Zika Virus-Induced Microcephaly and Its Possible Molecular Mechanism

Md Imam Faizan^a Mohd Abdullah^{a, c} Sher Ali^a Irshad H. Naqvi^b
Anwar Ahmed^d Shama Parveen^a

^aCentre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, New Delhi, India; ^bDr. M.A. Ansari Health Centre, Jamia Millia Islamia, New Delhi, India; ^cSchool of Life Sciences, Jaipur National University, Jaipur, India; ^dProtein Research Chair, Department of Biochemistry, College of Science, King Saud University, Riyadh, Saudi Arabia

Keywords

Zika virus · Microcephaly · Molecular mechanism

Abstract

Zika virus is an arthropod-borne re-emerging pathogen associated with the global pandemic of 2015–2016. The devastating effect of Zika viral infection is reflected by its neurological manifestations such as microcephaly in newborns. This scenario evoked our interest to uncover the neurotropic localization, multiplication of the virus, and the mechanism of microcephaly. The present report provides an overview of a possible molecular mechanism of Zika virus-induced microcephaly based on recent publications. Transplacental transmission of Zika viral infection from mother to foetus during the first trimester of pregnancy results in propagation of the virus in human neural progenitor cells (hNPCs), where entry is facilitated by the receptor (AXL protein) leading to the alteration of signalling and immune pathways in host cells. Further modification of the viral-induced TLR3-mediated immune network in the infected hNPCs affects viral replication. Downregulation of neurogenesis and upregulation of apoptosis in hNPCs leads to cell cycle arrest and death of the developing neurons. In addition, it is likely that the environmental, physiological, immunological, and ge-

netic factors that determine in utero transmission of Zika virus are also involved in neurotropism. Despite the global concern regarding the Zika-mediated epidemic, the precise molecular mechanism of neuropathogenesis remains elusive.

© 2017 S. Karger AG, Basel

Introduction

Zika virus is an arbovirus that belongs to the *Flavivirus* genus and *Flaviviridae* family [1]. It is an enveloped virus and its mature 3.8-Å resolution structure was determined by cryoelectron microscopy [2]. The structure of Zika virus is similar to that of other flaviviruses, such as Dengue and West Nile [3, 4]. The genome of Zika virus is positive sense linear, with monopartite RNA of 10,794 bases in length [5]. The genome encodes for a polyprotein that is cleaved into 3 structural proteins (capsid, pre-Membrane, envelope) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) [6]. Zika virus was first isolated from Rhesus monkey number 766 in 1947 from Zika forest near Lake Victoria in Uganda by a group of scientists who were conducting serosurveys on yellow fever viruses [7]. The first documented human infection of

Zika virus was reported from Uganda and the United Republic of Tanzania in 1952 [8]. Zika virus is transmitted by infected *Aedes* mosquitoes (*A. aegypti*, *A. albopictus*, *A. africanus*, and *A. luteocephalus*) [9, 10]. *Aedes* species is also responsible for the transmission of other arthropod-borne viral infections like Dengue and Chikungunya [11]. The other modes of transmission of Zika virus include sexual contact [12], saliva [13], urine [14], breast milk [15], blood transfusion [16], and the prenatal route from mother to foetus [17]. Zika fever caused by this virus has clinical symptoms similar to that of other arboviral infections like Dengue and Chikungunya [18, 19]. The clinical symptoms of Zika viral infection include fever, fatigue, myalgia, headache, rashes, conjunctivitis, and retro-orbital pain [20]. In some cases, stillbirth, microcephaly, intracranial calcifications, and Guillain-Barré syndrome are also manifested [21–23]. Zika virus infection can be diagnosed in the clinical samples by serology using IgM ELISA [24, 25] and RNA detection by RT-PCR [14, 26].

Between 1947 and 2007, a few sporadic human cases of Zika virus were reported from Africa and Asia [27–33]. A massive Zika virus outbreak was reported from Yap Island in 2007, affecting about 73% of the inhabitants [20]. Subsequent outbreaks were reported in 2013 and 2014 from the Pacific Islands, including Cook Island, French Polynesia, Easter Island, and New Caledonia [34–36]. Some of the cases identified during these outbreaks were also related to neurological complications and congenital malformations. For the first time, during the Pacific Island outbreak, Zika virus was found to be associated with microcephaly [36] and Guillain-Barré syndrome [37].

During 2015 and 2016, a global Zika virus pandemic was triggered in the Americas and Caribbean regions [38]. Around 153,322 confirmed, 507,851 suspected, and 14 death cases of Zika viral infection were reported by the Pan American Health Organization (PAHO) from this region up until October 13, 2016 [39]. According to the prediction of the World Health Organization (WHO), between 3 and 4 million Zika virus infections are expected to occur in 2016 in the Americas alone [40]. Up to October 13, 2016, people from 73 different countries had been affected by this virus since 2007 [41]. The frightening aspect of this global re-emerging viral threat is its association with a large number of neurological and autoimmune aberrations. In view of this viral pandemic, on February 1, 2016, the WHO declared a public health emergency of international concerns (PHEIC) for microcephaly and other neurological disorders in the context of the Zika viral infection [42]. The devastating effect of

this pandemic resulted in a large number of microcephaly and/or CNS malformations from different geographical regions during 2015 and 2016 (2,160 cases in total). This included 2,001 cases in Brazil, 42 cases in Columbia, and 27 cases in the USA up to October 13, 2016 [41].

Microcephaly

Microcephaly is a clinical condition in which the head size measured by the occipitofrontal circumference is less than –3 standard deviations. This is significantly smaller than normal for the person's age and sex [43, 44]. In humans, microcephaly represents a severe congenital defect [45]. Two types of microcephaly are recognized. The first occurs when the brain fails to grow to its appropriate size during pregnancy at around 32 weeks of the gestation period, and is caused by a gradual decrease in the neuron production. The other relates to a normal brain size at birth but failure to grow subsequently due to the loss of dendritic connections [46]. Some authors have classified 3 types of microcephaly on the basis of Giacomino's classification: (i) microcephalia vera, where the size of the brain remains small without any sign of injury or deformation, (ii) microcephalia spuria, which shows some pathological changes and injury to the brain, and (iii) microcephalia combinata, which reflects a small brain size with a trace of injury [45]. The etiology of microcephaly can be both genetic and non-genetic. The genetic aspects include ambiguous genitalia and the 14-3-3 epsilon gene that causes lissencephaly. The non-genetic factors include alcohol consumption during pregnancy, abnormal weight gain during the gestation period, poor parental care including malnutrition, incomplete placental development, systemic and metabolic disorders, exposure to teratogens during pregnancy, non-accidental head injury, Rubenstein-Taybi syndrome, and viral infections [46, 47]. Some microcephaly cases of unknown etiology reported earlier were known to be idiopathic [48]. A previous investigation has shown that microcephaly is probably caused by depletion of the founder population of radial glia and neural stem cells in the developing brain, either through cell death or premature differentiation [49].

Zika Virus-Induced Microcephaly

Neuro-developmental malformations have been previously linked to many viral infections, such as cytomegalovirus, rubella, West Nile virus, HIV (human immuno-

Fig. 1. Summary of the Zika virus (ZIKV) infection and proposed mechanism of microcephaly. **a** ZIKV infection and neuropathogenesis. [I] Transmission of ZIKV through an infected mosquito bite, sexual contact, or from mother to baby. [II] Enlarged image of ZIKV and the invasion of viral particles through dermal cells via an infected mosquito. [III] ZIKV crossing the placental barrier and

infecting the foetus. [IV] An enlarged view of the placental section (villus). [V] A section of terminal villi showing placental macrophages facilitating viral entry. [VI] A baby with a microcephalic head. [VII] A diagrammatic representation of hNPCs showing the proposed molecular mechanism of microcephaly.

deficiency virus), herpes simplex virus, and Chikungunya [50–53]. Significantly, neurological malformations caused by Zika virus are similar to those caused by cytomegalovirus [54]. Recent Zika virus infections in the Americas have been associated with neurological malformations in newborns known as microcephaly [17]. The first trimester of pregnancy is crucial for neurological development. Zika viral infection in the mother during this period is more likely to affect the central nervous system [36]. From such cases, Zika virus was isolated from hu-

man blood [55], amniotic fluid [17], breast milk [15], saliva [13], urine [14], semen [56], foetus brain [22], skin fibroblast [57], and placental tissue [58].

Possible Molecular Events Leading to Microcephaly

Dermal fibroblasts and epidermal keratinocytes are the primary targets of Zika virus infection. This is followed by the infection of dermal dendritic cells (Langer-

Fig. 1. Summary of the Zika virus (ZIKV) infection and proposed mechanism of microcephaly. **b** Proposed molecular mechanism of microcephaly in hNPCs based on recent publications. Binding of viral particles with the hNPCs via the AXL receptor facilitates viral entry with the formation of the endosome. The viral envelope degrades due to a decrease in pH and release of virion into the cytoplasm. Some viral RNA released in the cytoplasm replicates and forms the viral proteins using the host cell machinery. The viral RNA and proteins form progeny virions which are released from

the host cell. The viral RNA also binds to the TLR3 receptors present on the surface of the endosome. Hyperactivation of TLR3-mediated innate immune response occurs with the binding of viral RNA. Subsequent dysregulation in transcription occurs due to downregulation of about 41 specific genes responsible for NPC differentiation. This leads to impaired neurogenesis and activation of the proapoptotic pathway. Consequently, the downregulation of neurogenesis and upregulation of apoptosis leads to impaired brain growth culminating in microcephaly.

hans cells), which facilitates Zika virus dissemination to different organs through the circulatory system [57]. The transplacental transmission of Zika virus from mother to foetus may occur by the infection of placental macrophages (Hofbauer cells) and cytotrophoblasts [59]. Macrophages are the main target cells of Zika viral infection in the placenta [60]. Viral infection in placental macrophages induces the production of type I interferon and pro-inflammatory cytokines resulting in an antiviral gene expression [60].

The entry of Zika virus is mediated by cell surface receptors DC-SIGN, AXL, heat shock proteins, TYRO3, and TIM-1 [57, 61]. AXL, a phosphatidylserine protein, belongs to the TAM receptor family of phagocytic recep-

tors [62]. A recent investigation has shown that the AXL protein is overexpressed in developing human brain cells, including radial glia, astrocytes, endothelial, and microglia [61]. Interestingly, these cells with highly expressed AXL protein are particularly vulnerable to Zika virus infection. Entry of Zika virus through this receptor stimulates AXL-mediated signalling pathways and suppresses the innate immune response [57]. This leads to viral infection in neurons and their associated cells. Further investigations on the expression of AXL and other receptors will provide insight into the detailed molecular mechanism of Zika virus-induced neurovirulence.

A recent investigation has shown that Zika virus replicates in mouse embryonic brain mainly in the neural

progenitor cells (NPCs). Zika virus infection leads to an alteration in the regulation of genes associated with the immune response, cell cycle, differentiation, and apoptosis in NPCs, resulting in neurological malformations [63]. Furthermore, Tang et al. [64] demonstrated that Zika virus can also infect human (h) NPCs in the cell culture system. The authors concluded that Zika virus infection has been found to dysregulate the cell cycle, and transcription and apoptotic pathways in hNPCs, leading to the reduced growth of the cells. Although the precise molecular mechanism of microcephaly is still unknown, a model has recently been proposed for Zika virus-induced damage to the developing brain [65]. The authors developed a 3D model of a first-trimester human brain using embryonic stem cell-derived cerebral organoids. In normal cells, the TLR3 receptor serves as a defender against viral invasion or innate immune response. This study suggested that in Zika virus-infected NPCs, the TLR3-regulated immune network affects the expression of around 41 genes responsible for neurogenesis, the differentiation of NPCs, and apoptosis. Based upon these recent investigations, we describe a possible molecular mechanism of Zika virus-induced microcephaly involving hNPCs (Fig. 1a, b). Whilst the proposed mechanism seems to be of interest, it by no means authenticates the actual steps involved in microcephaly. Further identification of the putative genes in the context of Zika virus-induced microcephaly is likely to enrich our understanding on the menace of this infection.

It may appear to be out of context, but bioinformatics-based data has been reported supporting Zika virus-induced microcephaly [66]. Researchers have shown dysregulation of retinoic acid-dependent genes that may affect the formation of the neural tube in developing brain cells, causing neurological malformations. Additional

work on this line using elaborate experimental approaches will provide an insight into the mechanism of action of this neurotropic viral pathogen.

Conclusions

Zika virus-induced microcephaly is initiated in the first trimester of pregnancy due to its in utero transmission from mother to foetus. This virus targets the NPCs affecting gene expression, neurogenesis, the differentiation of developing neurons, and causing apoptosis leading to several neurological anomalies, including microcephaly. It would be rewarding if genes implicated in the regulation of physical and physiological anomalies are identified and the genotype-phenotype correlation is established beyond doubt. This would facilitate the identification of genes operating in the background besides the ones implicated in aberrant signalling and induced apoptosis. Analysis of neurotropic cases employing microarray, next generation sequencing, and exome sequencing, along with the elucidation of involvement of long non-coding RNA and siRNA would go a long way to determining the actual molecular pathways culminating in microcephaly.

Acknowledgements

Md Imam Faizan is supported by a research fellowship of the University Grants Commission, Government of India. Sher Ali acknowledges the award of a J.C. Bose National Fellowship from DST-SERB, New Delhi, India.

Work in our laboratory is supported by grants from the University Grants Commission and Council of Scientific and Industrial Research, Government of India. This study was supported by King Saud University, Deanship of Scientific Research, College of Science Research Centre.

References

- 1 Fields BN, Knipe DM, Howley PM: Fields Virology. Philadelphia, Wolters Kluwer Health, 2007.
- 2 Sirohi D, Chen Z, Sun L, Klose T, Pierson TC, Rossmann MG, et al: The 3.8 Å resolution cryo-EM structure of Zika virus. *Science* 2016;352:467–470.
- 3 Kuhn RJ, Zhang W, Rossmann MG, Pletnev SV, Corver J, Lenches E, et al: Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. *Cell* 2002; 108:717–725.
- 4 Zhang X, Ge P, Yu X, Brannan JM, Bi G, Zhang Q, et al: Cryo-EM structure of the mature dengue virus at 3.5-Å resolution. *Nat Struct Mol Biol* 2013;20:105–110.
- 5 Tova-Herrera OE, Villanueva-Segura OK, Dávila JM, Gomez-Govea MA, Medina-Sanchez A, de Jesús Zamudio-Osuna M, et al: Brief description of Zika virus. *Artrópodos y Salud* 2016;3:13–20.
- 6 Baronti C, Piorkowski G, Charrel RN, Boubis L, Leparac-Goffart I, de Lamballerie X: Complete coding sequence of Zika virus from a French Polynesia outbreak in 2013. *Genome Announc* 2014;2:e00500–e00514.
- 7 Dick G, Kitchen S, Haddow A: Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg* 1952;46:509–520.
- 8 Smithburn K: Neutralizing antibodies against certain recently isolated viruses in the sera of human beings residing in East Africa. *J Immunol* 1952;69:223–234.
- 9 Musso D, Gubler DJ: Zika virus. *Clin Microbiol Rev* 2016;29:487–524.
- 10 Hayes EB: Zika virus outside Africa. *Emerg Infect Dis* 2009;15:1347–1350.

- 11 Deeba F, Islam A, Kazim SN, Naqvi IH, Broor S, Ahmed A, et al: Chikungunya virus: recent advances in epidemiology, host pathogen interaction and vaccine strategies. *Path Dis* 2016;74:ftv119.
- 12 Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, et al: Probable non-vector-borne transmission of Zika virus, Colorado, USA. *Emerg Infect Dis* 2011;17:880–882.
- 13 Musso D, Roche C, Nhan T-X, Robin E, Teissier A, Cao-Lormeau V-M: Detection of Zika virus in saliva. *J Clin Virol* 2015;68:53–55.
- 14 Gourinat A-C, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M: Detection of Zika virus in urine. *Emerg Infect Dis* 2015;21:84–86.
- 15 Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D: Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. *Euro Surveill* 2014;19:20751.
- 16 Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, et al: Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. *Euro Surveill* 2014;19:20761.
- 17 Calvet G, Aguiar RS, Melo AS, Sampaio SA, de Filippis I, Fabri A, et al: Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. *Lancet Infect Dis* 2016;16:653–660.
- 18 Mardekian SK, Roberts AL: Diagnostic options and challenges for Dengue and Chikungunya viruses. *Biomed Res Int* 2015;2015:834371.
- 19 Ios S, Mallet H-P, Goffart IL, Gauthier V, Cardoso T, Herida M: Current Zika virus epidemiology and recent epidemics. *Med Mal Infect* 2014;44:302–307.
- 20 Duffy MR, Chen T-H, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al: Zika virus outbreak on Yap Island, federated states of Micronesia. *New Engl J Med* 2009;360:2536–2543.
- 21 Araujo LM, Ferreira MLB, Nascimento OJ: Guillain-Barré syndrome associated with the Zika virus outbreak in Brazil. *Arq Neuropsiquiatr* 2016;74:253–255.
- 22 Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, Mraz J, et al: Zika virus associated with microcephaly. *New Engl J Med* 2016;374:951–958.
- 23 Sarno M, Sacramento GA, Khouri R, do Rosário MS, Costa F, Archanjo G, et al: Zika virus infection and stillbirths: a case of hydrops fetalis, hydranencephaly and fetal demise. *PLoS Negl Trop Dis* 2016;10:e0004517.
- 24 Huzly D, Hanselmann I, Schmidt-Chanasit J, Panning M: High specificity of a novel Zika virus ELISA in European patients after exposure to different flaviviruses. *Euro Surveill* 2016;21:30203.
- 25 EUROIMMUN AG: First commercial antibody tests for Zika virus diagnostics now available. Press release, Luebeck, January 29, 2016. <https://www.euroimmun.com/index.php?eID=dumpFile&t=f&f=3013&token=6c0bb2eb7c10f484ae50ad91e69cd6424088529>.
- 26 Balm MN, Lee CK, Lee HK, Chiu L, Koay ES, Tang JW: A diagnostic polymerase chain reaction assay for Zika virus. *J Med Virol* 2012;84:1501–1505.
- 27 Macnamara F: Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. *Trans R Soc Trop Med Hyg* 1954;48:139–145.
- 28 Bearcroft W: Zika virus infection experimentally induced in a human volunteer. *Trans R Soc Trop Med Hyg* 1956;50:442–448.
- 29 Simpson D: Zika virus infection in man. *Trans R Soc Trop Med Hyg* 1964;58:335–338.
- 30 Moore Dá, Causey O, Carey D, Reddy S, Cooke A, Akinkugbe F, et al: Arthropod-borne viral infections of man in Nigeria, 1964–1970. *Ann Trop Med Parasitol* 1975;69:49–64.
- 31 Fagbami A: Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State. *J Hyg* 1979;83:213–219.
- 32 Filipe A, Martins C, Rocha H: Laboratory infection with Zika virus after vaccination against yellow fever. *Arch Gesamte Virusforsch* 1973;43:315–319.
- 33 Olson J, Ksiazek T: Zika virus, a cause of fever in Central Java, Indonesia. *Trans R Soc Trop Med Hyg* 1981;75:389–393.
- 34 Cao-Lormeau V-M, Musso D: Emerging arboviruses in the Pacific. *Lancet* 2014;384:1571–1572.
- 35 Roth A, Mercier A, Lepers C, Hoy D, Duituraga S, Benyon E, et al: Concurrent outbreaks of dengue, chikungunya and Zika virus infections – an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012–2014. *Euro Surveill* 2014;19:20929.
- 36 Cauchemez S, Besnard M, Bompard P, Dub T, Guillemette-Artur P, Eyrolle-Guignot D, et al: Association between Zika virus and microcephaly in French Polynesia, 2013–15: a retrospective study. *Lancet* 2016;387:2125–2132.
- 37 Cao-Lormeau V-M, Blake A, Mons S, Lastère S, Roche C, Vanhomwegen J, et al: Guillain-Barré syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* 2016;387:1531–1539.
- 38 Pan American Health Organization: Regional Zika epidemiological update (Americas): October 6, 2016. http://www.paho.org/hq/index.php?option=com_content&id=11599&Itemid=41691.
- 39 Pan American Health Organization: Zika cases and congenital syndrome associated with Zika virus reported by countries and territories in the Americas, 2015–2016, cumulative cases. http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=36555&lang=en.
- 40 Samarasekera U, Triunfol M: Concern over Zika virus grips the world. *Lancet* 2016;387:521–524.
- 41 World Health Organization: Situation report, Zika virus microcephaly Guillain-Barre syndrome. October 13, 2016. <http://apps.who.int/iris/bitstream/10665/250512/1/zikasi-trep13Oct16-eng.pdf?ua=1>.
- 42 World Health Organization: WHO Director-General summarizes the outcome of the Emergency Committee regarding clusters of microcephaly and Guillain-Barré syndrome. February 1, 2016. <http://www.who.int/media-centre/news/statements/2016/emergency-committee-zika-microcephaly/en/>.
- 43 Mochida GH, Walsh CA: Molecular genetics of human microcephaly. *Curr Opin Neurol* 2001;14:151–156.
- 44 Leviton A, Holmes LB, Allred EN, Vargas J: Methodologic issues in epidemiologic studies of congenital microcephaly. *Early Hum Dev* 2002;69:91–105.
- 45 Komai T, Kishimoto KI, Ozaki Y: Genetic study of microcephaly based on Japanese material. *Am J Hum Genet* 1955;7:51.
- 46 Woods CG: Human microcephaly. *Curr Opin Neurobiol* 2004;14:112–117.
- 47 Woods CG, Bond J, Enard W: Autosomal recessive primary microcephaly (MCPH): a review of clinical, molecular, and evolutionary findings. *Am J Hum Genet* 2005;76:717–728.
- 48 Woods CG, Parker A: Investigating microcephaly. *Arch Dis Child* 2013;98:707–713.
- 49 Barkovich AJ, Guerrini R, Kuzniecky RI, Jackson GD, Dobyns WB: A developmental and genetic classification for malformations of cortical development: update 2012. *Brain* 2012;135:1348–1369.
- 50 Ahlfors K, Ivarsson S-A, Bjerre I: Microcephaly and congenital cytomegalovirus infection: a combined prospective and retrospective study of a Swedish infant population. *Pediatrics* 1986;78:1058–1063.
- 51 Hagen M, Pivarsci M, Liebe J, Bernuth H, Didonato N, Hennermann JB, et al: Diagnostic approach to microcephaly in childhood: a two-center study and review of the literature. *Dev Med Child Neurol* 2014;56:732–741.
- 52 Gérardin P, Sampéris S, Ramful D, Boumahni B, Bintner M, Alessandri J-L, et al: Neurocognitive outcome of children exposed to perinatal mother-to-child Chikungunya virus infection: the CHIMERE cohort study on Reunion Island. *PLoS Negl Trop Dis* 2014;8:e2996.
- 53 O'Leary DR, Kuhn S, Kniss KL, Hinckley AF, Rasmussen SA, Pape WJ, et al: Birth outcomes following West Nile Virus infection of pregnant women in the United States: 2003–2004. *Pediatrics* 2006;117:e537–e545.
- 54 Conboy TJ, Pass RF, Stagno S, Britt WJ, Alford CA, McFarland CE, et al: Intellectual development in school-aged children with asymptomatic congenital cytomegalovirus infection. *Pediatrics* 1986;77:801–806.

- 55 Lustig Y, Mendelson E, Paran N, Melamed S, Schwartz E: Detection of Zika virus RNA in whole blood of imported Zika virus disease cases up to 2 months after symptom onset, Israel, December 2015 to April 2016. *Euro Surveill* 2016;21:30269.
- 56 Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau V-M: Potential sexual transmission of Zika virus. *Emerg Infect Dis* 2015; 21:359–361.
- 57 Hamel R, Dejarnac O, Wichit S, Ekchariyawat P, Neyret A, Luplertlop N, et al: Biology of Zika virus infection in human skin cells. *J Virol* 2015;89:8880–8896.
- 58 Tabata T, Pettit M, Puerta-Guardo H, Michlmayr D, Wang C, Fang-Hoover J, et al: Zika virus targets different primary human placental cells, suggesting two routes for vertical transmission. *Cell Host Microbe* 2016;20: 155–166.
- 59 Quicke KM, Bowen JR, Johnson EL, McDonald CE, Ma H, O'Neal JT, et al: Zika virus infects human placental macrophages. *Cell Host Microbe* 2016;20:83–90.
- 60 Olganier D, Muscolini M, Coyne CB, Diamond MS, Hiscott J: Mechanisms of Zika virus infection and neuropathogenesis. *DNA Cell Biol* 2016;35:367–372.
- 61 Nowakowski TJ, Pollen AA, Di Lullo E, Sandoval-Espinosa C, Bershteyn M, Kriegstein AR: Expression analysis highlights AXL as a candidate Zika virus entry receptor in neural stem cells. *Cell Stem Cell* 2016;18:591–596.
- 62 Perera-Lecoin M, Meertens L, Carnec X, Amara A: Flavivirus entry receptors: an update. *Viruses* 2013;6:69–88.
- 63 Li C, Xu D, Ye Q, Hong S, Jiang Y, Liu X, et al: Zika virus disrupts neural progenitor development and leads to microcephaly in mice. *Cell Stem Cell* 2016;19:672.
- 64 Tang H, Hammack C, Ogden SC, Wen Z, Qian X, Li Y, et al: Zika virus infects human cortical neural progenitors and attenuates their growth. *Cell Stem Cell* 2016;18:587–590.
- 65 Dang J, Tiwari SK, Lichinchi G, Qin Y, Patil VS, Eroshkin AM, et al: Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3. *Cell Stem Cell* 2016;19:258–265.
- 66 Kumar A, Singh HN, Pareek V, Raza K, Dandam S, Kumar P, et al: A possible mechanism of Zika virus associated microcephaly: imperative role of retinoic acid response element (RARE) consensus sequence repeats in the viral genome. *Front Hum Neurosci* 2016;10: 403.