Form 2

Dissertation Abstract

Report no.	(Dissertation-based) No.243	Name	Rahman Khondoker Md Zulfiker (ID: 14DB053)
Dissertation title	**Analysis of Mechanism of Hikeshi Modulating Stress Sensitivity in HeLa and hTERT-RPE1 Cells** [Hikeshiヒト HeLa細胞とヒト不死化網膜上皮細胞のストレス応答機構の解析]		

Under heat shock-stress condition, the importin β-mediated nuclear transport pathway is downregulated and another pathway mediated by Hikeshi, a protein encoded by human C11orf73 is activated. Hikeshi is a protein that mediates the heat stress-induced nuclear import of heat-shock protein 70 (HSP70s: HSP70 /HSC70). Dysfunction of Hikeshi causes some serious hereditary diseases in humans; however, the cellular function of Hikeshi is largely unknown. Here, we investigated the effects of Hikeshi depletion on the survival of human cells after proteotoxic stress and found opposite effects in HeLa and hTERT-RPE1 (RPE) cells; depletion of Hikeshi reduced the survival of HeLa cells but increased the survival of RPE cells in response to proteotoxic stress. Hikeshi depletion sustained heat-shock transcription factor 1 (HSF1) activation in HeLa cells after recovery from stress, but introduction of a nuclear localization signal-tagged HSC70 in Hikeshi-depleted HeLa cells down-regulated HSF1 activity. In RPE cells, the HSF1 was efficiently activated, but the activated HSF1 was not sustained after recovery from stress, as in HeLa cells. Additionally, we found that p53 and subsequent up-regulation of p21 were higher in the Hikeshi-depleted RPE cells than in the wild-type cells. The above results indicate that depletion of Hikeshi renders HeLa cells proteotoxic

stress-sensitive through the abrogation of the nuclear function of HSP70s required for HSF1 regulation. Moreover, Hikeshi depletion up-regulates p21 in RPE cells, which could be a cause of its proteotoxic stress resistant. Functional modulation of HSP70 with the inhibitor YM-1 caused cell death in the HeLa cells but resulted in growth arrest of the RPE cells. Furthermore, YM-1 treatment dramatically up-regulated p53 and p21 proteins in RPE cells and down-regulated FoxM1 and survivin, which are regulators of cell cycle progression, in both RPE cells and HeLa cells. Our results showed that regardless of the presence or absence of Hikeshi, the p53-p21 pathway becomes active when RPE non-cancer cells are treated with YM-1, which contributes to protection against cell death. Hikeshi might function as an upstream regulator of HSP70, which affects activation of the p53-p21 pathway, especially during and after proteotoxic stress. Additionally, expression of HSP70 and Bag3 is up-regulated by Hikeshi depletion whereas remains unaffected after YM-1 treatment indicating that Hikeshi depletion and HSP70 inhibition byYM-1 have different functions. Understanding the mechanism by which Hikeshi and HSP70 regulate the p53-p21 pathway in response to various cellular stimuli as well as which types of cancer cells become stress-sensitive upon Hikeshi depletion could make Hikeshi a potential therapeutic target.