(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	EMRALINO,Francine Lianne Castaneda
Thesis Title	MDA5-mediated type I interferonopathy mouse model displays lethal response to immune stimulation		

(Thesis Summary)

MDA5 is the critical sensor for viral double-stranded RNA (dsRNA) to initiate antiviral innate immune responses by inducing antiviral cytokines including interferons. However, constitutive production of antiviral cytokines causes autoimmune diseases including Singleton-Merten Syndrome (SMS) and Aicardi-Goutières Syndrome (AGS). The c.2465G>A (p.R822Q) missense mutation in the MDA5 gene *IFIH1* has been identified as a gain-of-function mutation leading to SMS. This study aims to understand how this mutation affects the mechanism of disease progression and how external factors such as immune stimulation can impact the immune response on affected individuals. A transgenic mouse line carrying human gene encoding MDA5 (R822Q) was generated (hM-R822Q Tg mice) and its immune responses were analyzed. hM-R822Q Tg mice developed SMS-like bone abnormality, heart fibrosis, aortic valve enlargement and aortic calcification with a systemic interferon-stimulated gene signature. Administration of synthetic dsRNA, poly I：poly C (poly I:C) resulted in systemic inflammation, most notable in the intestines. Poly I:C-injected female C56BL/6J hM-R822Q Tg mice also developed lethal hypercytokinemia marked by massive IL-6 levels in the serum. Genetic deletion of the downstream signal molecules including mitochondrial antiviral signaling (MAVS) and type I interferon receptor (IFNAR1) interrupted the interferon signal and ameliorated hyperinflammation in hMR822Q Tg mice. Administration of a drug tofacitinib, an inhibitor for signaling from interferon receptor, cytokine production and mucosal damage were reduced. These findings demonstrate that the MDA5 R822Q introduces a critical risk factor for uncontrollable inflammation upon immune stimulation such as viral infection and vaccination.

(Form 2)

(Thesis evaluation summary)

The applicant aimed to delineate the mechanism of pathogeneses of gain of function mutation of MDA5 (R822Q) found in a SMS patient. A transgenic mouse line containing a fragment of human chromosome encompassing whole human *IFIH1* gene encoding MDA5 R822Q. The mice were analyzed for histologic and immunological phenotypes. hM-R822Q Tg mice developed SMS-like bone abnormality, heart fibrosis, aortic valve enlargement and aortic calcification with a systemic interferon-stimulated gene signature. The applicant demonstrated that the bone phenotype was accompanied with aberrant development of osteoclast. To analyze phenotypes upon immunological stimuli, the mice were injected with poly I:C, a mimic of viral dsRNA. The injection caused severe inflammation in eyes and intestines accompanied with acute production of inflammatory cytokines including IL-6 and IL-17 and decreased survival. To investigate the role of antiviral signaling, hM-R822Q Tg mice with genetic deletion of MAVS or IFNAR1 were generated. These mice exhibited attenuated responses to poly I:C injection, suggesting that the poly I:C-inducible phenotype results from signal amplification through RIG-I-like receptor and produced type I interferon. Furthermore, administration of tofacitinib, a chemical inhibitor for signaling from IFNAR, ameliorated poly I:C-induced inflammation. This study demonstrated that MDA5 R822Q missense mutation not only causes chronic interferonopathy but also virus- or vaccine-inducible acute inflammation and IFNAR inhibitor is a potential candidate for treatment of the acute inflammation.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of immunology, and presents new discoveries and concepts that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On November 7th, 2022, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary, and thesis evaluation summary will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date that the thesis can be published. (Please note, however, based on Article 8 of the Degree Regulations, that the thesis must be published within three months of the date that the degree is awarded.)

Thesis publication date :