Medical & healthcare, Drug development

Alternative splicing of Mff regulates AMPK-mediated phosphorylation, mitochondrial fission and antiviral response

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Abstract

Mitochondrial morphology and function change dynamically in response to intracellular signaling and the surrounding environment. The mitochondrial fission factor Mff, which localizes to the outer mitochondrial membrane, mediates not only mitochondrial fission by recruiting the dynamin-related GTPase Drp1 to mitochondrial fission sites but also the double-stranded RNA-induced antiviral response on mitochondria through mitochondrial antiviral signaling (MAVS). Mff is reported to be regulated by AMP-activated protein kinase (AMPK)-mediated protein phosphorylation and alternative pre-mRNA splicing; however, the relationships among RNA splicing, phosphorylation, and multiple functions of Mff have not been fully understood. Here, we showed that mouse Mff has a tissue-specific splicing pattern, and at least eight Mff splice isoforms were expressed in mouse embryonic fibroblasts (MEFs). We introduced single Mff isoforms into Mff knockout MEFs and found that insertion of exon 6 just after the phosphorylation site, by the alternative splicing, reduced its phosphorylation by AMPK and its functions in mitochondrial fission and the antiviral response. In addition, the underlying mechanism repressing these functions was independent of phosphorylation. These results indicate that multiple functions of Mff on mitochondria are regulated by AMPK-mediated phosphorylation and alternative splicing, under the control of energy metabolism and cellular differentiation.

Background & Results

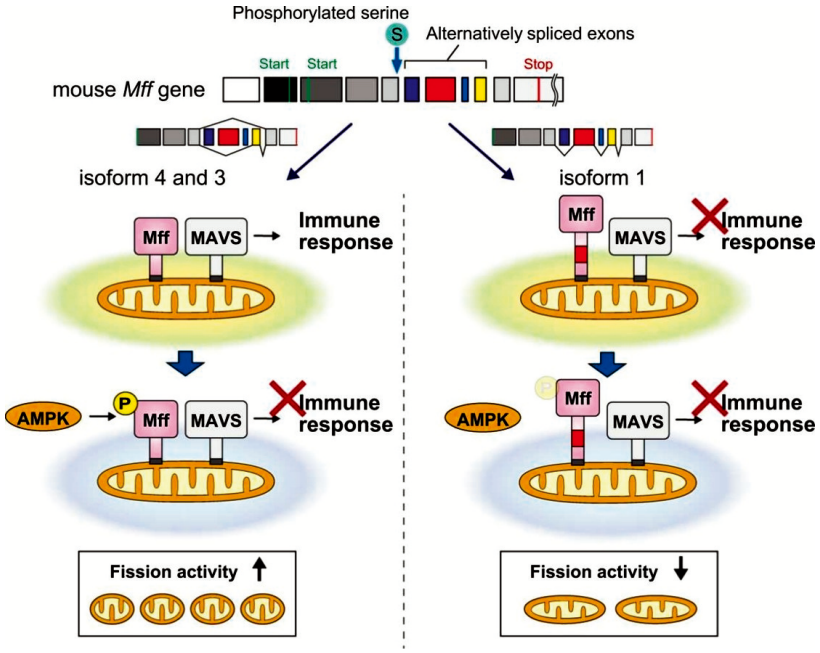
Mitochondria are multifunctional organelles involved in energy production through oxydative respiration and various cellular signaling pathways. While cellular energy and metabolism primarily occur in the inner membrane and matrix, many cellular signaling pathways are regulated on the outer membrane. Another role of the outer membrane is regulating innate immune responses. The mitochondrial antiviral signaling protein (MAVS) recognizes infected viral RNA and mediates signaling for cytokine induction.

In previous studies, we demonstrated that mitochondrial fission factor Mff, localized on mitochondrial outer membrane, also functions in innate immune response by regulating MAVS cluster formation on outer membrane. Furthermore, we found that mitochondrial dysfunction induces Mff phosphorylation via AMP-dependent kinase AMPK, thereby suppressing innate immune responses. However, the molecular mechanism remains poorly understood.

In this study, we analyzed AMPK-mediated phosphorylation and function of Mff splice isoforms. Our results confirmed that Mff splice isoforms exhibit different efficiencies of AMPK-mediated phosphorylation, as previously reported, and furthermore, we found that alternative splicing affects AMPK-mediated phosphorylation, mitochondrial fission, and antiviral responses. Our data also revealed that Mff alternative splicing occurs in a tissue-specific manner.

Significance of the research and Future perspective

We found that multiple functions of mitochondrial Mff are regulated by AMPK-mediated phosphorylation and alternative splicing. These findings should contribute to understanding how mitochondrial fission mechanisms and the mitochondrial-mediated antiviral innate immune response are controlled under the regulation of energy metabolism and cellular differentiation.



Effects of Mff alternative splicing on the innate immune response

Patent

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