ABSTRACT

Mitochondrial protein homeostasis is crucial for cellular function and integrity. It is ensured by many specific mitochondrial proteases with possible chaperone functions located across the various mitochondrial subcompartments.

In the first part, we have focused on characterization of functional overlap and cooperativity of proteolytic subunits AFG3L2 and YME1L of the mitochondrial inner membrane complexes m- and i-AAA in HEK293 cells. The double AFG3L2/YME1L knockdown cells showed severe alteration in OPA1 protein processing, marked elevation in OMA1 protease and severe reduction in SPG7. Our results reveal cooperative and partly redundant involvement of AFG3L2 and YME1L in the maintenance of mitochondrial protein homeostasis and further emphasize their importance for mitochondrial and cellular function and integrity.

The aim of the second part was to characterize the cellular function of LACE1 (lactation elevated 1) in mitochondrial protein homeostasis. LACE1 protein is a human homologue of yeast Afg1 (ATPase family gene 1) ATPase. We show that LACE1 is a mitochondrial integral membrane protein that exists as a part of three complexes of approximately 140, 400 and 500 kDa. We demonstrate that LACE1 mediates degradation of nuclear-encoded complex IV subunits COX4, COX5A and COX6A. Using affinity purification of LACE1-FLAG expressed in LACE1-knockdown background, we show that the protein interacts physically with COX4 and COX5A subunits, YME1L protease, and p53 protein. We demonstrate by ectopic expression of both K142A Walker A and E214Q Walker B mutants that an intact ATPase domain is essential for LACE-mediated degradation of nuclear-encoded complex IV subunits. We further show that LACE1 exhibits significant pro-apoptotic activity, which is dependent on p53, and is necessary for mitomycin c-induced translocation of p53 into mitochondria. Our work have identified that LACE1 has a role in protein turnover of subunits of the oxidative phosphorylation system and mediates mitochondrial translocation of p53 and its transcription-independent apoptosis.

Key words: AFG3L2 protease, apoptosis, complex IV, LACE1 protein, mitochondria, oxidative phosphorylation, p53 tumor suppressor protein, respiratory chain, translocation, YME1L protease