# 論　文　内　容　の　要　旨

## Synopsis of Thesis

| 氏　　名<br>Name | 崔　夢瑩（CUI MENGYING） |
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| 論文題名<br>Title | HKDC1, a target of TFEB, is essential to maintain both mitochondrial and lysosomal homeostasis, preventing cellular senescence<br>(TFEBの新規標的であるHKDC1は、ミトコンドリアとリソソームの両方の恒常性を維持し、細胞老化を防ぐために不可欠である) |

論文内容の要旨

〔目　的(Purpose)〕

Mitochondrial and lysosomal functions are intimately linked and are critical for cellular homeostasis, as evidenced by the fact that cellular senescence, aging, and multiple prominent diseases are associated with concomitant dysfunction of both organelles. However, it is not well understood how the two important organelles are regulated. Transcription factor EB (TFEB) is the master regulator of lysosomal function and is also implicated in regulating mitochondrial function; however, the mechanism underlying the maintenance of both organelles remains to be fully elucidated. The purpose is to clarify whether the homeostasis of the two organelles is coordinated simultaneously.

〔方法ならびに成績(Methods/Results)〕

By comprehensive transcriptome analysis and subsequent chromatin immunoprecipitation-qPCR, we identified hexokinase domain containing 1 (HKDC1), which is known to function in the glycolysis pathway as a direct TFEB target. Moreover, HKDC1 was upregulated in both mitochondrial and lysosomal stress in a TFEB-dependent manner, and its function was critical for the maintenance of both organelles under stress conditions. Mechanistically, the TFEB-HKDC1 axis was essential for PINK1 (PTEN-induced kinase 1)/Parkin-dependent mitophagy via its initial step, PINK1 stabilization. In addition, the functions of HKDC1 and voltage-dependent anion channels, with which HKDC1 interacts, were essential for the clearance of damaged lysosomes and maintaining mitochondria-lysosome contact. Interestingly, HKDC1 regulated mitophagy and lysosomal repair independently of its prospective function in glycolysis. Furthermore, loss function of HKDC1 accelerated DNA damage-induced cellular senescence with the accumulation of hyperfused mitochondria and damaged lysosomes.

〔総　括(Conclusion)〕

Our results show that HKDC1, a factor downstream of TFEB, maintains both mitochondrial and lysosomal homeostasis, which is critical to prevent cellular senescence.

# 論文審査の結果の要旨及び担当者

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## 論文審査の結果の要旨

In this research, the authors identified *HKDC1* as a target gene of TFEB, based on the RNA-seq analysis upon both mitochondrial and lysosomal stress. They clarified HKDC1' s important role in PINK1 stabilization during mitochondrial depolarization. By doing proteomics analysis with the sample expressing Full-length HKDC1 and that expressing ΔN20 HKDC1 which losing its mitochondria binding domain, they found several interactors, including Parkin, Ubiquitin and the known interactors VDAC1. In addition, TOM70 is a novel protein which is known to be required for PINK1 import into mitochondria. Through co-IP they confirmed HKDC1 interacted with TOM70. Moreover, the silencing of TOM70 certainly blunts PINK1 stabilization in HKDC1-overexpressing cells. Taken together, HKDC1 serves as a key factor in PINK1 stabilization through interaction with TOM70. Next, they examined whether HKDC1 maintains lysosomal homeostasis. They checked the turnover of damaged lysosomes by detecting galectin-3(Gal-3). After LLOMe washout 10h, in the siLuc-transfected cells, Gal-3 puncta were cleared up to almost no puncta, while knockdown of HKDC1 impeded the clearance of Gal3 puncta. So next they checked the lysosomal repair. Intriguingly, knockdown of HKDC1 impaired recruitment of CHMP4B, the component of ESCRT, indicating that HKDC1 is important for lysosomal repair. Why lysosomal repair requires a mitochondrial protein? Based on a previous report showing mitochondria-lysosome contact occurs through VDAC1, and HKDC1 can interact with VDAC1. They hypothesized HKDC1 can regulate mitochondria-lysosome contact sites. They used a split GFP-based contact sensor containing mitochondrial and lysosomal targeting sequence to detect their contact. When mitochondria and lysosomes locate in close proximity, GFP signals can be detected. The CLEM images exhibit GFP puncta exactly locate between mitochondria and lysosomes. LLOMe treatment increased GFP puncta, while HKDC1 knockdown decreased. Thus, HKDC1 is crucial for maintaining mitochondria-lysosome contact. Also, they examined which function of HKDC1 is required for maintaining mitochondrial and lysosomal homeostasis by expressing ΔN20 HKDC1 which makes HKDC1 unable to localize on mitochondria and SA HKDC1 which loses its hexokinase activity. Both Full Length HKDC1 and SA HKDC1 rescued PINK1 stabilization in mitophagy process and ESCRT component recruitment on damaged lysosomes, while ΔN20 HKDC1 did not restore PINK1 stabilization and ESCRT component recruitment, suggesting that HKDC1' s scaffolding function on mitochondria is critical for maintaining mitochondrial and lysosomal homeostasis. Lastly, HKDC1 increased in the DNA damage-induced senescent RPE1 cells. Importantly, after treating with doxorubicin, HKDC1 knockdown indeed showed many senescence-like phenotypes, increased p16 and p21, elevated SA-β-gal positive cells and upregulated IL-1α and IL-1β mRNA levels. Notably, IL-1α and IL-1β mRNA restoration also requires HKDC1 scaffolding function since only ΔN20 HKDC1 did not rescue IL-1α / IL-1β mRNA level.

This research is worth being granted a doctoral degree(medicine).