様式３

論　文　内　容　の　要　旨

氏　名　（Kim Junhyeong）	
論文題名	Atg5 plays crucial roles in naked mole-rat cell proliferation and maintenance of cellular homeostasis (Atg5はハダカデバネズミ細胞の増殖と恒常性維持に重要な役割を果たす)

論文内容の要旨

The naked mole-rat (NMR, *heterocephalus glaber*) is a eusocial subterranean rodent, native to Africa. NMRs are the longest-living rodent species with a maximum lifespan of over 30 years. While body size of NMRs is similar to that of house mouse (*Mus musculus*), NMRs live 10 times longer than house mouse. Furthermore, NMRs generally experience a greatly extended healthy lifespan within their total lifespan of 30 years. These extraordinary mammals also exhibit profound resistance to both spontaneous and experimentally induced cancer. A previous study identified that NMR fibroblasts exhibit hypersensitive contact inhibition termed early contact inhibition, which is regulated by $p16^{INK4a}$, p53 and Rb pathways. Moreover, NMRs have increased levels of basal macroautophagy compared with mouse.

Macroautophagy (hereafter, autophagy) is the evolutionarily conserved pathway that degrades intracellular components, including aggregated protein, organelles, macromolecules and invading pathogens via lysosomal degradation. Autophagy contributes to the maintenance of cellular homeostasis and fitness in both basal state a stressed state. Previous studies have suggested that autophagy is deeply implicated in animal aging. Many species display decreased autophagy activity with age. Furthermore, Studies in *C.elegans* have suggested that autophagy activation is implicated in lifespan extension. Brain-specific overexpression of Atg8a and neuron-specific upregulation of Atg1 activate and extend the lifespan in *Drosophila*. Atg5 overexpression in mice contributes to activation of autophagy and extension of lifespan. However, molecular mechanisms underlying high basal autophagy activity of NMRs and its physiological significance of this phenomenon remain to be elucidated.

In this study, I identified that the Atg12-Atg5 conjugate, a critical component of autophagosome formation, was highly expressed in NMR skin fibroblasts (NSFs) compared with that in mouse skin fibroblasts. I then generated Atg5 knockdown NSFs via lentiviral shRNA vectors to investigate the role of Atg5 in NSFs. Phenotypic analysis of Atg5 knockdown NSFs revealed that high basal autophagy activity in NSFs was associated with abundant expression of the Atg12-Atg5 conjugate. Atg5 knockdown in NSFs led to accumulation of dysfunctional

mitochondria, frequent appearance of abnormally large-sized cells, and suppressed cell proliferation and cell adhesion ability, promoting anoikis/apoptosis accompanied by upregulation of apoptosis-related genes, Bax and Noxa. Furthermore, inhibition of the p53/Rb pro-apoptotic pathway with SV40 large T antigen abolished the increase in cell size, cell cycle arrest and suppression of cell adhesion, the phenotypes related to anoikis/apoptosis induced by Atg5 knockdown. Taken together, these results suggest that high basal autophagy activity in NMR cells, mediated by Atg5, contribute to suppression of apoptosis by interfering with the activation of the p53/Rb pro-apoptotic pathway, potentially via degradation of stress-inducing factors. This mechanism could benefit the longevity of NMR cells.

様式 7

論文審査の結果の要旨及び担当者

氏　名	（Kim Junhyeong）

	（職）	氏　名
論文審査担当者		
主 査	教授	岡田　雅人
副 査	教授	三木　裕明
副 査	教授	石谷　太

論文審査の結果の要旨

ハダカデバネズミはマウスと同等の大きさながら最大寿命が 30 年以上であり、腫瘍形成にも非常に強い抵抗性を持っている齧歯類である。これらの特徴からハダカデバネズミはがんと老化研究のモデル動物として注目されている。オートファジーは細胞内の蛋白質、オルガネラ等を分解しリサイクルする分子機構である。最近オートファジーと老化の密接な関係性を示す報告が続いている。ハダカデバネズミは高いオートファジー活性を持つことも報告されているが、その分子メカニズムと生理的な機能に関してはまだ不明であった。そこで本論文において論文提出者は、まず Atg5 ノックダウン細胞を用いて Atg12-Atg5 の高発現がハダカデバネズミ細胞の高い基礎的なオートファジー活性に寄与することを明らかにした。また、Atg5 が細胞の恒常性維持に重要な役割を果たすことを確認し、恒常性が破綻した細胞には増殖抑制と細胞死が誘導されることを見出した。そして、がん抑制因子である p53/Rb がこの増殖抑制と細胞死誘導に関与することを示した。これらの発見はハダカデバネズミの長寿と抗がんのメカニズムに新たな識見を与えるものである。

よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。