Abstract 2599

AMPK functions as a non-canonical GEF for Arf6 activation in a kinase-independent manner upon energy deprivation

Fang-jen Lee, *National Taiwan Univ, College of Medicine*

Kuan-Jung Chen, Jia-Wei Hsu

AMP-activated protein kinase (AMPK) is a crucial cellular nutrient and energy sensor that maintains energy homeostasis. AMPK also governs cancer cell invasion and migration by regulating gene expression and activating multiple cellular signaling pathways. ADP-ribosylation factor 6 (Arf6) can be activated via nucleotide exchange by guanine nucleotide exchange factors (GEFs), and its activation also regulates tumor invasion and migration. By studying GEF-mediated Arf6 activation, we elucidated that AMPK functions as a noncanonical GEF for Arf6 in a kinase-independent manner. Moreover, by examining the physiological role of the AMPK-Arf6 axis, we determined that AMPK activates Arf6 upon glucose starvation and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) treatment. We further identified the binding motif in the C-terminal regulatory domain of AMPK that is responsible for promoting Arf6 activation and thus inducing cell migration and invasion of ovarian cancer cells. We previously also demonstrated that yeast Arf3 can be activated by Snf1, the yeast homologue of AMPK (Nature comms, 2015). These findings reveal an evolutionarily conserved role of AMPK in which its C-terminal regulatory domain serves as a non-canonical GEF for Arf6 activation during energy deprivation.

Ministry of Science and Technology, Taiwan (109-2636-B-002-015 and 110-2636-B-002-012).

104448, https://doi.org/10.1016/j.jbc.2023.104448

Abstract 2603

Human steroid sulfatase deficiency induces keratinization of HaCaT cells by improving the expression level of E-cadherin

Tae-uk Kwon, *Chung-Ang University College of Pharmacy*

Tae-uk Kwon, Yeo-Jung Kwon, Young-Jin Chun

X-linked ichthyosis (XLI) is a recessive genetic disorder of human skin caused by the genetic deletion of the steroid sulfatase (STS) gene. Deficiency of STS activity in XLI leads to intracellular lipid barrier malformation and delays keratinocyte degradation resulting in increasing corneodesmosomes. To understand how STS can regulate keratinization, the expression of cadherin proteins was determined because the cadherin proteins including E-cadherin may control the expression of keratinization-related proteins such as involucrin, loricrin, and TGM-1. In this study, we observed up-regulation of E-cadherin at mRNA and protein levels by knockdown of STS in human keratinocyte HaCaT cells. In addition, inhibition of STS enzymatic activity by STX64, a specific inhibitor, significantly enhanced E-cadherin expression. Furthermore, keratinization-related factors, including involucrin and loricrin, were increased in STS-knocked down cells. When cells were treated with TNF-α to induce STS expression, E-cadherin level was strongly repressed. Moreover, involucrin and loricrin were down-regulated when cells were treated with an E-cadherin antibody. Significant increases in epidermal and dermal levels were observed in STS-knockout mice compared to normal mice. Additionally, as the expression of E-cadherin increased, protein expression levels of markers for keratinization, involucrin, and loricrin were induced. In summary, these results suggest that STS deficiency may contribute to the desquamation which is induced by keratinization of human skin by inducing the expression of E-cadherin.

This research was supported by the National Research Foundation of Korea (NRF) funded by the Korean government (MSIP) (NRF-2021R1A2C201239512, NRF-2022R1A5A6000760).

104449, https://doi.org/10.1016/j.jbc.2023.104449