

EDITORIAL

Sex differences in antiviral immunity in SARS-CoV-2 infection: Mitochondria and mitomiR come into view

Mitochondria are multifaceted organelles representing the 'powerhouse of cells' for their function as bioenergetics and biosynthetic hubs. In addition, they play an essential role in the regulation of innate and adaptive immune responses, including host defences against viruses, as well as in inflammatory responses.¹ This peculiar role of mitochondria is principally because of the activation of adaptor mitochondrial proteins, known as mitochondrial antiviral signalling (MAVS) proteins. MAVS senses viral RNA and triggers the activation of the transcription factor NF- κ B or IFN pathways and autophagy, in order to clear the infection and avoid excessive inflammation respectively.¹

Several DNA or RNA viruses have evolved mechanisms to evade the interferon I-mediated host immune responses by targeting mitochondria and, in particular, MAVS.² A paradigmatic example of the host innate immunity evasion is that of SARS-CoV virus, closely related and homologous to SARS-CoV-2, whose open reading frame protein 9b (ORF9b) localized to the outer mitochondria membrane induces degradation of MAVS, with subsequent loss of the TNF receptor-associated factor 3 (TRAF3) and 6 (TRAF6), leading to an impaired IFN responses in the infected cells.³ Recent findings demonstrated that SARS-CoV-2, such as SARS-CoV virus, in addition to ORF7a and ORF8a, also expresses ORF9b⁴ that can associate with the mitochondrial protein TOM70, impairing antiviral responses.⁵ SARS-CoV-2 genome and RNA viral transcripts were also found localized into host mitochondria.⁴ These evidences supported the hypothesis that SARS-CoV-2 might modulate MAVS in order to evade host immune responses in favour of its replication cycle.

Innate and acquired immune responses are influenced by patient sex, with women having generally higher innate and cell-mediated immune responses to pathogens than men.⁶ It is well known that sex chromosomes and sex hormones (ie oestrogen, progesterone and testosterone) cooperate in determining sex dimorphism in immune responses.⁷ Interestingly, recent findings suggested that differences in immune responses between men and women might also be caused by mitochondria, whose correct functioning is important for an adequate immune response and is influenced by sex.⁸ In fact, in mammals, mitochondrial DNA are maternal transmitted,

and mitochondria are subjected to natural selection solely in females. Therefore, during the maturation of egg, defective mitochondria or those containing mutations harmful for the females are eliminated, whereas are ignored in males and can then become more deleterious for males than for females.⁸ This female-biased mitochondrial 'culling' might be responsible of a lower quality and functioning of male mitochondria that, in turn, might explain, at least in part, the observed lower immune response in men than in women.

Researchers also pointed mitochondria as potential mediators of inflammatory responses, as well as key players in the establishment of hyperinflammatory states in virus-infected cells. Inflammatory conditions, together with oxidative stress and cytokine storm, represent the main pathogenetic features of the ongoing global pandemic coronavirus disease 2019 (COVID-19), caused by the recently discovered coronavirus SARS-CoV-2.

A typical feature of COVID-19 is the oxidative stress conditions, created in the SARS-CoV-2-infected cells by an excessive activation of the immune response, leading to an exacerbated inflammatory responses described as 'cytokine storm' and culminating in mitochondrial dysfunctions. Under these stressful conditions, dysfunctional and damaged mitochondria induced in turn inflammation, with subsequent modulation of immune responses.⁹ Severely damaged mitochondria increase reactive oxygen species (ROS) as well as pro-inflammatory cytokine production, accompanied by release of mitochondrial DNA into the cytosol, leading to cell death, inflammation and tissue damage. Altogether these events contribute to exacerbate inflammation and lead to systemic damages, including ROS accumulation and oxidative stress, hyperferritinaemia, blood coagulation and thrombus formation,⁹ typically present in severe forms of COVID-19.

The worldwide epidemiological analyses of COVID-19 cases indicated that lethality is much higher in males than in females.¹⁰ Most of the COVID-19 patients died for a severe respiratory tract infection, mainly the aged patient population. Unfortunately, effective strategies to treat or prevent COVID-19 are lacking so far. Therefore, there is the need to consider and develop innovative approaches.

Therefore, taking into account these considerations, agents able to restore mitochondrial function could be useful at different level: (a) to better understand the COVID-19 pathogenesis; (b) to identify new COVID-19 diagnostic markers; (c) to antagonize the cascade of events after SARS-CoV-2 infection, responsible for the clinical picture, triggered by the imbalance towards oxidation, inflammation and cytokine storm; (d) and to develop potential new and sex-specific strategies to manage and control COVID-19.

Research data suggest that mitomiRs are a pool of miRNAs (cellular small non-coding RNAs) identified in the mitochondrial fraction and directly targeting mitochondrial functions. Growing evidence highlights miRNAs as new and important regulators of infections and pathogenesis induced by a wide variety of DNA or RNA viral pathogens, including coronaviruses. Indeed, coronaviruses, like other respiratory and non-respiratory viruses, have been reported to be able to alter the expression of several cellular miRNAs in favour of their replication cycle within the host, contributing to the pathogenesis of acute as well as chronic respiratory diseases, by eluding the cellular defence mechanisms.¹¹ Several of these miRNAs are associated with inflammation, aging and mitochondrial functions.¹² MitomiRs, in particular, have been demonstrated to regulate systemic energy homeostasis, oxidative capacity, ROS generation, inflammation and mitochondrial lipid metabolism. Thus, investigations on the potential modulation of immune and inflammatory responses in SARS-CoV-2-infected cells by mitomiR could be interesting and helpful to better elucidate the molecular mechanisms involved in immune evasion by SARS-CoV-2 and in COVID-19 pathogenesis.

The mitomiR 146a-5p might represent one possible candidate for further investigations. Indeed, evidence demonstrated that miR-146a-5p expression was modulated following several viral infections, such as that induced by Japanese encephalitis virus, Dengue virus, avian infectious bronchitis virus, hepatitis B virus, influenza A virus and Borna disease virus 1. Its viral-mediated overexpression has been reported to promote viral replication by inducing downregulation of IL-1 receptor-associated kinase-2 (IRAK2) and TRAF6, with consequent suppression of inflammatory cell responses and cytokine production. Interestingly, miR-146a has been found downregulated by oestrogens in murine splenic lymphocytes.¹³ Accordingly, a study conducted in Alzheimer's disease (AD) patients reported lower miR-146a levels in females compared to males, both in AD patients and healthy non-affected controls.¹⁴

Other intriguing candidates are the mitomiR-221 and 19b, both located on the human X chromosome and sex hormones sensitive.¹⁵ MiR-221 has been highlighted by researchers as a suppressor of innate antiviral immune responses. Indeed, overexpression of miR-221 inhibited the induction of IFN-I by MAVS.¹⁶ MiR-19b has been found to potentiate inflammation

in Japanese encephalitis virus (JEV)-infected human astrocytoma cell lines and in brain tissues of JEV-infected mice.¹⁷ Unfortunately, nothing is known about the potential role of these miRs in respiratory viral infections, including coronaviruses. Only one study reported that respiratory syncytial virus (RSV)-mediated inhibition of miR-221 favours viral replication by interfering with the apoptotic death of infected cells.¹⁸ Another study showed that miR-221 inhibition by the porcine epidemic diarrhoea coronavirus (PEDV) blocks NF- κ B pathway, enhancing virus replication.¹⁹

Therefore, in the light of the above, further studies on the role of mitomiRs would be indispensable to clarify both their possible role in the pathogenesis of COVID-19 and their potential as sex-specific disease biomarkers or therapeutic targets.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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