Zika Virus-Induced Microcephaly and Its Possible Molecular Mechanism

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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 genetic 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.

Introduction
Zika virus is an arbovirus that belongs to the \textit{Flavivirus} genus and \textit{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].

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

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

Fig. 1. Summary of the Zika virus (ZIKV) infection and proposed mechanism of microcephaly. a 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.
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 transcriptions 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.

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**References**


