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REVIEW

Insights into the molecular roles of Zika virus in human reproductive complications and congenital neuropathologies

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Summary

The recent upsurge in the association of congenital neurological disorders and infection by the Zika virus (ZIKV) has resulted in increased research focus on the biology of this flavivirus. Studies in animal models indicate that ZIKV can breach the placental barrier and selectively infect and deplete neuroprogenitor cells (NPCs) of the developing fetus, resulting in changes of brain structures, reminiscent of human microcephaly. *In vitro* and *ex vivo* studies using human cells and tissues showed that human NPCs and placental cells are targeted by ZIKV. Also of concern is the impact of ZIKV on human reproductive structures, with the potential to cause infertility, as the virus appears to remain in the genital tract for extended periods of time. This review discusses the putative roles of ZIKV on human reproductive complications and congenital neuropathologies.

Key words: Zika virus; neuropathology; fetus; placenta; microcephaly; congenital.

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INTRODUCTION

Zika virus (ZIKV), a positive-strand RNA virus, is an arbovirus that belongs to the *Flaviviridae* family and emerged as a mosquito-borne virus originally identified in Uganda in 1947.[1] Early studies indicated that ZIKV was infectious and pathogenic to humans,[2–4] resulting in headache, malaise, fever, cutaneous rash, arthralgia, and conjunctivitis in about 20% of patients and with 80% of infections being asymptomatic.[5] Thus, ZIKV was not considered a major public health concern; however, the recent explosion in ZIKV infection and its association with severe congenital neurological disorders in South America (mainly Brazil) in 2015–2016 prompted the World Health Organization (WHO) to declare the situation as a Public Health Emergency of International Concern.[6] Similar outbreaks and associated consequences occurred in the Pacific (specifically the island of Yap) in 2007[5] and in French Polynesia in 2012–2014.[7] The ZIKV-associated neurological disorders mainly include microcephaly in newborns,[8] chorioretinopathy in microcephalic infants,[9,10] and severe paralytic neuropathic symptoms of Guillain–Barré syndrome (GBS) in adults.[11–13]

Primary microcephaly is a paediatric neurological disorder characterised by significant reduction in head circumference, commonly associated with varying degrees of impaired sensory, motor, and cognitive functions.[14,15] Primary congenital microcephaly is caused by decreased neurogenesis in the brain *in utero*, resulting from depletion of cortical neural progenitor cells (NPCs) (a consequence of either apoptosis or reduced proliferation or a combination of these factors). The impaired cellular processes that limit neurogenesis in microcephaly arise from deregulated expression of a number of genes mostly associated with the expression of centrosomal proteins (reviewed by Tang[16] and Barbelanne and Tsang[17]). Congenital microcephaly can also arise from infections caused by *Toxoplasma*, rubella virus, cytomegalovirus, herpes virus and the syphilis bacteria (dubbed the TORCHS factors).[18–22]

An epidemiological association between ZIKV infection and microcephaly became obvious during the recent epidemic, mainly due to the unprecedented increase in infants born with this devastating neurological disorder.[23–26] Despite warning of an epidemic in February 2015 by WHO, there were still concerns about whether ZIKV was the cause of the sudden rise of microcephaly in infants. However, increased research quickly established ZIKV as a potential teratogenic agent, resulting from an initial infection. Whilst the principal mode of ZIKV transmission is by infected *Aedes* mosquitoes (*Ae. aegypti, Ae. albopictus, Ae. africanus, and Ae. luteocephalus*) (reviewed in Hayes),[27] other reported means of transmission include sexual contact,[28] the prenatal route from mother to fetus,[29] and through blood transfusion.[30] Although viral particles have been detected in other body fluids such as saliva,[31–33] urine,[34] and breastmilk,[35] there are no reports substantiating that ZIKV is spread by these fluids. Evidence suggests that ZIKV can breach the placental barrier in early gestation and disrupt neurogenesis, potentially resulting in severe congenital malformations, as well as giving rise to intra-uterine growth restriction (IUGR). In addition, emerging studies indicated that ZIKV could remain in the human genital tract for months, long after the virus was cleared from other body fluids,[36,37] and in mice ZIKV infection could lead to severe reproductive pathologies.

This review discusses the putative molecular roles of ZIKV in human reproductive complication and congenital neuropathologies.

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EPIDEMIOLOGICAL AND CLINICAL ASSOCIATION WITH NEUROCONGENITAL DEFECTS

ZIKV infection and associated neurological defects have now been reported for a number of countries in South America[38] and among pregnant travellers to ZIKV-affected areas.[39] Evidence of congenital infection is based on detection of ZIKV RNA in tissues, amniotic fluid and fetal brain tissue[29,40–42] and anti-ZIKV IgM antibodies in the cerebrospinal fluid (CSF) of infants born with microcephaly.[43] Several studies, including population-based analyses, revealed that there is a greater risk of congenital microcephaly during the first trimester of pregnancy in ZIKV-infected mothers.[44–46] However, ZIKV infection at the 36th week (third trimester) of pregnancy can still result in fetal brain injury, characterised by subependymal cysts and lenticulostriate vasculopathy in an otherwise normal sized brain.[47] Other clinical aspects associated with ZIKV infection include brainstem and cerebellar hypoplasia, delayed myelination/demyelination, severe ventriculomegaly due to loss of brain tissue, gross calcification of the brain parenchyma, and some cases of lissencephaly (absence or reduced neurocortical gyration).[48–51] Studies also reported the prevalence of sensorineural hearing loss in babies with ZIKV-associated microcephaly.[52] In adults, as stated, ZIKV infection is associated with GBS,[11,12] an autoimmune disease characterised by demyelination of peripheral motor axons, leading to muscle weakness and paralysis, and death.[11,53] Other flaviviruses are also associated with GBS.[54]

CELLULAR MECHANISMS OF ZIKV TROPISM: RECEPTOR-MEDIATED AND RECEPTOR-INDEPENDENT

It is now established that ZIKV tropism is mediated by cell surface receptors DC-SIGN, AXL, heat shock proteins, TYRO3, and TIM-1.[55,56] AXL is a phosphatidylserine protein that belongs to the TAM receptor family of phagocytic receptors[57] and it appears to play an important role in ZIKV infection of a wide range of cells of organ systems targeted by this virus, as discussed below. However, ZIKV may also use a combination of different cell surface/adhesion molecules to gain cellular entry.

Some studies have indicated that ZIKV can infect host cells in the absence of AXL expression. Genetic ablation of AXL failed to prevent ZIKV infection of NPCs and cerebral organoid.[58] In Axl-/- knockout mice, retinal cells were not protected against ZIKV infection *in vivo* and ZIKV RNA levels in the brains of infected Axl-/- mice were comparable to that of wild-type animals.[59] These studies suggest that cell surface receptor(s) other than AXL may be needed for ZIKV attachment to human cells. One such candidate is TYRO3. Hamel *et al.* showed that overexpression of TYRO3 in HEK293 cells, which are normally resistant to this flavivirus, resulted in ZIKV infection.[55] The co-expression of AXL and TYRO3 by human NPCs[60] may explain the extreme susceptibility of these cells to ZIKV. Therefore, inhibition of AXL in the presence of TYRO3 can still lead to ZIKV infection. However, induced pluripotent stem cells (iPSCs) recalcitrant to ZIKV infection express high levels of TYRO3 (>50 TPM) RNA,[58] leading to concerns about the role of this receptor in viral attachment and cellular entry. Similar concerns may also exist for placental cells targeted by ZIKV, although more research is needed. However, it is possible that other unknown or undiscovered receptors mediate cellular entry of ZIKV.

ZIKV may also invade target cells by receptor-independent mechanisms, which may be related to exosomes and extracellular vesicles. Exosomes are cell-derived, membrane-bound, nano-sized vesicles, used in cell-cell communication. Exosome-derived cargo, which may include DNA, RNA, proteins, and lipids, are capable of influencing cellular responses of target cells. Studies showed that cells infected by viruses, including the Dengue virus which is closely related to ZIKV, released exosomes that shuttled viral RNA and proteins and other genetic elements and regulatory factors to target cells, effectively spreading infection.[61–64] Several molecular players (syndecan-1, ALIX, syntenin) involved in exosome biogenesis[65,66] are expressed by subsets of cellular components of the placenta where they may be involved in immune-modulation.[67,68] *In vitro* studies have shown that trophoblast-derived exosomes deliver pro-autophagic microRNA (miRNA) to bystander cells, thereby conferring resistance to viral infection.[69] However, it remains to be determined whether ZIKV utilises a similar mechanism for viral infection of cells of placental tissues. In the nervous system, neural stem cell-derived exosomes significantly enhanced cellular entry of adenovirus type 5 (Ad5) into Coxsackie virus and adenovirus receptor (CAR)-deficient cells.[70] The uptake of exosomes may be facilitated by either interaction with the membrane lipid raft or through mechanisms that do not involve TAM receptors.

Upon cellular entry, ZIKV triggers the upregulation of Toll-like receptor 3 (TLR3), RIG-I, and MDA5, and genes involved in innate immune response as well as several interferon-stimulated genes, including OAS2, ISG15, and MX1.[55] Most studies have shown that ZIKV limits cell growth. ZIKV infection of human neuroepithelia stem cells resulted in translocation of centrosomal and cytosolic protein pTBK1 (phosphorylated TANK binding kinase 1) to the mitochondria leading to deregulated mitosis and increased cell death.[60] Increased cell death may also be a result of ZIKV-induced upregulation of the tumor suppressor gene TP53[71] and of TLR3,[72] and suppression of Akt-mTOR,[73] which results in autophagy (Fig 1).

PUTATIVE MECHANISM(S) OF ZIKV SUPPRESSION OF CELLULAR DEFENSES

It has been speculated that once ZIKV infection is fully established, the virus becomes resistant to interferon treatment, suggesting that the virus develops a set of countermeasures against the host cell defenses.[74] STAT2, which is the transcriptional activator of interferon responses,[75] appears to be targeted by the ZIKV proteins.[76] ZIKV NS5 protein binds to STAT2, leading to proteosomal degradation of STAT2.[74] Also, ZIKV NS1, NS4A, and NS5 proteins inhibit the induction of type-I IFNs by suppressing IRF3 expression and NF-κB anti-viral signalling.[74] The loss of critical antiviral defense measures (e.g., proteosomal degradation of STAT2) can contribute to the detriment of the host cells.

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