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# Zika Says No Dice to Dicer

**Hengli Tang**[1,*]
[1]Department of Biological Science, Florida State University, Tallahassee, FL 32306, USA
*Correspondence: tang@bio.fsu.edu
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**Among the common flaviviruses that infect humans, Zika virus, and the contemporary Asian strain in particular, is distinctively associated with microcephaly. Zeng et al. (2020) report in this issue of *Cell Stem Cell* that strong binding and inhibition of human Dicer enzyme by the capsid protein is a potential mechanism for this unique pathogenic potential.**

Just 4 years ago, a virus not named coronavirus gripped the headlines worldwide and caused international concern. Zika virus (ZIKV) has not impacted as many lives as SARS-CoV-2 has, but it too appeared to target a specific vulnerable population, in this case pregnant women and their babies. The recognition of the large-scale outbreak in South America in 2015 was indeed triggered by the observation of an increase in microcephaly rates in newborns. Placental and brain infections in fetuses represent two critical facets of ZIKV pathogenesis. In the central nervous system (CNS), the effects of ZIKV infection on multiple cell types likely contribute to the collective mechanism by which ZIKV disrupts fetal brain development. For a direct link between ZIKV infection and impaired early neurogenesis, the neural progenitor cell is an attractive target (Tang et al., 2016). Although multiple mechanisms have been proposed regarding how ZIKV would inhibit the neurogenesis potential of these neural stem cells (NSCs), a key question remains as to why ZIKV appears to be the only virus among the common flaviviruses to cause the microcephaly phenotype. In addition, whether the more recent epidemic strains differ from the older strains of ZIKV by gaining mutations conferring this unique ability is unknown.

A study published in the current issue of *Cell Stem Cell* (Zeng et al., 2020) presents important clues to both puzzles. In this elegant paper, Qiming Liang and colleagues present a series of well-executed and meticulously controlled experiments to demonstrate that ZIKV capsid-mediated inhibition of Dicer function is a strong contributor to the unique ability of ZIKV in causing the microcephaly phenotype in an animal model; they further show that stronger Dicer binding and inhibition may have bestowed the epidemic ZIKV strains their increased pathogenic potential in the CNS.

Starting with a ZIKV-NSC interactome, the authors identified human Dicer protein as the top binding partner for the capsid protein of ZIKV. The binding was direct and independent of RNA. Strikingly, none of the capsid proteins from seven other flaviviruses was able to bind Dicer in the same assays. Partly based on this information, the authors identified a single amino acid change, H41R, that can abolish the capsid-Dicer interaction. In the form of individual capsid protein expressed either in mammalian cells or as recombinant protein, this mutant abrogated the ability of ZIKV capsid to inhibit Dicer-mediated processing of small hairpin RNA and pre-microRNA (pre-miRNA), the natural substrates of the Dicer enzyme. In the full-length genome background, the H41R mutation led to a virus that replicated less efficiently than the wild-type (WT) virus, but only in Dicer-competent cells. When Dicer is knocked out, the WT virus replicated at a lower rate similar to that of the H41R virus. Furthermore, trans-complementation of WT capsid was able to rescue the replication defect of the H41R virus in fetal NSCs, which are Dicer competent. These results, which strongly argue that capsid-mediated binding and inhibition of Dicer is an important determinant of ZIKV replicative potential, were next validated in an *in utero* infection mouse model of ZIKV neuropathogenesis. The capsid protein was shown to be both necessary and sufficient for causing neurogenesis defects *in vivo*.

Convinced of the critical role of the capsid-Dicer interaction in ZIKV pathology, the authors turned to a more speculative question, which is whether the strength of this interaction contributes to any pathogenic difference between an epidemic strain of the 2015–2016 outbreak and an older African strain that has not been associated with microcephaly. Remarkably, the capsid proteins from these two strains showed differential binding and Dicer inhibition *in vitro*, and *in vivo* experiments with chimeric viruses and capsid-swapping constructs support the hypothesis that this difference is a major contributor to the distinct pathogenic aptitudes of the two strains. The two amino acid differences between the capsids of the two strains were not tested individually, but they add to the collection of mutations in NS1 (nonstructural protein 1), prM (precursor membrane protein), and E (envelope protein) that have been reported to associate with the increased epidemic potential of the contemporary ZIKV strains (Liu et al., 2017; Yuan et al., 2017, Shan et al., 2020). Mechanistically, the authors demonstrate that the WT and H41R viruses differentially regulate miRNA profiles and that capsid expression perturbs the maturation of miRNAs involved in neurogenesis, consistent with the hypothesis that part of the impact of ZIKV infection on NSCs is mediated by interfering with miRNA biogenesis (Dang et al., 2019).

In addition to providing answers to significant questions regarding ZIKV neuropathology, the establishment of ZIKV capsid as an inhibitor of Dicer function *in vivo* and the availability of molecular tools such as the viable H41R virus can provide the impetus to re-kindle the debate on whether flavivirus genomes are true substrates of Dicer for producing virus-derived miRNAs or miRNA-like small RNAs (Skalsky et al., 2014). The data in the current study by Zeng et al. showing that the replicating defect of the H41R mutant as compared to WT virus

can be normalized by either Dicer1 KO or ectopic expression of WT expression is remarkable and strong evidence that Dicer modulates ZIKV replication. In a similar vein, a previous study reported production of virus-derived small interfering RNAs (viRNAs), which was reduced in Dicer KO cells (Xu et al., 2019), although the key data in that study were limited to a single probe with a northern blot as opposed to the viRNA global profiling similar to what was done in WT cells. Interestingly, Zeng et al. suggest in the epilogue of the current *Cell Stem Cell* paper that they have observed capsid-mediated suppression of viRNA production, presumably via interference of Dicer-mediated cleavage of ZIKV RNA. If Dicer can indeed digest ZIKV genomic RNA and the capsid counteracts this action, additional questions will arise such as how other flaviviruses that can't inhibit Dicer survive this cleaving action and what might be the specific substrates (secondary structures, subgenomic RNAs) for Dicer cleavage.

Of the two other ZIKV proteomic studies using a cell line SK-N-BE2 (Scaturro et al., 2018) or HEK293T (Shah et al., 2018), Scaturro et al. list Dicer1 as a capsid-binding partner in a large network of capsid-binding proteins, but Dicer was not among the top hits and not characterized further in that study. The choice of using human induced pluripotent stem cell (iPSC)-derived NSCs for the interactor screen in the current study may have contributed to Zeng et al.'s success in uncovering Dicer as a consequential ZIKV interactor with far-reaching implications for ZIKV pathogenesis and epidemic potential.

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