEAN², E. D. PETERSEN^{2,1}, B. SRINAGESHWAR^{1,3}, U. HOCHGESCHWENDER^{2,1}, G. L. DUNBAR^{1,5,4,3}, M. J. CONWAY², J. ROSSIGNOL^{2,3,1};

¹Neurosci., ²Col. of Med., ³Field Neurosciences Inst. for Restorative Neurosci., ⁴Psychology, Central Michigan Univ., Mount Pleasant, MI; ⁵Field Neurosciences Inst., Saginaw, MI

TECHNICAL ABSTRACT: The recent emergence of Zika virus (ZIKV) has been linked to severe nervous system abnormalities in infants, such as microcephaly, and the autoimmune disorder, Guillain-Barré, in adults. The World Health Organization predicts that by the end of this year three to four million people will be infected with the Zika virus, a virus now known to be transmitted by mosquitoes and through sexual contact in humans. Very little is known regarding the basic biology of this virus and its implications in the developing brain, fueling the need to investigate the interactions of ZIKV with neural cells. So far, studies have shown that ZIKV affects the morphology and survival of neural stem cells and neural progenitor cells. In our study, we sought to assess the effect of ZIKV on neural stem cells (NSCs) and mature neurons using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and multielectrode arrays (MEAs), respectively. We were able to determine the impact of infection on the secreted proteome “secretome” of NSCs as well as how the infection impacts the neuronal firing capabilities of mature neurons. Our results show that the ZIKV strain of African lineage is able to replicate in mouse NSCs and leads to a significant downregulation in secretion of proteins into the extracellular environment. These studies provide mechanistic detail on how ZIKV impacts the differentiation, survival, development, and function of neural stem cells and neuronal cultures.

Speaker Summary 495.14

Speaker: Xuyu Qian
Johns Hopkins University
Baltimore

(508) 373-3500
xqian6@jhmi.edu

Lab-Made ‘Mini-Brains’ Allow Researchers to Study Zika’s Effects on Developing Brains

Tuesday, Nov. 15, 9-10 a.m., Halls B-H

Our research using lab-grown “mini-brains” suggests that Zika virus infection causes abnormally small brains in fetuses by targeting and damaging neural progenitor cells (NPCs). The World Health Organization recently declared the clustered incidents of neurological birth defects in Zika virus outbreak regions a public health emergency of international concern. Recent clinical studies examining fetal tissues have shown that upon infection during pregnancy, Zika virus can pass through placenta to gain access to the developing fetal brain and cause damage, potentially leading to an abnormally small head, a condition termed microcephaly. However, it remains unclear how Zika virus can cause this damage.

Previous studies from our group and others, using human NPCs and neurosphere cultures, showed infection by Zika virus leads to increased cell death and inhibited growth. However, these initial studies were performed in 2D cell culture systems that lack many structural features of the human brain, and could not directly address the causal link between Zika virus and microcephaly. In the present study, we instead used 3D “mini-brain” organoids grown from human stem cells to investigate the effects of Zika virus exposure in a more relevant context.

Human induced pluripotent stem cells (hiPSCs) were grown in 3D suspension culture supplemented with specific growth factors and chemical cues to facilitate self-assembly of the cells to form brain organoids resembling fetal brain at both cellular and organizational levels. An interdisciplinary team engineered a miniaturized spinning bioreactor, using 3-D printing technology, that allowed efficient culturing of organoids with reduced cost. This mini-bioreactor made it easier to find optimal growth conditions and establish a highly consistent method to generate brain organoids to model human fetal brain development. The organoids were then transiently exposed to Zika virus at different growth stages and allowed to grow for an additional two weeks before examining the effects. Results showed Zika virus efficiently infects organoids, with a specific preference for infecting NPCs, the cell type that gives rise to other neural cells in developing brain. In addition, Zika virus infection in NPCs is productive as infected cells were transformed into viral factories to produce more virus to infect neighboring cells. Zika infection causes increased cell death and reduced proliferation of NPCs, resulting in significantly smaller organoids compared to non-infected organoids, due to a dramatic reduction in the size of the neuronal layer.

These results provide compelling evidence that upon access to the fetal brain, productive and preferential infection of NPCs by Zika virus leads reduced brain volume as identified in fetuses and newborns with microcephaly. The next step will be to use the brain organoids as a platform to test drugs that might provide protection against Zika virus. Furthermore, the organoids provide us with a unique system to investigate the underlying mechanisms of our findings. We are currently introducing Zika virus genes one at a time into organoids, in order to determine which component of the Zika virus is responsible for the detrimental effects observed.

Moreover, our brain organoid system proves a reliable platform to model human diseases beyond Zika virus. Compared to other existing models, these organoids better recapitulate developing human brains and offer an opportunity to investigate embryonic human brain development as a continuous dynamic process, and in turn allow for pharmacological and genetic manipulations to investigate underlying mechanisms.

Research was supported with funds from the National Institute of Neurological Disorders and Stroke, the National Institute of Mental Health, the National Institute of Environmental Health Sciences, the National Institute of Allergy and Infectious Diseases Extramural Activities, the Maryland Stem Cell Research Fund, and the Simons Foundation Autism Research Initiative.

Speaker Summary 573.06

Speaker: Erica McGrath
University of Texas Medical Branch
Galveston, Texas

(443) 293-2559
elmgrat@utmb.edu

Sorting Out Why Some Babies' Brains Are More Susceptible to Zika Than Others

Tuesday, Nov. 15, 2:15-2:30 p.m., SDCC 30B

Our research shows that human fetuses may have different susceptibility to neurological problems caused by a Zika virus infection. Zika virus has recently emerged as a public health threat in several regions worldwide, with 65 countries and territories reporting active Zika virus transmission. The Zika virus can be transmitted by a certain type of mosquito, as well as sexually. While the Zika virus typically results in mild or asymptomatic infections in healthy adults or children, the risk of microcephaly and other serious neurological disorders in a developing fetus is an alarming consequence of a Zika virus infection, and thus raises a huge concern for a serious health problem worldwide.

A Zika virus infection is known to cause microcephaly in a subset of infants born to infected pregnant mothers. It is important to understand why not all children born to Zika-infected mothers develop microcephaly. If we can understand what makes a fetus more or less vulnerable to a Zika virus infection, we may be able to design more effective treatments and prevention strategies.

Our study is the first to show individual differences in human fetal neural stem cells taken from independent donors. Our results confirm what other studies have reported: that a Zika virus infection is associated with detrimental changes in neural stem cells.

There are two main types of Zika viruses that have been reported to infect humans, African and Asian lineages. But only the Asian Zika virus has been linked to microcephaly. We used an Asian Zika virus isolated from the 2015 Mexican outbreak (Mex1-7) to infect native human brain-derived neural stem cells. These human cells were originally derived from three individual fetal brains, and neural stem cells are known to be critical during fetal brain development. All three lines of human neural stem cells were infected at similar rates and had similar reductions in cell proliferation. However, Mex1-7 caused cells to die and impaired the ability to produce neurons in a human origin-dependent manner. Specifically, two of the cell lines had a decreased number of neural stem cells becoming neurons, while one cell line showed no change after infection. Our pilot study also found that the Zika virus disturbed the expression of more than 900 genes, for example increasing genes associated with innate immunity and glial cells while decreasing cell cycle genes.

Our next step is to understand how the genes that the Zika virus changes are able to impact normal function and growth of human neural stem cells. By understanding how the Zika virus changes the cell, we can then move forward and work on designing ways to prevent the detrimental consequences of Zika.

Research was supported with funds from the John S. Dunn Foundation and the University of Texas Medical Branch.