Nrf2 negatively regulates CMA to rescue macroautophagy deficiency induced liver dysfunction

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As an evolutionarily conserved metabolic process autophagy functions in transporting intracellular components by the autophagosome to the lysosome for degradation, to meet metabolic needs and to relief stress. Three main forms of autophagy have been identified: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA), which also affect each other by regulation. CMA, a pathway present in all mammalian cells, cytosolic proteins targeted for degradation are identified by the chaperone HSC70 that recognizes a pentapeptide motif (KFERQ) in their amino acid sequence. These KFERQ motif – substrate proteins are transported to, and docks at the surface of lysosomes via interacting with lysosome-associated membrane protein type 2A (LAMP-2A). Translocation of the substrate across the lysosomal membrane also requires the presence of a luminal form of HSC70 (Lys-HSC70), then substrate proteins are rapidly degraded by the lysosomal enzymes. Different from macroautophagy, CMA does not require the ATG molecules and autophagosomes.

Our previous study found that macroautophagy deficiency in livers causes severe hepatomegaly and liver injury, accompanied by inflammation, fibrosis, and tumorigenesis, which can be rescued by *Nrf2* co-deletion. Thus, we hypothesis that CMA plays a key role during this rescue process. Our data showed that the molecular weight of LAMP-2A increased in both *Atg7* knockout and *Atg7/Nrf2* double knockout livers. Meanwhile, the expression level of LAMP-2A was higher and substrate proteins of CMA, including HSC70, IκBα and GAPDH, were lower in Atg7/Nrf2 double knockout livers than those in Atg7 knockout livers. In the livers of *Atg7/Nrf2* double knockout mice injected with leupeptin, an inhibitor of lysosomal proteases, the expression of LAMP-2A and Annexin I increased significantly. These data indicates that CMA may be enhanced in Atg7 knockout livers, which is more significant in livers with *Atg7* and *Nrf2* co-deletion. To further confirm this result, we analyzed by immunoblotting the P10 fraction of the livers, which mainly contain the lysosomes and the mitochondria. The data showed that the molecular weight of LAMP-2A and expression level of HSC70 were altered in lysosomes of both *Atg7* knockout and *Atg7/Nrf2* double knockout livers, compared to that in *Nrf2* knockout livers.

The substrate proteins, IκBα and GAPDH, also decreased significantly. In hepatic lysosomes of *Atg7/Nrf2* double knockout mice injected with leupeptin, the expression level of LAMP-2A and substrate proteins increased significantly, compared to that in liver lysosomes of Atg7 knockout mice injected with leupeptin. Then we investigated the role of Nrf2 on CMA by an *in vitro* model. Upon treatment of AML-12 and Huh-7 cells with CDDO-ME, an activator of Nrf2, the expression of LAMP-2A decreased, and that of CMA substrate Annexin I was increased, in a dose dependent manner. Thus, Nrf2 activation seems to inhibit CMA. Conversely, we found that CMA activity could be enhanced by Nrf2 knockdown in Huh-7 cells. The in vitro data showed that Nrf2 plays a negatively regulatory role on CMA activity.

Overall, our results demonstrate that CMA activity in Atg7 and Nrf2 co-deletion livers is enhanced, which may contribute to the rescue of the liver dysfunction caused by macroautophagy deficiency.