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CORRESPONDENCE

Running interference on miR-33: a new amplification loop for type I interferon in the host antiviral response

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Innate immunity is the first line of host defense against viral infections. Mitochondria, best known as the cell's "power plant" for ATP generation, have recently been shown to serve as essential platforms for the signaling events that induce antiviral type I interferon (IFN). Localization of the adaptor mitochondrial antiviral signaling protein (MAVS) to the mitochondrial surface via a C-terminal transmembrane domain is required for RIG-I-like receptors (RLRs) to transduce antiviral signaling upon detection of double-stranded RNA in the cytosol. Mitochondrial quality control through autophagy (i.e., mitophagy) can impact RLR/MAVS signaling through effects on mitochondrial mass and function.[1] Previous studies have generally indicated that mitophagy promotes virus infection by decreasing RLR/MAVS signaling and downstream type I IFN production.[2] Viruses can interestingly coopt this housekeeping function for their benefit, as viral proteins translocate to mitochondria, accelerate mitophagy through attenuation of mitochondrial membrane potential, and thereby suppress RIG-I signaling.[3] MAVS signaling to type I IFN can also be regulated by mitochondrial dynamics through fusion and fission, whereby MAVS expression promotes fission, while fission inhibits MAVS.[4] Finally, type I IFNs have recently themselves been shown to prime antiviral responses in part through reprogramming mitochondrial metabolism, suggesting the existence of complex feedback loops.[5]

MicroRNA (miR)-33, which is located within an intron of the cholesterol synthesis transcriptional regulator sterol-regulatory element–binding factor–2 (*Srebf2*), has recently been identified as a master coordinator of cell metabolism and host defense. First identified for its inhibition of cellular cholesterol efflux through its targeting of lipid efflux transporters for degradation,[6] miR-33 has since been shown to skew cellular bioenergetics away from mitochondrial fatty acid oxidation and toward glycolysis.[6,7] miR-33 also directly targets for degradation ATG5, ATG12, MAP1LC3B, LAMP1, and PRKAA1 in the autophagy pathway.[7,8] Interestingly, in addition to its co-induction along with *Srebf2* in response to metabolic cues (i.e., low cholesterol), miR-33 is responsive to inflammatory and infectious stimuli, including bacterial lipopolysaccharide and IFN-γ. *M. tuberculosis* (Mtb) upregulates miR-33 in airway macrophages, thereby preventing autophagic killing (i.e., xenophagy) and accumulating lipid bodies for its own nutrient source; conversely, pharmacological silencing of miR-33 promotes Mtb clearance from the host.[8]

In this issue, Liu et al. report an exciting new connection between miR-33/33* and mitochondrial quality control during vesicular stomatitis virus (VSV) infection in macrophages.[9] Viral infection induces type I IFN receptor-dependent suppression of miR-33, whereas overexpression of miR-33/33* downregulates VSV-induced type I IFN production. The latter phenomenon occurs via miR-33-dependent inhibition of MAVS aggregation on the mitochondrial surface, thereby impairing RIG-I signaling. The authors reveal that miR-33/33* inhibition of MAVS aggregation is through direct miR-33-mediated degradation of *Prkaa1* mRNA, which encodes the AMP-activated protein kinase (AMPK) catalytic subunit. As AMPK is a master driver of autophagy, its degradation impairs autophagic quality control of mitochondria (i.e., mitophagy), thereby impairing mitochondrial function. These findings are consistent with a previous report showing that miR-33 antagonists increase *Prkaa1* levels in nonhuman primates.[10] Finally, the authors show that in vivo treatment of mice with miR-33/33* agomirs prior to systemic infection with VSV impairs the type I IFN response, increases VSV burden in tissues, and worsens survival.

The study by Liu et al. thus identifies miR-33 as an indirect, inverse IFN-stimulated gene product and extends the biological scope of miR-33 to antiviral host defense. Overall, the authors identify an intriguing, indirect feedforward loop whereby type I IFN sustains further type I IFN induction by ensuring mitochondrial quality control via suppression of miR-33. Moving forward, it will be interesting for future studies to test whether inhibition of miR-33/33* (e.g., with antagomirs) reduces VSV viral load in vivo and accelerates the clearance of virus from the host. While this seems plausible, miR-33 may already be effectively suppressed by endogenous type I IFN in the infected host. On the other hand, prior studies have indicated that autophagy enhances oncolytic measles virus replication through the inhibition of type I IFN.[2] Given this observation, autophagy, miR-33, and/or AMPK may have context- (i.e., cell type- and/or virus type-) dependent roles in antiviral host defense that need to be better delineated. It will also be important to determine the identity of the AMPK effectors (e.g., TFEB, FOXO3[8]) in the miR-33-mitophagy-MAVS pathway. Whether miR-33 affects MAVS function through peroxisome proliferator-activated receptor-γ coactivator (PGC)1α-dependent mitochondrial biogenesis, and whether some of the effects of miR-33 upon mitochondria and/or viral replication could be through its control of cellular cholesterol levels are additional important questions. Regarding the latter, several viruses are known to rely on lipid-rich "replication organelles" in the infected cell for their life cycle.

Finally, several interesting translational questions arise at the level of the infected host. Given that inhibition of macrophage miR-33 reportedly promotes differentiation of T regulatory cells (Tregs)[7], one wonders whether suppression of miR-33 may also promote Treg-dependent resolution of inflammation and tissue

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injury in vivo during viral infection. Conversely, it is intriguing to consider whether miR-33/33* agomirs might prove useful as an adjunct to oncolytic virus treatment for cancer. While confirmation of the miR-33-MAVS pathway in humans will be required for translational approaches to move forward, it is encouraging to note that miR-33 has been confirmed to target AMPK in vivo in nonhuman primate models.[10] In closing, pharmacological inhibition of miR-33 has already shown early promise as a potential treatment in a number of preclinical disease models, including atherosclerosis and lung infection.[7,8] The study by Liu et al. now adds viral infection as yet another promising avenue for therapeutic targeting of miR-33.

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ADDITIONAL INFORMATION

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