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Letter to the Editor

TRIF and MAVS signaling pathways regulate RAB27A induction and melanosome transfer by TLR3 signaling in human epidermal melanocytes

Human epidermis is a barrier defending human internal organs from outer environment such as microbes, ultraviolet, dryness, etc. Epidermal melanocytes localize in basal layer of the epidermis and express Toll-like receptors (TLR) to sense innate immune stimuli [1,2]. We previously reported that TLR2 and TLR3 are involved in melanocyte functions such as melanogenesis, intra/extra cellular melanosome transfer, and melanosome transfer to keratinocytes [3]. TLR3 agonist Poly(I:C) increases RAB27 A mRNA and protein expression and enhances co-localization of RAB27 A and Gp100 +melanosome at cell periphery in human melanocyte. Correlatively, the knockdown of RAB27 A decreases the melanosome transfer by Poly(I:C). Although Chiaverini et al. reported that microphthalmia associated transcription factor (MITF) regulates RAB27 A transcription in melanoma cells [4], the knockdown of MITF did not affect the RAB27 A expression and function in normal human melanocytes stimulated by TLR3 agonist Poly(I:C) [3]. There are two key intracellular signaling molecules involving in TLR3 signals; TIR-domain-containing adaptor-inducing interferon-β (TRIF) and Mitochondrial antiviral signaling protein (MAVS). TLR3 utilize the intracellular molecule TRIF to activate IFN-β expression [5]. MAVS is activated by retinoic acid-inducible gene-I (RIG-I)-like RNA helicases bound to viral RNA, and MAVS affects the TLR3 signaling through transcription factors IRF3 and NF-κB to induce type I interferons [6]. To understand insights how TLR3 signaling regulates melanogenesis in human melanocytes, we examined in this study if TLR3 agonist Poly(I:C) activates TRIF and MAVS in melanocytes and if these pathways are involved in RAB27 A induction as well as melanosome transfer by TLR3 stimuli.

Normal human epidermal melanocytes (Kurabo, Osaka, Japan) were cultured and stimulated by TLR3 agonist Poly(I:C) as described in the supplementary information. After 24 h stimulation by Poly(I:C), TRIF and MAVS mRNA and protein were increased in melanocytes (Fig. 1A-D). Next, we examined if Poly (I:C) affects the intracellular localization of TRIF and MAVS. To examine the intracellular TRIF and MAVS localization and interaction with melanosomes in melanocytes, the immunofluorescence staining were performed. In the absence of Poly(I:C), TRIF and MAVS were diffusely distributed throughout the cytosol in human melanocytes and accumulated at peri-nuclear area by Poly(I:C) (Fig. 1E,F). Although we observed that TRIF and MAVS were partially co-localized with Gp100+melanosomes in human melanocytes, Poly(I:C) induced Gp100+melanosomes translocation to cell periphery while Poly(I:C) induced TRIF and MAVS accumulation to peri-nuclear area (Fig. 1E,F), suggesting that majority of Gp100+melanosomes did not directly interact with TRIF and MAVS. To examine the TRIF and MAVS involvement in RAB27 A induction by Poly(I:C), we employed siRNA for TRIF (siTRIF) and MAVS (siMAVS). Confirming TRIF and MAVS knock down by each siRNA (Fig. 2A,B), we observed that SiTRIF and siMAVS suppressed the RAB27 A induction by Poly(I:C) (Fig. 2C). We next examined if TRIF and MAVS knockdown suppresses melanosome transfer. Both of siTRIF and siMAVS suppressed transfer of GP100-positive melanosomes to human keratinocytes induced by Poly(I:C) (Fig. 2D,E). Since siTRIF and siMAVS suppressed both RAB27 A induction and transfer of GP100-positive melanosomes to human keratinocytes similarly to the effect of RAB27 A knockdown as we previous reported [3], these results suggested that TRIF and MAVS regulated RAB27 A induction and melanosome transfer in human melanocytes.

Human melanocytes can sense pathogen-associated molecular patterns (PAMPs) via TLRs as well as damage-associated molecular patterns (DAMPs) including IL-1 and IL-8 [7]. We revealed that Poly (I:C) increased the expression of TRIF and MAVS, two main downstream signaling adaptors of TLR3, and enhanced TRIF and MAVS accumulation at peri-nuclear area in human melanocytes (Fig. 1). We observed both siTRIF and siMAVS suppressed the RAB27 A induction in melanocytes and reduced GP100-positive melanosome transfer to human keratinocytes induced by Poly(I:C) (Fig. 2). In HeLa cells, TRIF localizes diffusely in the cytosol and forms the speckle-like structures at low expression level, and overexpressed TRIF localizes in peri-nuclear area [8]. MAVS forms clusters that partially overlapped with mitochondria in peri-nuclear area in response to Sendai virus infection [9]. Thus, the perinuclear accumulation of TRIF and MAVS by Poly(I:C) suggested that Poly(I:C) activates TRIF and MAVS in melanocytes.

The external stimuli include ultraviolet (UV) induce α-melanocyte-stimulating hormone (α-MSH) production and release from keratinocytes. α-MSH activates the melanocortin-1 receptor (MC1R) and induces MITF gene transcription in melanocytes. MITF is a key transcriptional factor in melanogenesis and also directly binds to the RAB27 A promoter region to enhance RAB27 A expression [4]. However, knockdown of MITF did not affect the RAB27 A induction by TLR3 agonist Poly(I:C) stimuli in human melanocytes [3]. In this study, we showed that siTRIF and siMAVS suppressed the RAB27 A induction and following melanosome transfer to keratinocytes by Poly(I:C). The activation of TRIF and MAVS leads to NF-κB signaling pathway, and activated NF-κB molecules translocate to nucleus and act as a transcriptional factor [5,6]. We previously reported that curcumin, a NF-κB inhibitor, suppressed RAB27 A induction by Poly(I:C) in human melanocytes [3]. Similar to our findings, an increase and phosphorylation of NF-κB p65 subunit enhances NF-κB transcriptional activity and leads NF-κB binding to the RAB27 A promoter region to induce the RAB27 A transcription in colon cancer cells [10]. Although this study does not provide exact data to proof a direct interaction

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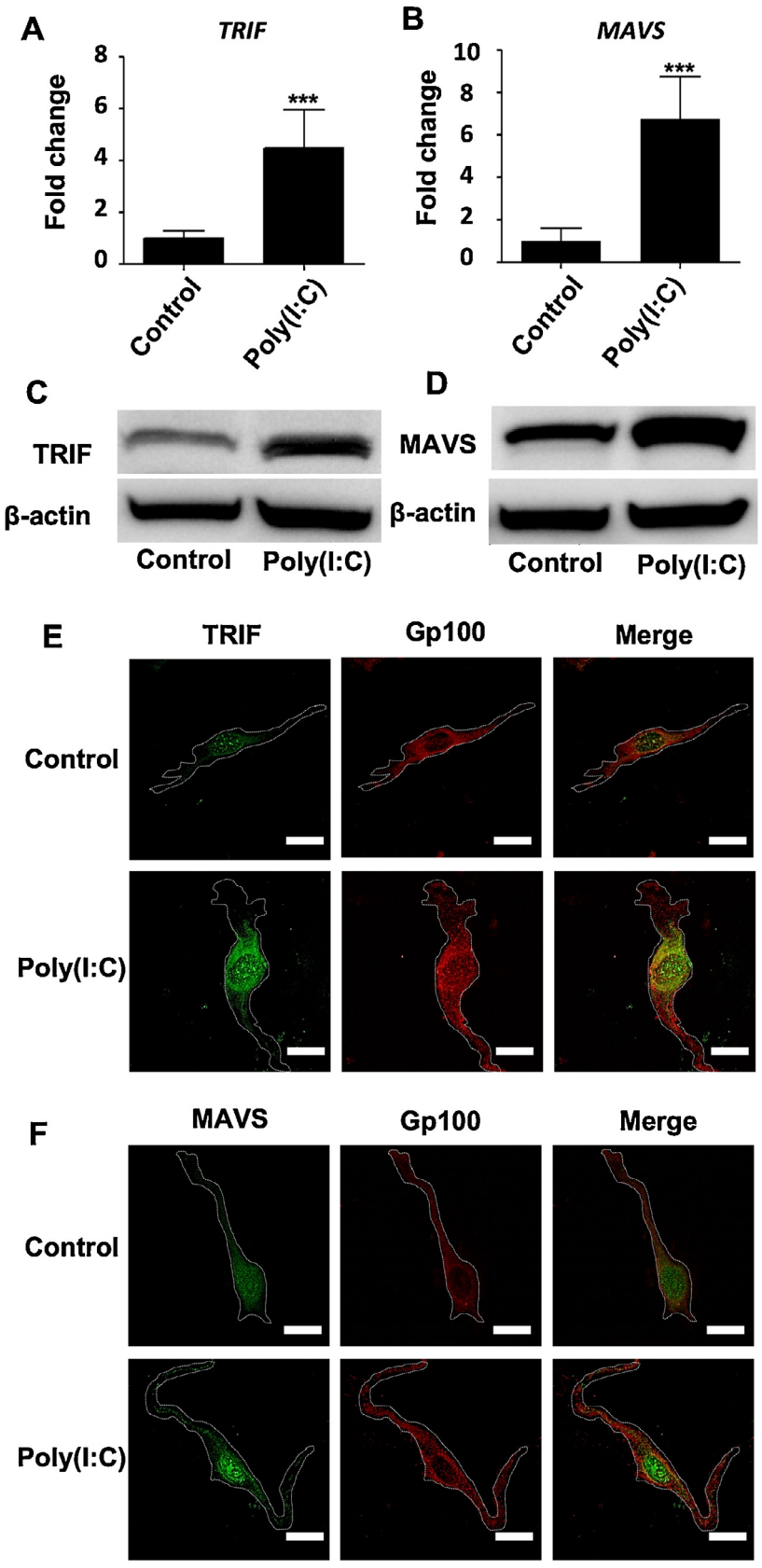

Fig. 1. Poly(I:C) increased the TRIF and MAVS mRNA and protein.
Melanocytes were treated with TLR3 agonist Poly(I:C) with the concentration described in materials and methods. After 24 h incubation, the expressions of TRIF and MAVS were examined expression was confirmed by real-time PCR (A,B) and western-blotting (C,D) in melanocytes. The results of real time PCR are shown as relative values to the control. ***$P < 0.001$. There are representative images in 3 independent experiments for western blotting. (E) Melanocytes treated with Poly(I:C) (1 μg ml^{-1}) for 24 h, and intracellular localization of Gp100 (red) and TRIF (green) or MAVS (green) was visualized by immunofluorescence staining. Scale bars = 20 μm.

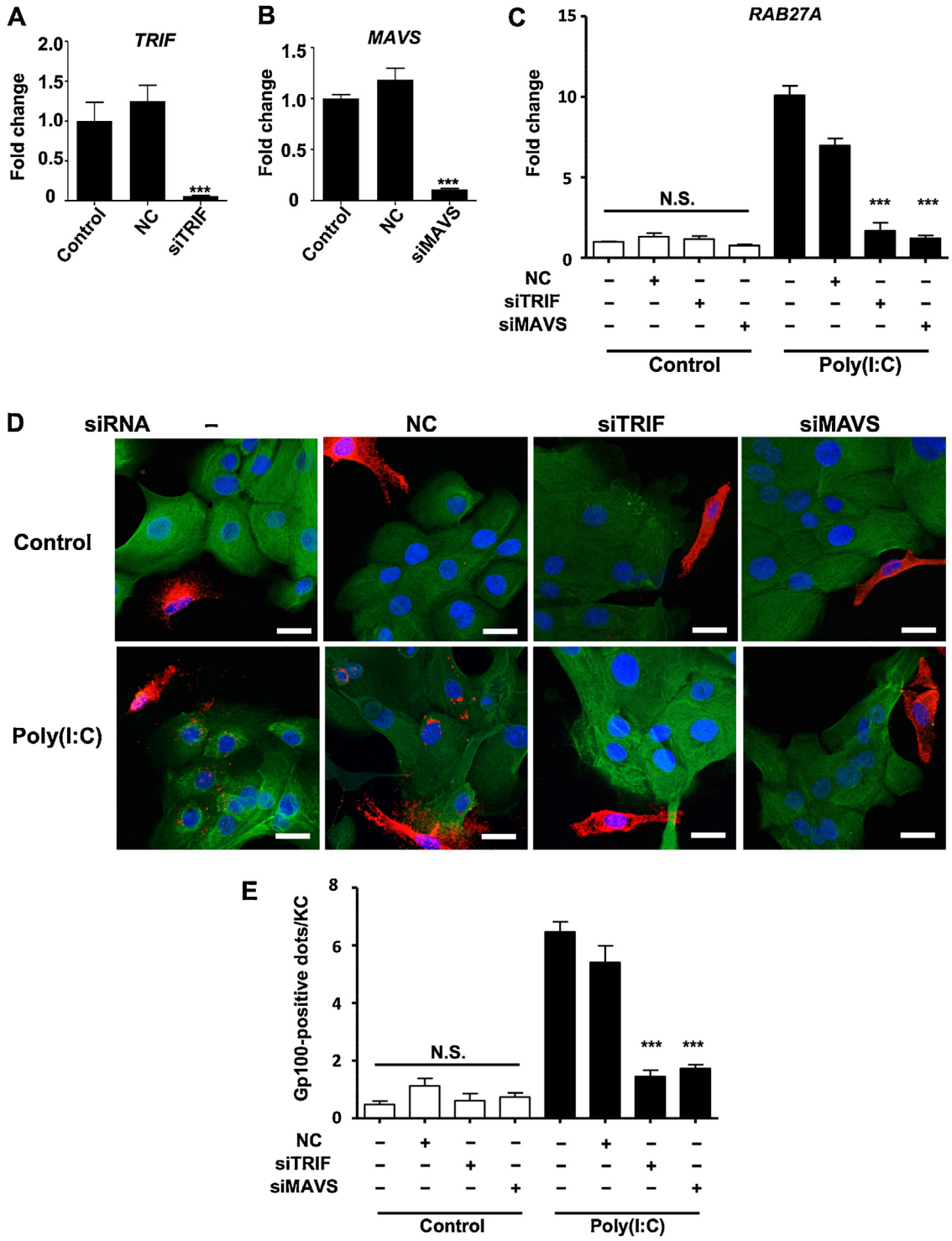

Fig. 2. Knockdown of TRIF and MAVS suppressed RAB27 A induction by Poly(I:C).
(A,B) Twenty-four hours after siTRIF and siMAVS transfections, the decrease of target factor expression was confirmed by real-time PCR in melanocytes. (C) After siRNA transfection, RAB27 A mRNA expression was examined in melanocyte with or without Poly(I:C) stimulation. (D,E) Melanocytes co-cultured with keratinocytes were treated with siRNA of TRIF and MAVS for 24 h, and then stimulated by Poly(I:C) (1 μg ml^{-1}). After 24 h incubation, PMEL/Gp100 (red) and keratin (green) were visualized by immunofluorescence staining (D, Scale bars = 50 μm). The number of PMEL/Gp100-positive dots per keratinocyte were counted as described in the methods and shown as mean ± SD in the graph (E). The result was shown as relative values to the control. '–' and '+' mean samples treated without and with siTRIF and siMAVS, respectively. 'N.S.' indicates 'not significant. ***$P < 0.001$.

between TRIF and MAVS in melanocytes, both TRIF and MAVS as well as downstream NF-κB activation are required to enhance RAB27 A activation by TLR3 signals. Combining these data and knowledge, we concluded that TLR3 agonist Poly(I:C) induces TRIF and MAVS signaling pathway, and the following NF-κB activation influences RAB27 A expression and the melanosome dynamics independent of MITF. In conclusion, normal human epidermal melanocytes utilize common innate immune system to enhance melanogenesis and affect melanosome transfer to neighboring keratinocytes.

Author contributions

S.K. and K.Y. designed the project and wrote the manuscript; S.K., T.Y., R.S.O. and K.T. performed experiments; S.K. and K.Y. performed statistical data evaluation; S.A. contributed to the data interpretation; K.Y. conceived and supervised all aspects of this work.

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Conflicts of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.jdermsci.2019.04.004.

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