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Avaliação da infecção do Zika vírus em astrócitos

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RESUMO

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O Zika vírus (ZIKV) é um flavivírus capaz de causar infecção em humanos após a contaminação através da picada por mosquitos fêmeas do gênero Aedes, por transmissão sexual ou vertical (da mãe para o feto). Em 2015, a linhagem brasileira deste vírus - $ZIKV^{BR}$ - produziu uma epidemia no Brasil, gerando diversos casos de infecção congênita por transmissão vertical, caracterizada por malformações cerebrais. Estudos *in vitro* em células progenitoras neurais, neurônios e organoides derivados de células-tronco pluripotentes induzidas (iPSC) revelaram o neurotropismo do $ZIKV^{BR}$ e ajudaram a clarificar sua relação com o Sistema Nervoso Central (SNC), mostrando que essas células são preferencialmente afetadas pela infecção. Neste projeto analisamos os efeitos da infecção do $ZIKV^{BR}$ em astrócitos, células da glia responsáveis pela homeostase e defesa do SNC. Para tanto, produzimos astrócitos a partir de duas linhagens de iPSC humanas para identificar aspectos moleculares e celulares decorrentes da infecção com o vírus. Os resultados mostraram que os astrócitos são susceptíveis e permissíveis à infecção por $ZIKV^{BR}$ e respondem à presença do vírus sofrendo apoptose e produzindo citocinas. Ademais, os astrócitos apresentaram aumento da expressão gênica de receptores TAM, receptores envolvidos com a regulação de resposta imune e também candidatos à entrada viral, e de transportadores de glutamato. Adicionalmente, a captação de glutamato foi prejudicada, bem como a sinaptogênese, quando cultivamos neurônios com astrócitos infectados ou com o sobrenadante de cultura de astrócitos livre de vírus, indicando que os produtos metabólicos gerados por astrócitos infectados afetam o fenótipo neuronal. Por fim, mostramos que mesmo infectadas, NPC se diferenciam em astrócitos. Os resultados gerados neste trabalho auxiliam a compreender os efeitos biológicos causados pelo $ZIKV^{BR}$ em astrócitos derivados de iPSC humanas, o que pode contribuir para o melhor entendimento da patogênese do ZIKV no SNC.

Palavras-chave: Zika vírus. Astrócitos. Células-tronco pluripotentes induzidas. Microcefalia. Sistema nervoso central.

ABSTRACT

OHKI, C.M.Y. **Evaluation of Zika virus infection in astrocytes.** 2019. 84 p. Dissertation (Master thesis in Microbiology) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2019.

Zika Virus (ZIKV) is a flavivirus able to promote human infection through bites of *Aedes* female mosquitoes, sexual or vertical transmission (from expectant mothers to fetuses). In 2015, the Brazilian strain of this virus - $ZIKV^{BR}$ - caused a significant outbreak in Brazil, which led to numerous cases of congenital infection after vertical transmission. *In vitro* studies with neural progenitor cells (NPC), neurons and brain organoids derived from human induced pluripotent stem cells (iPSC), have reported $ZIKV^{BR}$ neurotropism and helped clarify its correlation with the Central Nervous System (CNS) by showing that these cells are significantly affected by infection. In this project, we analyzed the effects of $ZIKV^{BR}$ infection in astrocytes, glial cells responsible for CNS homeostasis and defense. In order to do so, we have generated astrocytes from two human iPSC lineages to verify molecular and cellular changes regarding virus infection. Our results have shown that astrocytes are susceptible and permissive to $ZIKV^{BR}$ and respond to viral presence by undergoing apoptosis and releasing cytokines. In addition, after infection, astrocytes had increased expression of TAM receptors, which are involved with suppression of immune response and also candidates for viral entry, and glutamate transporters. Additionally, we have also verified impaired glutamate uptake by these astrocytes, as well as synaptogenesis when mature neurons were cultivated in the presence of infected astrocytes or with their virus-free supernatant, indicating that metabolic products of infected astrocytes affected neuronal phenotype. At last, we have demonstrated that generation of astrocytes from infected NPC is possible. This work pursued to understand the biological effects caused by $ZIKV^{BR}$ in human iPSC-derived astrocytes, which could provide a better comprehension about ZIKV pathogenesis in CNS.

Keywords: Zika virus. Astrocytes. Induced pluripotent stem cells. Microcephaly. Central nervous system.

INTRODUÇÃO

O ZIKV é um vírus da família *Flaviviridae* que recebeu enorme atenção mundial a partir de 2015, quando então surgiram vários casos de recém-nascidos com microcefalia na região Nordeste do Brasil (BRASIL. MINISTÉRIO DA SAÚDE. SECRETARIA DE VIGILÂNCIA EM SAÚDE., 2015). Desde seu primeiro isolamento em macacos Rhesus (DICK; KITCHEN; HADDOW, 1952), as infecções em humanos só foram observadas em nível mundial no início do século XXI, com sintomatologia branda como febre, erupções maculopapulares e dores de cabeça e nas articulações (como revisto por PETERSEN et al., 2016). As consequências da infecção pelo ZIKV que estavam sendo observadas nos recém-nascidos, na forma de uma síndrome denominada Síndrome Congênita do Zika (SCZ), com especial comprometimento do SNC (MIRANDA-FILHO et al., 2016), nunca haviam sido descritas anteriormente.

A capacidade de infecção do ZIKV no SNC já havia sido demonstrada experimentalmente, com a injeção direta do vírus em cérebros de camundongos neonatos (BELL; FIELD; NARANG, 1971). Apesar dos vírus terem sido inoculados diretamente no cérebro dos camundongos, as células do SNC se mostraram susceptíveis e permissíveis ao ZIKV, revelando inclusive um efeito citopático (ECP) específico, com o aumento da relação núcleo/citoplasma, com núcleo de aspecto globoso e vazio à observação histológica. Após esse artigo de 1971, em 2016 foi observado semelhante ECP à histologia do cérebro de camundongos neonatos, porém após a transmissão vertical em camundongos prenhes infectados pela linhagem brasileira do ZIKV, comprovando não apenas o tropismo do vírus pelo SNC, mas também sua capacidade de atravessar a placenta (CUGOLA et al., 2016). Apesar do neurotropismo do ZIKV estar postulado e evidenciado, ainda faltam informações acerca dos mecanismos envolvidos na fisiopatologia da infecção no SNC, os quais estão ainda estão sendo avaliados pela comunidade científica nos diferentes tipos celulares.

Os astrócitos são as células mais abundantes e mais heterogêneas da glia no SNC, promovendo suporte estrutural, metabólico e trófico aos neurônios. Além disso, os astrócitos parecem estar envolvidos em mecanismos neuroprotetores, de neurogênese e de sinaptogênese durante o processo de maturação neuronal (BECERRA-CALIXTO; CARDONA-GÓMEZ, 2017; JOHNSON et al., 2007). Existem evidências de que astrócitos seriam células susceptíveis e permissíveis ao ZIKV (LANKO et al., 2017a; LIMONTA et al.,

2018; SIMONIN et al., 2016; STEFANIK et al., 2018). Além disso, astrócitos também têm alta expressão do receptor AXL (MEERTENS et al., 2017; NOWAKOWSKI et al., 2016), um membro da família de receptores TAM (juntamente com TYRO3 e MERTK), que já foram implicados na entrada de ZIKV e Dengue na célula hospedeira (HAMEL et al., 2015; MEERTENS et al., 2012).

Considerando o exposto, se faz necessário entender e explorar os mecanismos envolvidos na fisiopatologia da doença congênita causada pelo ZIKV, e a tecnologia utilizada na modelagem de doenças através de produção de tipos celulares específicos do SNC *in vitro* a partir das iPSC têm se mostrado valiosa, tanto para modelar a infecção no SNC causada pelo ZIKV (CUGOLA et al., 2016; TANG et al., 2016), quanto para a produção de tipos celulares de interesse de outras doenças infecciosas, como na infecção pelo vírus da Herpes (D'AIUTO et al., 2019) e da hepatite E (TODT et al., 2018). De maneira mais ampla, o uso de iPSC permitiu a geração de modelos celulares para o estudo de doenças em células-alvo, preservando ainda o *background* genético do paciente, denominando-se esse tipo de abordagem de modelagem de doenças. Esta também fornece a possibilidade de testar medicamentos específicos *in vitro* (BELTRÃO-BRAGA et al., 2011, 2013). Importante ressaltar que estratégias como essa tem sido largamente utilizadas para estudar doenças neurodegenerativas e do neurodesenvolvimento, justamente pela dificuldade de ter acesso a células do SNC vivas e em funcionamento (EBERT et al., 2009; IMAIZUMI et al., 2012; KONDO et al., 2013; RUSSO et al., 2018; TAKAHASHI; YAMANAKA, 2006; YU et al., 2014).

Neste contexto, como mencionado anteriormente, nosso grupo demonstrou que células progenitoras neurais (NPC), neurônios e organóides cerebrais derivados de iPSC são susceptíveis e permissíveis ao ZIKV, cuja infecção gera alterações morfológicas e morte celular significativa (CUGOLA et al., 2016). Entretanto, este trabalho não incluiu a abordagem de astrócitos e possíveis fenótipos que essas células pudessem apresentar frente à infecção pelo ZIKV. Portanto, considerando a importância dos astrócitos para a homeostase e regulação imune no SNC e a dimensão dos prejuízos causados pela infecção pelo ZIKV em termos de saúde pública, visamos entender os efeitos biológicos que este vírus pode gerar em duas linhagens de astrócitos derivados de iPSC humanas.

CONCLUSÕES

- Não são observadas mudanças morfológicas, em termos de ECP, entre astrócitos infectados e astrócitos MOCK, mas nota-se que há aumento de morte celular nas primeiras 48 horas de infecção, o que tende a decrescer até o ponto de 96 h.p.i.;
- Astrócitos infectados aparentam expressar mais citocinas e quimiocinas majoritariamente pró-inflamatórias quando comparados aos astrócitos controle. No entanto, há diferenças de resposta entre os pacientes;
- Astrócitos infectados tendem a expressar mais receptores AXL e o transportador de glutamato EAAT2, porém, mais uma vez, há diferenças entre linhagens quanto a expressão gênica de receptores TAM e transportadores de glutamato;
- Astrócitos infectados demonstram prejuízo em sua função de captação de glutamato, ao contrário da condição MOCK. Quanto à sua participação no estabelecimento do fenótipo neuronal, neurônios parecem apresentar um déficit na sinaptogênese tanto quando cultivados na presença de astrócitos infectados ou apenas de seus produtos metabólicos;
- O processo de astrogliogênese infectada, por sua vez, parece ser menos afetado em L2, uma vez que há diferenciação para esse tipo celular, o que não é observado em L1. Entretanto, o $ZIKV^{BR}$ afeta o processo de astrogliogênese, tanto em causando diminuição do diâmetro de neuroesferas quanto na migração dessas células, o que poderiam colaborar para o desenvolvimento da microcefalia.

REFERÊNCIAS*

ABBINK, P. et al. Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. **Science**, v. 353, n. 6304, p. 1129–1132, 9 set. 2016.

ALIMONTI, J. B. et al. Zika virus crosses an in vitro human blood brain barrier model. **Fluids and Barriers of the CNS**, v. 15, n. 1, p. 15, 15 dez. 2018.

ANDERSON, C. M.; SWANSON, R. A. Astrocyte glutamate transport: Review of properties, regulation, and physiological functions. **Glia**, v. 32, n. 1, p. 1–14, out. 2000.

ARGAW, A. T. et al. VEGF-mediated disruption of endothelial CLN-5 promotes blood-brain barrier breakdown. **Proceedings of the National Academy of Sciences**, v. 106, n. 6, p. 1977–1982, 2009.

BECERRA-CALIXTO, A.; CARDONA-GÓMEZ, G. P. The Role of Astrocytes in Neuroprotection after Brain Stroke: Potential in Cell Therapy. **Frontiers in Molecular Neuroscience**, v. 10, n. April, p. 1–12, 2017.

BELL, T. M.; FIELD, E. J.; NARANG, H. K. Zika virus infection of the central nervous system of mice. **Archiv fur die gesamte Virusforschung**, v. 35, n. 2, p. 183–193, 1971.

BELTRÃO-BRAGA, P. C. B. et al. Feeder-Free Derivation of Induced Pluripotent Stem Cells From Human Immature Dental Pulp Stem Cells. **Cell Transplantation**, v. 20, n. 11, p. 1707–1719, 2011.

BELTRÃO-BRAGA, P. C. B. et al. In-a-dish: induced pluripotent stem cells as a novel model for human diseases. **Cytometry. Part A : the journal of the International Society for Analytical Cytology**, v. 83, n. 1, p. 11–17, jan. 2013.

BESNARD, M. et al. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. **Eurosurveillance**, v. 19, n. 13, p. 20751, 3 abr. 2014.

BHOWMICK, S. et al. Induction of IP-10 (CXCL10) in astrocytes following Japanese encephalitis. **Neuroscience Letters**, v. 414, n. 1, p. 45–50, 2007.

BRAHMACHARI, S. Induction of Glial Fibrillary Acidic Protein Expression in Astrocytes by Nitric Oxide. **Journal of Neuroscience**, v. 26, n. 18, p. 4930–4939, 2006.

BRASIL. MINISTÉRIO DA SAÚDE. SECRETARIA DE VIGILÂNCIA EM SAÚDE. **Monitoramento dos casos de microcefalia no Brasil até a Semana Epidemiológica 50**. Disponível em: <http://portalarquivos2.saude.gov.br/images/pdf/2015/dezembro/28/2015-boletim-microcefalia-se50-vol46-n46.pdf>. Acesso em: 24 fev. 2019.

BRASIL. MINISTÉRIO DA SAÚDE. SECRETARIA DE VIGILÂNCIA EM SAÚDE. **Situação epidemiológica da infecção pelo vírus Zika no Brasil, de 2015 a 2017**. Disponível em: <http://portalarquivos2.saude.gov.br/images/pdf/2018/novembro/12/2018-034.pdf>. Acesso em: 24 fev. 2019a.

BRASIL. MINISTÉRIO DA SAÚDE. SECRETARIA DE VIGILÂNCIA EM SAÚDE. **Monitoramento integrado de alterações no crescimento e desenvolvimento**

relacionadas à infecção pelo vírus Zika e outras etiologias infecciosas, até a Semana Epidemiológica 45 de 2018. Disponível em: <http://portalarquivos2.saude.gov.br/images/pdf/2018/dezembro/14/2018-061.pdf>. Acesso em: 25 fev. 2018b.

BULLARD-FEIBELMAN, K. M. et al. The FDA-approved drug sofosbuvir inhibits Zika virus infection. **Antiviral Research**, v. 137, p. 134–140, jan. 2017.

CAIRES-JÚNIOR, L. C. et al. Discordant congenital Zika syndrome twins show differential in vitro viral susceptibility of neural progenitor cells. **Nature Communications**, v. 9, n. 1, p. 475, 2018.

CAMPBELL, I. L. et al. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. **Proceedings of the National Academy of Sciences**, v. 90, n. 21, p. 10061–10065, 1993.

CAO-LORMEAU, V.-M. et al. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. **The Lancet**, v. 387, n. 10027, p. 1531–1539, 9 abr. 2016.

CARPENTIER, P. A.; GETTS, M. T.; MILLER, S. D. Pro-inflammatory functions of astrocytes correlate with viral clearance and strain-dependent protection from TMEV-induced demyelinating disease. **Virology**, v. 375, n. 1, p. 24–36, 2008.

CHAI, Q. et al. Enhancement of Blood-Brain Barrier Permeability and Reduction of Tight Junction Protein Expression Are Modulated by Chemokines/Cytokines Induced by Rabies Virus Infection. **Journal of Virology**, v. 88, n. 9, p. 4698–4710, 2014.

CHANG, C. Y. et al. Disruption of in vitro endothelial barrier integrity by Japanese encephalitis virus-infected astrocytes. **Glia**, v. 63, n. 11, p. 1915–1932, 2015.

CHEN, C. et al. Upregulation of RANTES Gene Expression in Neuroglia by Japanese Encephalitis Virus Infection Upregulation of RANTES Gene Expression in Neuroglia by Japanese Encephalitis Virus Infection. **Journal of Virology**, v. 78, n. 22, p. 12107–12119, 2004.

CHEN, J. et al. AXL promotes Zika virus infection in astrocytes by antagonizing type I interferon signalling. **Nature Microbiology**, 2018.

CHOI, D. W.; ROTHMAN, S. M. The Role of Glutamate Neurotoxicity in Hypoxic-Ischemic Neuronal Death. **Annual Review of Neuroscience**, v. 13, n. 1, p. 171–182, mar. 1990.

CUGOLA, F. R. et al. The Brazilian Zika virus strain causes birth defects in experimental models. **Nature**, v. 534, n. 7606, p. 1–15, 2016.

D'AIUTO, L. et al. Modeling HSV-1 infections in human CNS neuronal cells using two-dimensional and three- dimensional cultures derived from induced pluripotent stem cells. **Journal of Virology**, 20 fev. 2019.

D'ORTENZIO, E. et al. Evidence of Sexual Transmission of Zika Virus. **New England Journal of Medicine**, v. 374, n. 22, p. 2193–2195, 2016.

DANG, J. et al. Zika Virus Depletes Neural Progenitors in Human Cerebral Organoids through Activation of the Innate Immune Receptor TLR3. **Cell Stem Cell**, v. 19, n. 2, p.

258–265, ago. 2016.

DE OLIVEIRA, W. K. et al. Infection-related microcephaly after the 2015 and 2016 Zika virus outbreaks in Brazil: a surveillance-based analysis. **The Lancet**, v. 390, n. 10097, p. 861–870, 2017.

DEVHARE, P. et al. Zika virus infection dysregulates human neural stem cell growth and inhibits differentiation into neuroprogenitor cells. **Cell Death and Disease**, v. 8, n. 10, p. 1–8, 2017.

DICK, G. W. A.; KITCHEN, S. F.; HADDOW, A. J. Zika Virus. I. Isolations and Serological Specificity. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, v. 46, n. 5, p. 509–520, 1952.

DOWD, K. A. et al. Rapid development of a DNA vaccine for Zika virus. **Science**, v. 354, n. 6309, p. 237–240, 14 out. 2016.

EBERT, A. D. et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. **Nature**, v. 457, n. 7227, p. 277–80, 15 jan. 2009.

FAISSNER, A. et al. Contributions of astrocytes to synapse formation and maturation - Potential functions of the perisynaptic extracellular matrix. **Brain Research Reviews**, v. 63, n. 1–2, p. 26–38, 2010.

FARIA, N. R. et al. Zika virus in the Americas: Early epidemiological and genetic findings. **Science**, v. 352, n. 6283, p. 345–349, 15 abr. 2016.

FARINA, C.; ALOISI, F.; MEINL, E. Astrocytes are active players in cerebral innate immunity. **Trends in Immunology**, v. 28, n. 3, p. 138–145, mar. 2007.

FERREIRA, A. C. et al. Sofosbuvir protects Zika virus-infected mice from mortality, preventing short- and long-term sequelae. **Scientific Reports**, v. 7, n. 1, p. 1–9, 2017.

FISCHER, C. et al. External Quality Assessment for Zika Virus Molecular Diagnostic Testing, Brazil. **Emerging Infectious Diseases**, v. 24, n. 5, p. 888–892, maio 2018.

GABRIEL, E. et al. Recent Zika Virus Isolates Induce Premature Differentiation of Neural Progenitors in Human Brain Organoids. **Cell Stem Cell**, p. 1–10, 2017.

GARCEZ, P. P. et al. Zika virus disrupts molecular fingerprinting of human neurospheres. **Scientific Reports**, p. 1–10, 2017.

GARCIA, R.; MARCHETTE, N. J.; RUDNICK, A. Isolation of Zika Virus from Aedes Aegypti Mosquitoes in Malaysia *. **The American Journal of Tropical Medicine and Hygiene**, v. 18, n. 3, p. 411–415, 1 maio 1969.

GURDON, J. B. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. **Journal of embryology and experimental morphology**, v. 10, n. December, p. 622–40, dez. 1962.

HAMEL, R. et al. Biology of Zika Virus Infection in Human Skin Cells. **Journal of Virology**, v. 89, n. 17, p. 8880–8896, 1 set. 2015.

HAMEL, R. et al. African and Asian Zika virus strains differentially induce early antiviral responses in primary human astrocytes. **Infection, Genetics and Evolution**, v. 49, p.

134–137, 2017.

HASTINGS, A. K. et al. TAM Receptors Are Not Required for Zika Virus Infection in Mice. **Cell Reports**, v. 19, n. 3, p. 558–568, 2017.

HUANG, Y. et al. Zika virus propagation and release in human fetal astrocytes can be suppressed by neutral sphingomyelinase-2 inhibitor GW4869. **Cell Discovery**, v. 4, n. 1, p. 19, 24 dez. 2018.

HUGHES, E. G. et al. Loss of glial fibrillary acidic protein results in decreased glutamate transport and inhibition of PKA-induced EAAT2 cell surface trafficking. **Molecular Brain Research**, v. 124, n. 2, p. 114–123, 2004.

HUGHES, R. A. C.; REES, J. H. Clinical and Epidemiologic Features of Guillain-Barré Syndrome. **Oxford Journals**, v. 176, n. May, p. S92–S98, 2017.

IMAIZUMI, Y. et al. Mitochondrial dysfunction associated with increased oxidative stress and alpha-synuclein accumulation in PARK2 iPSC-derived neurons and postmortem brain tissue. **Molecular Brain**, v. 5, n. 1, p. 35, 2012.

JOHNSON, M. A. et al. Functional Neural Development from Human Embryonic Stem Cells: Accelerated Synaptic Activity via Astrocyte Coculture. **Journal of Neuroscience**, v. 27, n. 12, p. 3069–3077, 21 mar. 2007.

KAM, K.; NICOLL, R. Excitatory Synaptic Transmission Persists Independently of the Glutamate Glutamine Cycle. **Journal of Neuroscience**, v. 27, n. 34, p. 9192–9200, 2007.

KAM, Y. W. et al. Specific biomarkers associated with neurological complications and congenital central nervous system abnormalities from Zika virus-infected patients in Brazil. **Journal of Infectious Diseases**, v. 216, n. 2, p. 172–181, 2017.

KAMIYAMA, N. et al. Ribavirin inhibits Zika virus (ZIKV) replication in vitro and suppresses viremia in ZIKV-infected STAT1-deficient mice. **Antiviral Research**, v. 146, p. 1–11, 2017.

KANG, S.; BROWN, H. M.; HWANG, S. Direct Antiviral Mechanisms of Interferon-Gamma. **Immune Network**, v. 18, n. 5, 2018.

KONDO, T. et al. Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Aβ and differential drug responsiveness. **Cell Stem Cell**, v. 12, n. 4, p. 487–496, 2013.

KOZAK, R. A. et al. MicroRNA and mRNA dysregulation in astrocytes infected with Zika virus. **Viruses**, v. 9, n. 10, p. 1–18, 2017.

KUMAR, A. et al. Human sertoli cells support high levels of zika virus replication and persistence. **Scientific Reports**, v. 8, n. 1, p. 1–11, 2018.

LANCASTER, M. A. et al. Cerebral organoids model human brain development and microcephaly. **Nature**, v. 501, n. 7467, p. 373–379, 2013.

LANCASTER, M. A.; KNOBLICH, J. A. Generation of cerebral organoids from human pluripotent stem cells. **Nature Protocols**, v. 9, n. 10, p. 2329–2340, 2014.

LANCIOTTI, R. S. et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. **Emerging Infectious Diseases**, v. 14, n. 8, p.

1232–1239, 2008.

LANKO, K. et al. Replication of the Zika virus in different iPSC-derived neuronal cells and implications to assess efficacy of antivirals. **Antiviral Research**, v. 145, p. 82–86, 2017a.

LANKO, K. et al. Replication of the Zika virus in different iPSC-derived neuronal cells and implications to assess efficacy of antivirals. **Antiviral Research**, v. 145, p. 82–86, 2017b.

LAROCCA, R. A. et al. Vaccine protection against Zika virus from Brazil. **Nature**, v. 536, n. 7617, p. 474–478, 25 ago. 2016.

LEMKE, G. Biology of the TAM receptors. **Cold Spring Harbor Perspectives in Biology**, v. 5, n. 11, p. 1–17, 2013.

LENTING, K. et al. Glioma: experimental models and reality. **Acta Neuropathologica**, v. 133, n. 2, p. 263–282, 2017.

LI, C. et al. Chloroquine, a FDA-approved Drug, Prevents Zika Virus Infection and its Associated Congenital Microcephaly in Mice. **EBioMedicine**, v. 24, p. 189–194, out. 2017.

LI, F. et al. Viral Infection of the Central Nervous System and Neuroinflammation Precede Blood-Brain Barrier Disruption during Japanese Encephalitis Virus Infection. **Journal of Virology**, v. 89, n. 10, p. 5602–5614, 2015.

LIMONTA, D. et al. Human Fetal Astrocytes Infected with Zika Virus Exhibit Delayed Apoptosis and Resistance to Interferon: Implications for Persistence. **Viruses**, v. 10, n. 11, p. 1–19, 2018.

LOSSIA, O. V. et al. Zika virus induces astrocyte differentiation in neural stem cells. **Journal of NeuroVirology**, p. 1–10, 2017.

LUM, F. M. et al. Zika virus infects human fetal brain microglia and induces inflammation. **Clinical Infectious Diseases**, v. 64, n. 7, p. 914–920, 2017.

MANSUY, J. M. et al. Zika virus: High infectious viral load in semen, a new sexually transmitted pathogen? **The Lancet Infectious Diseases**, v. 16, n. 4, p. 405, 2016.

MARCHETTO, M. C. N. et al. Transcriptional signature and memory retention of human-induced pluripotent stem cells. **PLoS ONE**, v. 4, n. 9, 2009.

MARCHETTO, M. C. N. et al. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. **Cell**, v. 143, n. 4, p. 527–539, 12 nov. 2010.

MARCHETTO, M. C. N.; WINNER, B.; GAGE, F. H. Pluripotent stem cells in neurodegenerative and neurodevelopmental diseases. **Human Molecular Genetics**, v. 19, n. R1, p. 71–76, 2010.

MARTINES, R. B. et al. Notes from the Field : Evidence of Zika Virus Infection in Brain and Placental Tissues from Two Congenitally Infected Newborns and Two Fetal Losses — Brazil, 2015. **MMWR. Morbidity and Mortality Weekly Report**, v. 65, n. 06, p. 159–160, 19 fev. 2016.

MCGRATH, E. L. et al. Differential Responses of Human Fetal Brain Neural Stem Cells to Zika Virus Infection. **Stem Cell Reports**, v. 8, p. 715–727, 2017.

MEERTENS, L. et al. The TIM and TAM families of phosphatidylserine receptors mediate dengue virus entry. **Cell Host and Microbe**, v. 12, n. 4, p. 544–557, 2012.

MEERTENS, L. et al. Axl Mediates ZIKA Virus Entry in Human Glial Cells and Modulates Innate Immune Responses. **Cell Reports**, v. 18, n. 2, p. 324–333, 2017.

MENESES, J. D. A. et al. Lessons Learned at the Epicenter of Brazil's Congenital Zika Epidemic: Evidence from 87 Confirmed Cases. **Clinical Infectious Diseases**, v. 64, n. 10, p. 1302–1308, 2017.

MESCI, P. et al. Modeling neuro-immune interactions during Zika virus infection. **Human Molecular Genetics**, v. 27, n. 1, p. 41–52, 2018a.

MESCI, P. et al. Blocking Zika virus vertical transmission. **Scientific Reports**, v. 8, n. 1, p. 1218, 19 dez. 2018b.

MIRANDA-FILHO, D. DE B. et al. Initial Description of the Presumed Congenital Zika Syndrome. **American Journal of Public Health**, v. 106, n. 4, p. 598–600, 9 mar. 2016.

MISHRA, M. K.; KUMAWAT, K. L.; BASU, A. Japanese encephalitis virus differentially modulates the induction of multiple pro-inflammatory mediators in human astrocytoma and astroglioma cell-lines. **Cell Biology International**, v. 32, n. 12, p. 1506–1513, 2008.

MLAKAR, J. et al. Zika Virus Associated with Microcephaly. **New England Journal of Medicine**, v. 374, n. 10, p. 951–958, 10 mar. 2016.

MOTTA, I. J. F. et al. Evidence for Transmission of Zika Virus by Platelet Transfusion. **New England Journal of Medicine**, v. 375, n. 11, p. 1101–1103, 15 set. 2016.

MUFFAT, J. et al. Human induced pluripotent stem cell-derived glial cells and neural progenitors display divergent responses to Zika and dengue infections. **Proceedings of the National Academy of Sciences**, v. 115, n. 27, p. 7117–7122, 3 jul. 2018.

NGUYEN, S. M. et al. Highly efficient maternal-fetal Zika virus transmission in pregnant rhesus macaques. **PLoS Pathogens**, v. 13, n. 5, p. 1–22, 2017.

NOWAKOWSKI, T. J. et al. Expression Analysis Highlights AXL as a Candidate Zika Virus Entry Receptor in Neural Stem Cells. **Cell Stem Cell**, v. 18, n. 5, p. 591–596, 2016.

PALUS, M. et al. Infection and injury of human astrocytes by tick-borne encephalitis virus. **Journal of General Virology**, v. 10, n. 2014, p. 2411–2426, 2018.

PAPA, M. P. et al. Zika Virus infects, activates, and crosses brain microvascular endothelial cells, without barrier disruption. **Frontiers in Microbiology**, v. 8, n. DEC, 2017.

PETERSEN, E. et al. Rapid Spread of Zika Virus in The Americas - Implications for Public Health Preparedness for Mass Gatherings at the 2016 Brazil Olympic Games. **International Journal of Infectious Diseases**, v. 44, n. May 2015, p. 11–15, 2016.

RAMESH, G.; MACLEAN, A. G.; PHILIPP, M. T. Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. **Mediators of Inflammation**, v. 2013, 2013.

RANSOHOFF, R. M.; BROWN, M. A. Innate immunity in the central nervous system. **The**

Journal of Clinical Investigation, v. 122, n. 4, p. 1164–1171, 2012.

RAPONI, E. et al. S100B expression defines a state in which GFAP-expressing cells lose their neural stem cell potential and acquire a more mature developmental stage. **Glia**, v. 55, n. 2, p. 165–177, 15 jan. 2007.

RETALLACK, H. et al. Zika virus cell tropism in the developing human brain and inhibition by azithromycin. **Proceedings of the National Academy of Sciences**, v. 113, n. 50, p. 14408–14413, 2016.

RIBEIRO, M. R. et al. Zika-virus-infected human full-term placental explants display pro-inflammatory responses and undergo apoptosis. **Archives of Virology**, v. 163, n. 10, p. 2687–2699, 2018.

RICHARD, A. S. et al. AXL-dependent infection of human fetal endothelial cells distinguishes Zika virus from other pathogenic flaviviruses. **Proceedings of the National Academy of Sciences**, v. 114, n. 8, p. 2024–2029, 2017.

RIDET, J. L. et al. Reactive astrocytes: Cellular and molecular cues to biological function. **Trends in Neurosciences**, v. 20, n. 12, p. 570–577, 1997.

ROSA-FERNANDES, L. et al. Zika Virus Impairs Neurogenesis and Synaptogenesis Pathways in Human Neural Stem Cells and Neurons. **Frontiers in Cellular Neuroscience**, v. 13, n. March, p. 1–16, 2019.

RUSSO, F. B. et al. Modeling the Interplay Between Neurons and Astrocytes in Autism Using Human Induced Pluripotent Stem Cells. **Biological Psychiatry**, v. 83, n. 7, p. 569–578, 2018.

SAAD, T. et al. Neurological manifestations of congenital Zika virus infection. **Child's Nervous System**, v. 34, n. 1, p. 73–78, 10 jan. 2018.

SACRAMENTO, C. Q. et al. The clinically approved antiviral drug sofosbuvir inhibits Zika virus replication. **Scientific Reports**, v. 7, p. 40920, 18 jan. 2017.

SHAN, C. et al. A single-dose live-attenuated vaccine prevents Zika virus pregnancy transmission and testis damage. **Nature Communications**, v. 8, n. 1, 2017.

SHIRYAEV, S. A. et al. Repurposing of the anti-malaria drug chloroquine for Zika Virus treatment and prophylaxis. **Scientific Reports**, v. 7, n. 1, p. 15771, 2017.

SHUAIB, W. et al. Re-Emergence of Zika Virus: A Review on Pathogenesis, Clinical Manifestations, Diagnosis, Treatment, and Prevention. **American Journal of Medicine**, v. 129, n. 8, p. 879.e7-879.e12, 2016.

SIMONIN, Y. et al. Zika Virus Strains Potentially Display Different Infectious Profiles in Human Neural Cells. **EBioMedicine**, v. 12, p. 161–169, 2016.

SIMPSON, D. I. H. Zika virus infection in man. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, v. 58, n. 4, p. 339–348, 1964.

SOFRONIEW, M. V.; VINTERS, H. V. Astrocytes: Biology and pathology. **Acta Neuropathologica**, v. 119, n. 1, p. 7–35, 2010.

SOUZA, B. S. F. et al. Zika virus infection induces mitosis abnormalities and apoptotic cell

death of human neural progenitor cells. **Scientific Reports**, v. 6, n. 1, p. 39775, 2016.

STEFANIK, M. et al. Characterisation of Zika virus infection in primary human astrocytes. **BMC Neuroscience**, v. 19, n. 1, p. 5, 20 dez. 2018.

TAKAHASHI, K.; YAMANAKA, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. **Cell**, v. 126, n. 4, p. 663–676, 25 ago. 2006.

TANG, H. et al. Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth. **Cell Stem Cell**, v. 18, n. 5, p. 587–590, maio 2016a.

TANG, W. W. et al. A Mouse Model of Zika Virus Sexual Transmission and Vaginal Viral Replication. **Cell Reports**, v. 17, n. 12, p. 3091–3098, 20 dez. 2016b.

THEEL, E. S.; HATA, D. J. Diagnostic Testing for Zika Virus: a Postoutbreak Update. **Journal of Clinical Microbiology**, v. 56, n. 4, p. 1–12, 31 jan. 2018.

TODT, D. et al. The natural compound silvestrol inhibits hepatitis E virus (HEV) replication in vitro and in vivo. **Antiviral Research**, v. 157, p. 151–158, set. 2018.

TRICARICO, P. M. et al. Zika virus induces inflammasome activation in the glial cell line U87-MG. **Biochemical and Biophysical Research Communications**, v. 492, n. 4, p. 597–602, out. 2017.

UCHIL, P. D.; KUMAR, A. V. A.; SATCHIDANANDAM, V. Nuclear Localization of Flavivirus RNA Synthesis in Infected Cells. **Journal of Virology**, v. 80, n. 11, p. 5451–5464, 2006.

VALADÃO, A. L. C.; AGUIAR, R. S.; DE ARRUDA, L. B. Interplay between Inflammation and Cellular Stress Triggered by Flaviviridae Viruses. **Frontiers in Microbiology**, v. 7, n. August, p. 1–19, 25 ago. 2016.

VAN DEN POL, A. N. et al. Zika virus targeting in the developing brain. **The Journal of Neuroscience**, p. 3124–16, 2017.

VASUDEVAN, J. et al. Zika Virus. **Reviews in Medical Microbiology**, v. 29, n. 2, p. 43–50, 2018.

VENTURA, C. V. et al. Zika virus in Brazil and macular atrophy in a child with microcephaly. **The Lancet**, v. 387, n. 10015, p. 228, 2016.

VERKHRATSKY, A.; NEDERGAARD, M. Physiology of Astroglia. **Physiological Reviews**, v. 98, n. 1, p. 239–389, 2018.

VERMA, S.; KUMAR, M.; NERURKAR, V. R. Cyclooxygenase-2 inhibitor blocks the production of West Nile virus-induced neuroinflammatory markers in astrocytes. **Journal of General Virology**, v. 92, n. 3, p. 507–515, 2011.

WANG, K. et al. IP-10 Promotes Blood–Brain Barrier Damage by Inducing Tumor Necrosis Factor Alpha Production in Japanese Encephalitis. **Frontiers in Immunology**, v. 9, n. May, p. 1148, 2018.

WATANABE, M. et al. Self-Organized Cerebral Organoids with Human-Specific Features Predict Effective Drugs to Combat Zika Virus Infection. **Cell Reports**, v. 21, n. 2, p. 517–

532, out. 2017.

WELLS, M. F. et al. Genetic Ablation of AXL Does Not Protect Human Neural Progenitor Cells and Cerebral Organoids from Zika Virus Infection. **Cell Stem Cell**, v. 19, n. 6, p. 703–708, 2016.

WU, K.-Y. et al. Vertical transmission of Zika virus targeting the radial glial cells affects cortex development of offspring mice. **Cell Research**, v. 26, n. 6, p. 645–654, 2016.

YU, D. X. et al. Modeling hippocampal neurogenesis using human pluripotent stem cells. **Stem Cell Reports**, v. 2, n. 3, p. 295–310, 2014.

YUAN, L. et al. A single mutation in the prM protein of Zika virus contributes to fetal microcephaly. **Science**, v. 358, n. 6365, p. 933–936, 17 nov. 2017.

ZHANG, X. et al. Tick-borne encephalitis virus induces chemokine RANTES expression via activation of IRF-3 pathway. **Journal of Neuroinflammation**, v. 13, n. 1, p. 1–18, 2016.

ZHOU, B. Y. et al. Astrocyte activation and dysfunction and neuron death by HIV-1 Tat expression in astrocytes. **Molecular and Cellular Neuroscience**, v. 27, n. 3, p. 296–305, nov. 2004.

ZUKOR, K. et al. Zika virus-induced acute myelitis and motor deficits in adult interferon αβ/γ receptor knockout mice. **Journal of NeuroVirology**, p. 1–18, 2018.

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